Food Allergy:

Adverse Reactions to Foods and Food Additives

Third Edition

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Third Edition

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*Dr. Metcalfe's work as an editor and author was done in a personal capacity, not as part of his official Government duties. Thus the views expressed do not necessarily represent the views of the National Institutes of Health, the Department of Health and Human Services, or the United States.

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Preface

It is the privilege of the editors to present the third Edition of Food Allergy: Adverse Reactions to Foods and Food Additives. As in the first two editions, we have attempted to create a book that in one volume would cover pediatric and adult adverse reactions to foods and food additives, stress efforts to place adverse reactions to foods and food additives on a sound scientific basis, select authors to present subjects on the basis of their acknowledged expertise and reputation, and reference each contribution thoroughly. The growth in knowledge in this area continues to be gratifying, and is reflected in the increased length of this edition. Again, this book is directed toward clinicians, nutritionists, and scientists interested in food reactions, but we also hope that others interested in such reactions will find the book to be a valuable resource.

The text is divided into sections covering basic and clinical perspectives of adverse reactions to food antigens; adverse reactions to food additives; and contemporary topics. The number of chapters addressing these areas has been increased from 29 chapters in the first Edition and 38 chapters in the second Edition to 42 chapters in the third Edition. Basic science begins with overview chapters on immunology of particular relevance to the gastrointestinal tract as a target organ in allergic reactions and the properties that govern reactions initiated at this site. Two chapters are now devoted to food biotechnology and genetic engineering.

The section on clinical adverse reactions to foods begins with separate overview chapters on immediate reactions to foods in infants and children as well as in adults, and then presents chapters dealing with distinct clinicopathologic entities (eczema, urticaria, respiratory diseases, anaphylaxis, gluten-sensitive enteropathy, exercise and pressure-induced syndromes, and occupational reactions to food allergens). Other chapters deal with eosinophilic syndromes and infantile colic.

Adverse reactions to food additives are covered in a separate division. Chapters address specific additive sensitivities, including those to sulfites, monosodium glutamate, tartrazine, benzoates, and parabens. Other chapters address food colorings and flavors, and skin reactions and asthma related to additives.

The final division of the book is devoted to contemporary topics in adverse reactions to foods. This includes discussions of the pharmacologic properties of food, the history and prevention of food allergy, diets and nutrition, neurologic reactions to foods and food additives, psychiatry and adverse reactions to foods, connective tissue and inflammatory bowel disease, and a review of unproven diagnostic and therapeutic techniques. New chapters have been added on seafood toxins, future approaches to therapy, and hidden food allergens.

Each of the chapters in this book is capable of standing alone, but when placed together they present a mosaic of the current ideas and research on adverse reactions to foods and food additives. Overlap is unavoidable but, we hope, is held to a minimum. Ideas of one author may sometimes differ from those of another, but in general there is remarkable agreement from chapter to chapter. We, the editors, thus present the third Edition of a book that we believe represents a fair, balanced, and defensible review of adverse reactions of foods and food additives.

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Part 1

Adverse Reactions to Food Antigens
Basic Science

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1

Mucosal Immunity

Lloyd Mayer

Introduction

An allergic response is thought to be an aberrant, misguided, systemic immune response to an otherwise harmless antigen. Therefore, an allergic response to a food antigen can be thought of as an aberrant mucosal immune response. The magnitude of this reaction is multiplied severalfold when one looks at this response in the context of normal mucosal immune responses, that is, responses that are suppressed or down-regulated. The current view of mucosal immunity is that it is the antithesis of a typical systemic immune response. In the relatively antigen-pristine environment of the systemic immune system, foreign proteins, carbohydrates, or even lipids are viewed as potential pathogens. A coordinated reaction seeks to localize and subsequently rid the host of the foreign invader. The micro- and macroenvironment of the gastrointestinal (GI) tract is quite different, with continuous exposure to commensal bacteria in the mouth, stomach, and colon, and to dietary substances (proteins, carbohydrates, and lipids) that, if injected subcutaneously, would surely elicit a systemic response. Yet pathways have been established in the mucosa to allow such non-harmful antigens/organisms to be tolerated (1, 2). In fact, it is believed that the failure to tolerate commensals and food antigens is at the heart of a variety of intestinal disorders (e.g., celiac disease and gluten [3, 4]; inflammatory bowel disease and normal commensals [4–7]). Thus, it makes sense that some defect in mucosal immunity predisposes a person to food allergy. This chapter will lay the groundwork for the understanding of mucosal immunity, and subsequent chapters will focus on the specific pathology seen when the normal immunoregulatory pathways involved in this system are altered.

Mucosal Immunity is Associated with Suppression: the Phenomena of Controlled Inflammation and Oral Tolerance

As stated in the introduction, the hallmark of mucosal immunity is suppression. Two linked phenomena symbolize this state: controlled/physiologic inflammation and oral tolerance. The mechanisms governing these phenomena are not completely understood, because the dissection of factors governing mucosal immunoregulation is still evolving. It has become quite evident that the systems involved are complex and that the rules governing systemic immunity frequently do not apply in the mucosa. These tissues possess unique compartmentalization, cell types, and routes of antigen trafficking, all of which come together to produce the immunosuppressed state.

Controlled/Physiologic Inflammation (Fig. 1–1)

The anatomy of the mucosal immune system underscores its unique aspects. A single layer of columnar epithelia separates a lumen replete with dietary, bacterial, and viral antigens from the lymphocyte-rich environment of the underlying loose connective tissue stroma called the lamina propria. Histochemical staining of this site reveals an abundance of plasma cells, T cells, B cells, mac-

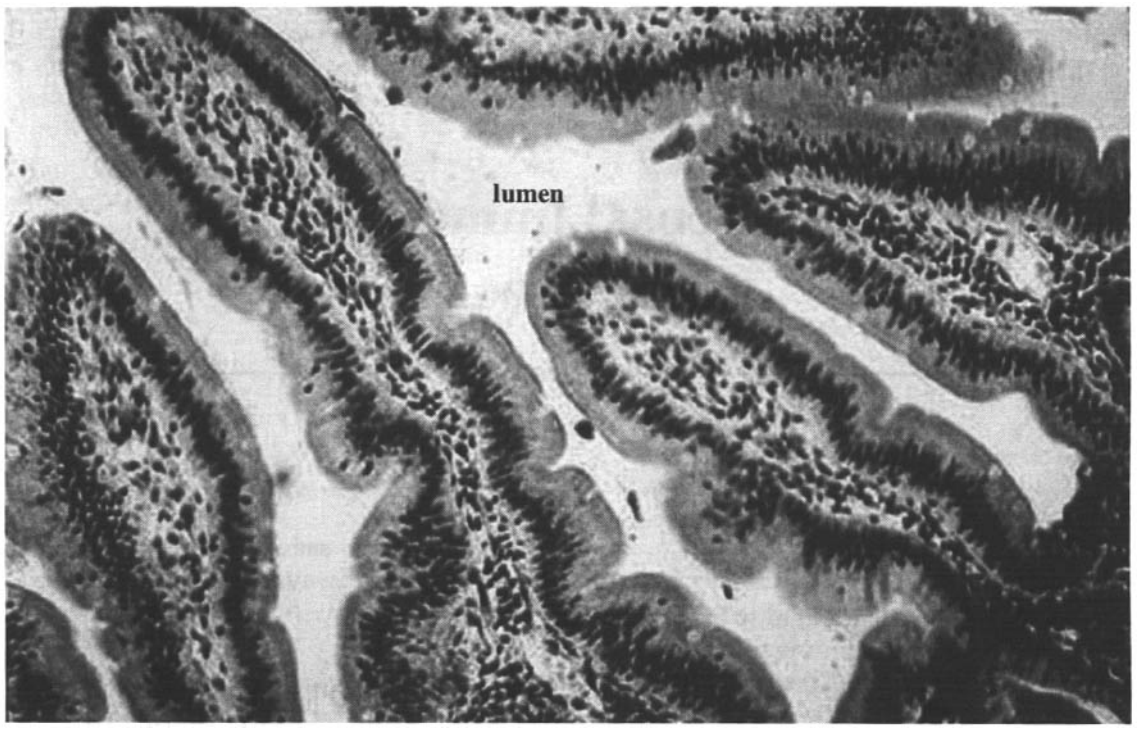

Figure 1–1. Hematoxylin and eosin stain of a section of normal small intestine (20×). Depicted are the villi lined with normal absorptive epithelium. The loose connective tissue stroma (lamina propria) is filled with lymphocytes, macrophages, and DCs. This appearance has been termed controlled or physiologic inflammation.

rophages, and dendritic cells (DCs) (2, 8–10). The difference between the lamina propria and a systemic lymph node is that there is no clear-cut organization in the lamina propria, and its cells are virtually all activated memory cells. Although the cells remain activated, they do not cause destruction of the tissue or severe inflammation. The cells appear to reach a certain stage of activation but never make it to the next step. This phenomenon has been called controlled/physiologic inflammation. The entry and activation of the cells into the lamina propria is antigen driven. Germ-free mice have few cells in their lamina propria, but within hours to days of colonization with normal intestinal flora (no pathogens), there is a massive influx of cells (11–14). Despite the persistence of an antigen drive (luminal bacteria), these cells fail to develop into aggressive, inflammation-producing lymphocytes and macrophages. Interestingly, many research groups have noted that cells activated in the systemic immune system tend to migrate to the gut, possibly due to the likelihood of re-exposure to a specific antigen at a mucosal rather than a systemic site. Activated T cells and B cells express the mucosal integrin $\alpha_4\beta_7$, which recognizes its ligand, the adhesion molecule MAdCAM (11–18), on high endothelial venules (HEV) in the lamina propria. They exit the venules into the stroma and remain activated in the tissue. Bacteria or their products play a role in this persistent state of activation. Conventional ovalbumin-T cell receptor (OVA-TCR) transgenic mice have activated T cells in the lamina propria even in the absence of antigen (OVA), whereas OVA-TCR transgenic mice crossed to a recombinase activating gene-2 (RAG-2) deficient background (hence no B or T cells) fail to have activated T cells in the lamina propria (19). In the former case, the endogenous TCR can rearrange or associate with the transgenic TCR, generating receptors that recognize luminal bacteria. This tells us that the drive to recognize bacteria is quite strong. In the latter case, the only TCR expressed is that which recognizes OVA, and even in the presence of bacteria, no activation occurs. If OVA is administered orally to such mice, activated T cells do appear in the lamina propria.

So antigen drive is clearly the important mediator. The failure to produce pathology despite the activated state of the lymphocytes is the consequence of suppressor mechanisms at work. Whether these mechanisms involve regulatory cells, cytokines, or other, as yet undefined, processes, remains to be determined. It may reflect a combination of events. It is well known that lamina propria lymphocytes (LPLs) respond poorly when activated via the TCR (20, 21). They fail to proliferate, although they still produce cytokines. This phenomenon may also contribute to controlled inflammation; cell populations cannot expand, but the cells can be activated. Conventional cytolytic T cells (class I restricted) are not easily identified in the mucosa, and macrophages respond poorly to bacterial products such as lipopolysaccharide (LPS) because they down-regulate a critical component of the LPS receptor, CD14, which associates with Toll-like receptor-4 (TLR4) and MD2 (22). In the OVA-TCR transgenic mouse mentioned above, OVA feeding results in the influx of cells but there is no inflammation, even when the antigen is expressed on the overlying epithelium (23). All of these observations support the existence of control mechanisms that tightly regulate mucosal immune responses.

Clearly, there are situations in which the inflammatory reaction is intense, such as with infectious diseases or ischemia. However, even in the setting of invasive pathogens such as *Shigella* or *Salmonella,* the inflammatory response is limited, and restoration of the mucosal barrier following eradication of the pathogen is quickly followed by a return to the controlled state. Suppressor mechanisms are thought to be a key component of this process as well.

Oral Tolerance (Fig. 1–2)

Perhaps the best-recognized phenomenon associated with mucosal immunity and equated with suppression is oral tolerance (24–29). Oral tolerance can be defined as the active, antigen-specific non-response to antigens that are administrated orally. Many factors play a role in tolerance induction, and it may be that there are multiple forms of

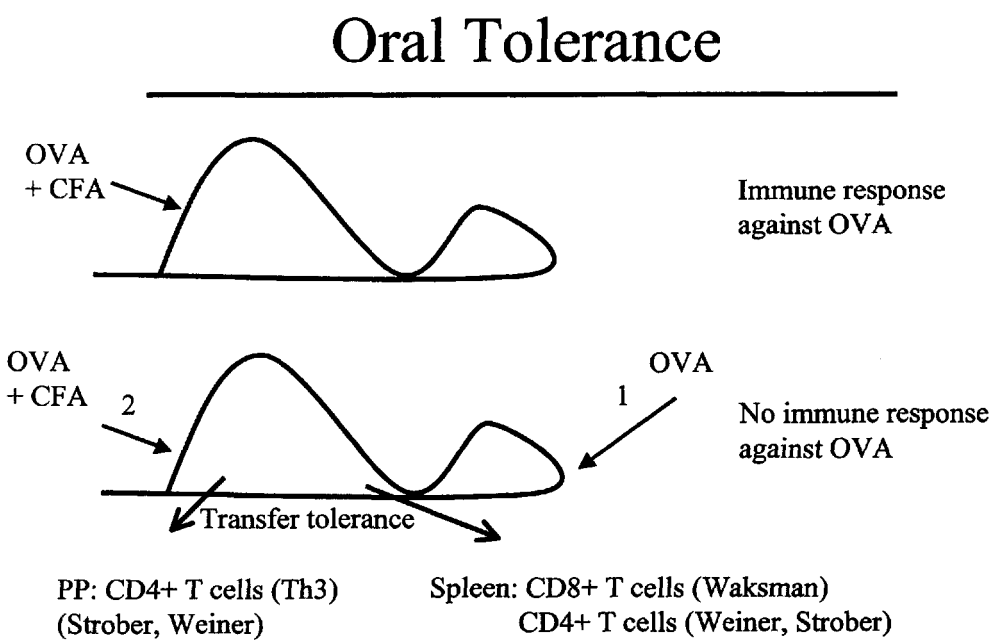

Figure 1–2. Comparison of immune responses elicited by changing the route of administration of the soluble protein antigen ovalbumin (OVA). The upper panel represents the response to systematically administered antigen. There is both a T and B cell response. However, if the mouse is initially administered OVA orally, systemic immunization fails to generate a T or B cell response. Tolerance is an active process—it can be transferred by either Peyer's patch $CD4^+$ T cells or splenic $CD8^+$ T cells. These findings suggest that there are multiple mechanisms involved in tolerance induction.

tolerance elicited by these different factors. The concept of oral tolerance arose from the recognition that immune responses do not normally arise to foods that are eaten, despite the fact that they can be quite foreign to the host. Part of the explanation for this observation is trivial, relating to the properties of digestion. These processes take large macromolecules and, through aggressive proteolysis and carbohydrate and lipid degradation, render potentially immunogenic substances non-immunogenic. In the case of proteins, digestive enzymes break down large polypeptides into non-immunogenic di- and tri-peptides that are too small to bind to major histocompatibility complex (MHC) molecules. However, several groups have reported that upwards of 2% of dietary proteins enter the draining enteric vasculature intact (30). Two percent is not a trivial amount, given the fact that, for example, Americans eat 40–120 grams of protein per day in the form of beef, chicken, or fish.

The key question, then, is this: How do we regulate the response to antigens that have bypassed complete digestion? The answer is oral tolerance. Its mechanisms are complex (Table 1–1) and depend on age, genetics, nature of the antigen, form of antigen, dose of antigen, and the state of the mucosal barrier.

Several groups have noted that oral tolerance is difficult to achieve in the neonate (31). This may relate to the relatively permeable barrier that exists in the newborn or the immaturity of the mucosal immune system. Within 3 months of age (in the mouse), oral tolerance can be induced, and many previous antibody responses to food antigens are suppressed. The limited diet in the newborn may further serve to protect the infant from generating a vigorous response to food antigens. However, as alluded to above, for many of these issues there are still more questions than answers.

The next factor involved in tolerance induction is the genetics of the host. Lamont and coworkers (32) published a study detailing tolerance induction using the same protocol in different mouse strains. Balb/c mice tolerize easily whereas some other strains failed to tolerize at all. Furthermore, some of the failures to tolerize were antigen specific; upon oral feeding, a mouse could be rendered tolerant to one antigen but not another. This finding suggested that the nature and form of the antigen play a significant role in tolerance induction. Protein antigens are the most tolerogenic, whereas carbohydrate and lipids are much less effective in inducing tolerance (33). The form of the antigen is also critical; for example, a protein given in soluble form (e.g., OVA) is quite tolerogenic whereas, once aggregated, it loses its potential to induce tolerance. The mechanisms underlying these observations have not been completely defined but appear to reflect the nature of the antigen-presenting cell (APC) and the way in which antigen traffics to the underlying mucosal lymphoid tissue. Insolubility or aggregation may also render a luminal antigen incapable of being sampled (2). In this setting, non-immune exclusion of the antigen would lead to immunological ignorance from lack of exposure of the mucosa-associated lymphoid tissue (MALT) to the antigen in question.

The dose of antigen administered is also critical to the form of oral tolerance generated. In mouse models, low doses of antigen appears to activate regulatory/suppressor T cells (34, 35). There are an increasing number of such cells identified, of both CD4 and CD8 T cell lineages. Th3 cells were the initial regulatory/suppressor cells described in oral tolerance (35–37). These cells appear to be activated in the Peyer's patch (PP) (see below) and secrete transforming growth factor beta (TGF-β). This cytokine plays a dual role in mucosal immunity; it is a potent suppressor of T and B cell responses while promoting the production of IgA (it is the IgA switch factor) (31, 38–40). TGF-β is the most potent immunosuppressive cytokine defined and its activities are broad and nonspecific. The production of TGF-β by Th3 cells elicited by low-dose antigen administration helps to explain a phenomenon associated with oral tolerance, bystander suppression. As mentioned earlier, oral tolerance is antigen specific, but if a second antigen is co-administered systemically with the tolerogen, T and B cell responses to that antigen will be suppressed as well. The participation of other regulatory T cells in oral tolerance is less well defined. Tr1 cells produce interleukin (IL)-10 and appear to be involved in the suppression of graft-versus-host disease (GVHD) and colitis in

Table 1–1.
Factors Affecting the Induction of Oral Tolerance

Age of host (reduced tolerance in neonate)
Genetics of host
Nature of antigen (protein >>> carbohydrate >>>>> lipid)
Form of antigen (soluble > particulate)
Dose of antigen (low dose → regulatory T cells; high dose → clonal deletion or anergy)
State of the barrier (decreased barrier → decreased tolerance)

mouse models, but their activation during oral antigen administration has not been as clear-cut (41–43). There is more evidence for the activation of $CD4^+CD25^+$ regulatory T cells during oral tolerance induction protocols, but the nature of their role in the process is still being studied (44–47). Lastly, initial studies suggested that antigen-specific $CD8^+$ T cells were involved in tolerance since transfer of splenic $CD8^+$ T cells following feeding of protein antigens could transfer the tolerant state to naïve mice (48–51). Minimal work in this area has been done since the description of the $CD4^+$ regulatory T cells. However, like the various forms of tolerance described, it is likely that the distinct regulatory T cells defined might work either alone (depending on the nature of the tolerogen) or in concert, to orchestrate the suppression associated with oral tolerance, and more globally, mucosal immunity.

Higher doses of antigen lead to a different response: either clonal anergy or deletion (52). In this setting, transfer of T cells from such tolerized animals does not lead to the transfer of tolerance. Clonal deletion may be a common mechanism given the enormous antigen load in the GI tract.

The last factor affecting tolerance induction is the state of the barrier. This was alluded to earlier in the discussion relating to the failure to generate tolerance in the neonate. However, several states of barrier dysfunction are associated with aggressive inflammation and a lack of tolerance. While the exact mechanisms explaining this have not been defined, it is speculated that barrier disruption leads to altered pathways of antigen uptake and failure of conventional mucosal sampling and regulatory pathways. For example, treatment of mice with interferon-gamma (IFN-γ) can disrupt the mucosal barrier. These mice fail to develop tolerance to OVA feeding (53, 54). IFN-γ disrupts the interepithelial tight junctions, allowing for paracellular access by fed antigens. IFN-γ affects many different cell types, so mucosal barrier disruption may be only one of several defects induced by such treatment. N-cadherin is a component of the epithelial cell barrier, and N-cadherin dominant-negative mice develop mucosal inflammation (loss of controlled inflammation) (55). These mice are immunologically intact yet fail to suppress inflammation, possibly because of the enormous antigenic exposure produced by a leaky barrier. Although no oral tolerance studies have been performed in these animals, the concept that controlled inflammation and oral tolerance are linked suggests that defects in tolerance would exist in these animals as well.

Do these phenomena relate to food allergy? There is no clear answer yet. No studies of oral tolerance to protein antigens have been done in food-allergic individuals, and data conflict in studies on the integrity of the mucosal barrier in children with various GI diseases (56–60). The studies required are reasonably straightforward and an answer is critically important for our understanding of food allergy. Oral tolerance does exist in humans, although the studies describing it are limited (61). One clear difference between humans and mice is that tolerance is induced for T cells but not for B cells. This difference may have relevance in human antibody-mediated diseases.

The Nature of Antibody Responses in the Gut-Associated Lymphoid Tissue (GALT)

IgE is the antibody most responsible for food allergy. In genetically predisposed individuals, an environment favoring IgE production in response to an allergen is established. The generation of T cell responses promoting a B cell class switch to IgE has been described (i.e., Th2 lymphocytes secreting IL-4). The next question, therefore, is whether such an environment exists in the GALT, and what types of antibody responses predominate in this system.

The production of a unique antibody isotype—secretory IgA (sIgA)—was the first difference noted between systemic and mucosal immunity. In fact, given the surface area of the GI tract (the size of two tennis courts), the cell density and overwhelming number of plasma cells within the GALT, IgA produced by the mucosal immune system far exceeds the quantity of any other antibody in the body. Secretory IgA is a dimeric form of IgA produced in the lamina propria and transported into the lumen by a specialized pathway through the intestinal epithelium (Fig. 1–3) (62). Secretory IgA is also unique in that it is anti-inflammatory. It does not bind classical complement components, but instead binds to luminal antigens, preventing their attachment to the epithelium or promoting agglutination and subsequent removal of the antigen in the mucous layer of the epithelium. These latter two events reflect "immune exclusion," as opposed to the nonspecific mechanisms of exclusion described earlier (the epithelium, the mucous bar-

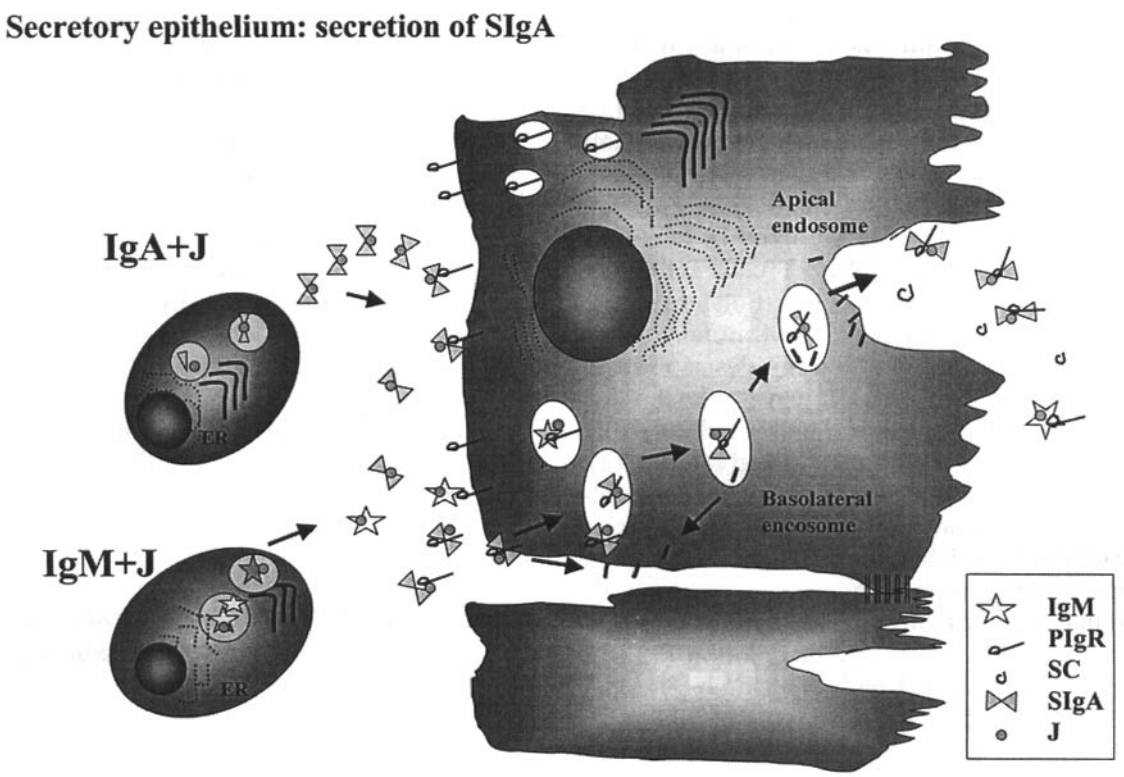

Figure 1–3. Depiction of the transport of secretory IgA (sIgA) and sIgM. Plasma cells produce IgA or IgM monomers, which polymerize after binding to the J chain. Polymerized immunoglobulins are secreted into the lamina propria and taken up by the polymeric immunoglobulin receptor (pIgR) or secretory component (SC) produced by intestinal epithelial cells and expressed on the basolateral surface. Bound sIgA or sIgM are internalized and transcytosed in vesicles across the epithelium and released, with SC, into the intestinal lumen. SC protects the sIg from degradation once in the lumen (Provided by Charlotte Cunningham-Rundles. Mount Sinai Medical Center).

rier, proteolytic digestion, etc). Secretory IgA has one additional unique aspect—its ability to bind to an epithelial cell-derived glycoprotein called secretory component (SC) or to the receptor for polymeric immunoglobulins (pIgR) (63–66). SC serves two functions: it promotes the transcytosis of secretory IgA from the lamina propria through the epithelium into the lumen, and, once in the lumen, it protects the antibody against proteolytic degradation. This role is critically important, because the enzymes that digest proteins are equally effective at degrading antibody molecules. For example, pepsin and papain in the stomach digest IgG into $F(ab)_2$' and Fab fragments. Further protection against trypsin and chymotrypsin in the lumen allows sIgA to exist in a hostile environment.

IgM is another antibody capable of binding SC (pIgR). Like IgA, IgM uses J chains produced by plasma cells to form polymers—in the case of IgM, a pentamer. SC binds to the Fc portion of the antibody formed by the polymerization. The ability of IgM to bind SC may be important in patients with IgA deficiency. Although not directly proven, secretory IgM (sIgM) may compensate for the absence of IgA in the lumen.

What about other Ig isotypes? The focus for years in mucosal immunity was sIgA. It was estimated that upwards of 95% of antibody produced at mucosal surfaces was IgA. Initial reports ignored the fact that IgG was present not only in the lamina propria but also in secretions (67, 68). These latter observations were attributed to leakage across the barrier from plasma IgG. However, recent attention has focused on the potential role of the neonatal Fc receptor (FcR_N), which might serve as a bidirectional transporter of IgG (69, 70). The FcR_N is expressed early on, possibly as a mechanism to take up maternal IgG in breast milk. Its expression was thought to be down-regulated after weaning, but recent studies suggest that it may still be expressed in adult lung and kidney,

and possibly gut epithelium. As suggested above, there are new data suggesting that it might serve to transport IgG both to and from the lumen. In some inflammatory diseases of the bowel, marked increases in IgG in the lamina propria and lumen have been observed (71).

We are left, then, with IgE. Given the modest amounts of this antibody in the serum, its detection in mucosal tissues or secretions has been more difficult than with the other antibodies. However, few studies have attempted to do so. Mucosal mast cells are well described in the gut tissue. The IgE Fc receptor FcεRI is present, and mast cell degranulation is reported (although not necessarily IgE related). FcεRI is not expressed by the intestinal epithelium, so it is unlikely that this molecule would serve a transport function in these cells. CD23, however, has been described on gut epithelial cells, and one model has suggested that it may play a role in facilitated antigen uptake and consequent mast cell degranulation (72, 73). In this setting, degranulation is associated with fluid and electrolyte loss into the luminal side of the epithelium, an event clearly associated with an allergic reaction in the lung and gut. Thus the initial concept that IgA was the be-all and end-all in the gut may be shortsighted, and roles for other isotypes in health and disease require further study.

The Anatomy of the GALT; Antigen Trafficking Patterns (Fig. 1–4)

The final piece of the puzzle is probably the most critical for regulating mucosal immune responses: the cells involved in antigen uptake and presentation. As alluded to earlier, antigen in the GI tract is treated very differently than it is in the systemic immune system. There are additional hurdles for it to jump. Enzymes, detergents (bile salts), and extremes of pH can alter the nature of the antigen before it comes into contact with the GALT. If the antigen survives this onslaught, it has to deal with a thick mucous barrier, and dense epithelium membranes and intercellular tight junctions. Mucin produced by goblet cells and trefoil factors produced by epithelial cells provide a vis-

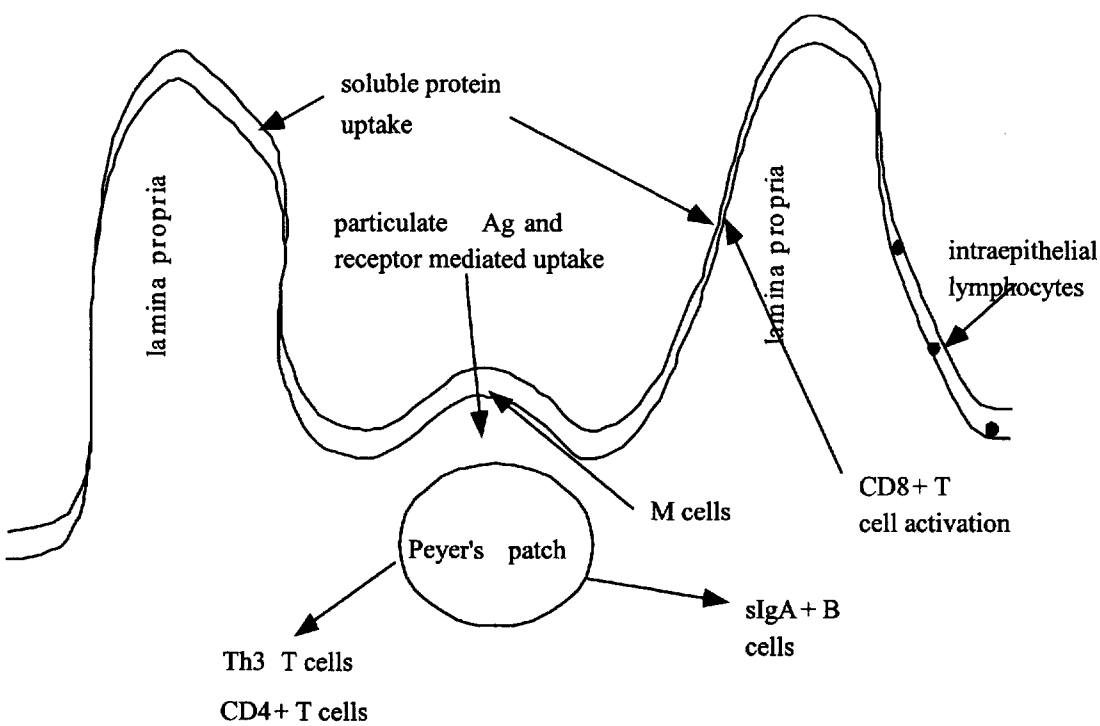

Figure 1–4. Sites of antigen uptake in the gut. Antigens taken up by M cells travel to the underlying Peyer's patch, where Th3 (TGF-β-secreting) cells are activated and isotype switching to IgA occurs (B cells). This pathway favors particulate or aggregated Ag. Antigen taken up by IECs may activate $CD8^+$ T cells, which suppress local (and possibly systemic—tolerance) responses. This pathway favors soluble Ag.

cous barrier to antigen passage. However, despite these obstacles, antigens manage to find their way across the epithelium, and immune responses are elicited.

Probably the best defined pathway of antigen traffic is through a specialized epithelium overlying the only organized lymphoid tissue of the GALT; the Peyer's patch. This specialized epithelium has been called follicle-associated epithelium (FAE) or microfold cell (M cell). The M cell is unique in contrast to the adjacent absorptive epithelium. It has few microvilli, a limited mucin overlayer, a thin, elongated cytoplasm, and it forms a pocket around subepithelial lymphocytes, macrophages, and DCs. The initial description of the M cell not only documented its unique structure but also its ability to take up large particulate antigens from the lumen into the subepithelial space (74–77). M cells contain few lysosomes, so little or no processing of antigen can occur (78). M cells protrude into the lumen, pushed up by the underlying PP. This provides a larger area for contact with luminal contents. The surface of the M cell is also special in that it expresses a number of lectin-like molecules, which helps promote binding to specific pathogens. For example, poliovirus binds to the M cell surface via a series of glycoconjugate interactions (79). Interestingly, antigens that bind to the M cell and are transported to the underlying PP generally elicit a positive (sIgA) response. Successful oral vaccines bind to the M cell and not to the epithelium. Thus, this part of the GALT appears to be critical for the positive aspects of mucosal immunity.

The M cell is a conduit to the PP. Antigens transcytosed across the M cell and into the subepithelial pocket are taken up by macrophages and DCs, and are carried into the PP. Once in the patch, TGF-β-secreting T cells promote B cell isotype switching to IgA (40). These cells leave the patch and migrate to mucosal sites—the lamina propria—where they undergo terminal maturation to dimeric IgA-producing plasma cells.

Several groups have suggested that M cells are involved in tolerance induction as well. The same TGF-β-producing cells activated in the PP that promote IgA switching also suppress IgG and IgM production and T cell proliferation. These are the Th3 cells described by Weiner's group initially (34). There are some problems with this scenario, however. First, M cells are more limited in their distribution, so that antigen sampling by these cells may be modest in the context of the whole gut. Second, M cells are rather inefficient at taking up soluble proteins. As stated earlier, soluble proteins are the best tolerogens, so these two factors together suggest that sites other than PPs are important for tolerance induction. Recent studies have attempted to more clearly define the role of M cells and the PP in tolerance induction. Work initially performed by Kerneis et al (80) documented the requirement of the PP for M cell development. M cell differentiation depended on direct contact between the epithelium and PP lymphocytes (B cells).

In the absence of the PP there are no M cells. In B cell-deficient animals (where there are no PP), M cells have not been identified (81). Several groups looked at tolerance induction in controlled experiments to assess the role of M cells in this process. In most cases, there appeared to be a direct correlation between the presence of PP and tolerance; however, each manipulation (LTβ-/-, LTβR-/-, treatment with LTβ-Fc fusion protein in utero) (82–84) is associated with abnormalities in systemic immunity as well (e.g., no spleen, altered mesenteric LNs, etc), so interpretation of these data is clouded.

The other cell type possibly involved in antigen sampling is the absorptive epithelium. This cell not only takes up soluble proteins but also expresses MHC class I, Ib, and II molecules to serve as restriction elements for local T cell populations (Fig. 1–5). Indeed, a number of groups have documented the capacity of intestinal epithelial cells (IECs) to serve as antigen-presenting cells for both CD4 and $CD8^+$ T cells (85–92). In man, in vitro studies have suggested that normal IECs used as APCs selectively activate $CD8^+$ suppressor T cells (90). Activation of such cells could be involved in controlled inflammation and possibly oral tolerance. Epithelial cells could interact with intraepithelial lymphocytes (IELs) ($CD8^+$ in the small intestine) or LPLs. Mucosal lymphocytes differ from their systemic counterparts in their failure to be activated via the TCR and their preferential use of co-stimulatory pathways (CD2 for IELs; CD2/CD28 for LPLs) (93–96) and cytokines (IL7 for IELs; IL7 and IL15 for LPLs). There is little evidence for a strict Th1 or Th2 microenvironment in the gut. In the normal state there is IFN-γ, IL-10, IL-5, and some TGF-β. Even the Th1/Th2 dogma does not fit in the GALT (except, perhaps, for disease states).

Once again, how does this fit into the process of food allergy? Do allergens traffic differently in predisposed individuals? Is there a Th2-dominant environment in the GALT of food-

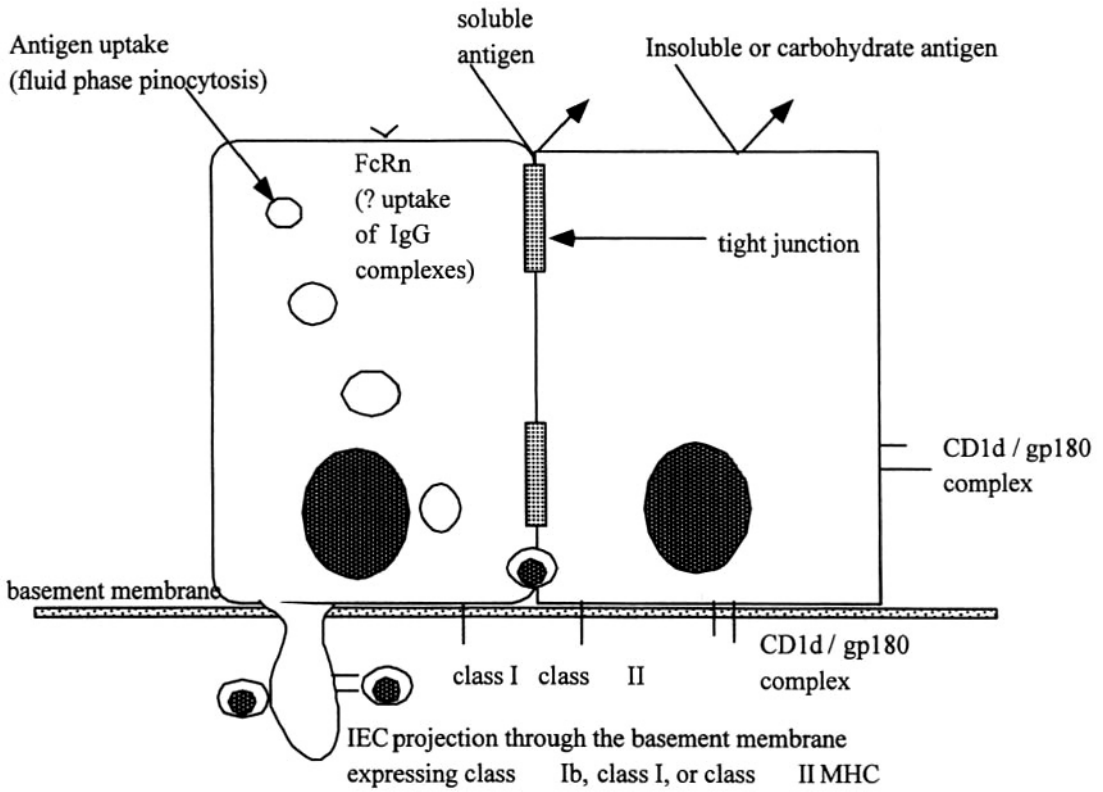

Figure 1–5. Antigen uptake by intestinal epithelial cells (IECs). Soluble proteins are taken up by fluid-phase endocytosis and pursue a transcellular pathway (endolysosomal pathway). Particulate and carbohydrate antigens are either not taken up or are taken up with slower kinetics. Paracellular transport is blocked by the presence of tight junctions. In the case of antigen presentation by the IEC, a complex of a molecule (CD1d) and a CD8 ligand gp180 is recognized by a subpopulation of T cells in the lamina propria (and possibly the intraepithelial space as well). The interaction of IEC with lamina propria lymphocytes occurs by foot processes extruded by the IECs into the lamina propria through fenestrations in the basement membrane. Antigens can also be selectively taken up by a series of Fc receptors expressed by IECs (neonatal FcR for IgG, or CD23 for IgE). The consequences of such uptake may affect responses to food antigens (e.g., food allergy).

allergic patients? As mentioned earlier, IECs do express CD23 induced by IL-4, so there may be a pathway for allergen/IgE complexes to enter from the lumen. However, these are secondary events. The real key is how the initial IgE is produced, and what pathways are involved in its dominance. The answers to these questions will provide major insights in the pathogenesis of food allergy.

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2

Immune Mechanisms in Food-Induced Disease

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Introduction

Food intolerance is a common problem in children and adults. Twenty percent to 45% of the general population complain about adverse reactions to food, and the number is increasing (1). The underlying mechanisms are heterogeneous and by far not restricted to IgE-mediated allergic reactions. Only during early childhood are a high percentage of adverse food reactions IgE mediated. The prevalence of food allergy decreases from about 4% in early childhood to 1%–2% in adults. There is a marked discrepancy between the prevalence of adverse reactions to food on the one hand, and confirmed food allergy on the other hand. Therefore, accurate diagnosis means that confirming the suspicion of food allergy objectively is mandatory in patients with adverse reactions to food.

During the last decade, it became evident that allergic diseases, such as seasonal rhinitis and allergic asthma, are increasing in industrialized countries, particularly in urban areas (2). It is likely that these observations also hold true for food allergy, although this has not been proven. The reasons for this increase are not yet clear, but it is probable that environmental rather than genetic factors are responsible (3). An increasing body of evidence from epidemiological studies suggests that hygiene standards decrease the incidence of infectious diseases and microbial burden, and that this in general favors the development of allergies (4–6). These findings give rise to new strategies for the prevention and therapy of allergies, such as the use of probiotics thought to alter the intestinal flora (7, 8), or the use of bacterial components for induction of an allergen-specific T cell tolerance (9). Recent studies showed that prophylactic treatment of pregnant women with a family history of atopic disease, and of their newborns, for a period of 6 months with *Lactobacillus GG* reduced the prevalence of atopic eczema by 50% in 2-year-old children (7). These findings represent not only a "proof of concept" but also offer new strategies for the prevention of allergic diseases.

The scientific societies for allergy in the USA (American Academy of Allergy, Asthma, and Immunology [AAAAI]) and in Europe (European Academy of Allergology and Clinical Immunology [EAACI]) have classified adverse reactions to food, also named "food intolerance," according to the underlying mechanisms (10–12). First, these classifications distinguish between toxic and non-toxic reactions (Fig. 2–1). Contamination with bacteria or chemicals induce toxic reactions to foods in all individuals, whereas non-toxic reactions only occur in selected individuals and may be based either on immunological alterations or other mechanisms. Within the category of non-toxic responses, examples of non-immune-mediated reactions include enzymatic defects (e.g., lactose intolerance), or abnormal pharmacological reactions toward food additives (Fig. 2–1). In patients suffering from chronic gastrointestinal (GI) disorders such as inflammatory bowel disease (IBD) or irritable bowel syndrome (IBS), the prevalence of non-specific food intolerance is markedly increased

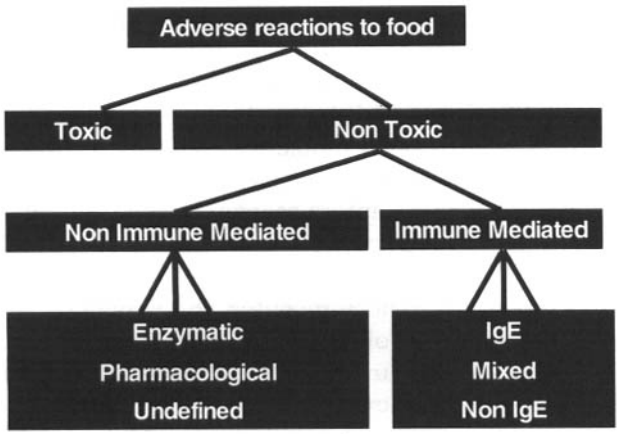

Figure 2–1. Classification of food intolerance. Modified from (10, 11).

(13, 14). At present, the reasons are unclear. Mucosal hypersensitivity caused by inflammation or neuronal dysfunction and bacterial overgrowth have been suggested to be of relevance, at least in some cases of food intolerance (15, 16).

Food allergy, defined as immune-mediated food intolerance of the non-toxic type, can be divided into disorders that are IgE-mediated (e.g., immediate type GI hypersensitivity, oral allergy syndrome, acute urticaria and angioedema, allergic rhinitis, and acute bronchospasm), and non-IgE-mediated (dietary protein-induced enterocolitis and proctitis, celiac disease and dermatitis herpetiformis). Some authors have extended this classification by supposing a third subgroup of mixed IgE- and non-IgE-mediated disorders such as allergic eosinophilic esophagitis and gastroenteritis, atopic dermatitis, and allergic asthma (11). The immune mechanisms of food allergy are not well understood—in particular, IgE-independent reactions. Food allergy can be a consequence of enhanced food antigen entry into the intestinal mucosa, abnormal antigen presentation to lymphocytes, or an uncontrolled inflammatory reaction of the gut or other organs. This chapter will focus on the mechanisms of food antigen uptake and allergic inflammation mediated by mast cells (MCs), basophils, eosinophils, and other effector cells.

The development of food allergy is a multistep process requiring repetitive challenges with a particular food antigen, in contrast to non-immune-mediated reactions, which can cause symptoms even after a single food exposure. Several factors, such as genetic polymorphisms, environmental conditions, mucosal barrier function, mucosal immune function, type and dose of food allergen, route of allergen administration, and age of the afflicted individual are relevant to the development of food allergy. The disease is preceded by a sensitization phase without symptoms, where specific IgEs are raised against selected food proteins to which the individual is exposed (most likely by the oral route) (17). This is of particular importance in newborns whose GI epithelium is not yet fully mature. However, in older children and adults, sensitization via the airways may also play a role, because pollen-associated food allergy becomes more important in these age groups. Inhaled pollen epitopes such as Bet v 1 may share structural similarities to fruit and vegetables epitopes and thereby induce sensitization (18, 19).

Allergen challenge of sensitized individuals results in an immune response inducing tissue inflammation. The sites of allergen sensitization, allergen uptake, and subsequent inflammatory reactions quite often vary, and the factors determining the selection of afflicted organs (e.g., gut, lung, skin, etc.) are unknown (11). In particular, the molecular mechanisms of extra-intestinal manifestations of food allergy are largely unclear. At least two hypotheses may be envisioned, which are not mutually exclusive. First, sensitized T cells or specific IgE may migrate from the site of sensitization to the affected organ. Second, food allergens penetrating the GI epithelium may enter into the blood circulation and so may be transported to other effector organs (Fig. 2–2). In the following text, the steps in the pathogenesis of food allergy (allergen absorption, immune deviation, and allergic inflammation) will be discussed , with particular focus on the GI mucosa.

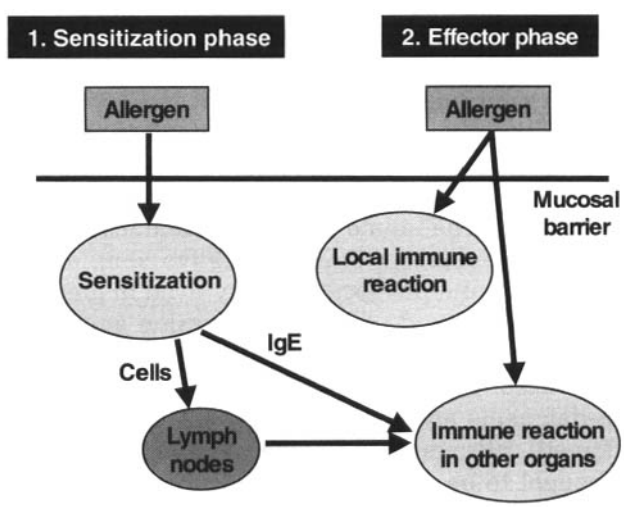

Figure 2–2. Fundamental mechanisms in food allergy.

Intestinal Allergen Absorption

Intestinal Barrier and the Innate Immune System

The intestinal mucosa is constantly challenged with food antigens, microbes, and toxins. The uncontrolled uptake of these potential pathogens through the epithelium may be harmful. Therefore, on one hand, the host has to protect itself against invading pathogens and possible allergens. On the other hand, absorption of nutrients and controlled uptake of antigens is crucial for the development of immunological defense and tolerance (20).

Innate and adoptive mechanisms have evolved to prevent uncontrolled antigen penetration (Fig. 2–3). Gastric acid, mucus, an intact epithelial layer, digestive enzymes, and intestinal peristalsis are nonspecific factors, also named nonimmunological mechanisms (17). The breakdown of such defense mechanisms may result in an increased antigen load in the intestinal tract. For example, an increased mucosal transport of albumin was shown in rats when gastric acidity was neutralized by ingestion of bicarbonate, most likely because gastric proteolysis was less effective at neutral pH (21). The high capacity of the GI epithelium to regenerate itself is of particular importance in maintaining barrier function. Epithelial stem cells are found in the crypts, allowing a renewal of the epithelium every 30–100 hours under normal conditions. Moreover, epithelial proliferation has been shown to be increased in ulcerative colitis and parasitic infection (22). Several factors, such as transforming growth factor-beta (TGF-β), hepatocyte growth factor, and trefoil peptides regulate both the maintenance of barrier function and repair after injury (23, 24).

Similarly, immunological defense mechanisms are of crucial importance in preventing uncontrolled antigen uptake and pathogen invasion in the gut. The innate immune system, consisting of antimicrobial peptides, alternative complement pathways, and phagocytes, is involved in preventing infection and controlling invasion and replication of pathogens and possibly of commensal microbes. Antimicrobial peptides, such α- and β-defensins and cathelicidins, are produced by Paneth cells and granulocytes. These peptides are thought to be effective at the luminal side of the gut (25, 26). Macrophages and neutrophils may be the most important effector cells of the innate immune system, but evidence suggests that other cells, such as MCs and eosinophils, are also involved (27, 28) These cells recognize highly conserved molecular structures present in a large group of microorganisms, and therefore are named pathogen-associated molecular patterns (PAMPs) and commensal-associated molecular patterns (CAMPs). The receptors recognizing these structures are called pattern recognition receptors (PRRs). PRRs consist of the well-characterized macrophage mannose receptor, the scavenger receptors, and the Toll-like receptor family (29, 30). The specific immune system in the gut consists of certain T lymphocyte subsets and of B lymphocytes producing secretory IgA (sIgA) and IgM (sIgM). Most of the lymphocytes are located in the mucosal lamina propria. Immunoglobulins are released into the GI lumen and protect the host by binding and neutralizing luminal antigens and pathogens. It has been shown that sIgA can even bind antigens within the lamina propria and subsequently transport them back to the lumen (20, 31).

Permeability and Uptake of Allergens

The notion was questioned for years that undigested macromolecules such as food allergens pass the intestinal barrier as intact proteins, interact with the local intestinal immune system, and are transported to other body sites such as the skin or the lung. However, several studies indicate that undigested macromolecules such as ovalbumin are taken up by the intestinal mucosa and can be detected in peripheral blood (32). Moreover, the famous so-called "Prausnitz-Küstner-experiment" provides indirect evidence that food allergens may be transported to the skin, where they interact with specific IgE and thereby induce an allergic inflammatory response in sensitized individuals (33).

Persorption, or the absorption of intact proteins, seems to occur at limited rates under normal conditions and may be a factor in intestinal immunoregulatory reactions. However, due to their immature intestinal mucosa, in infants increased uptake of macromolecules may have clinical consequences (34). The enhanced absorption of macromolecules in newborns also includes maternal proteins such as immunoglobulins and growth factors thought to have important physiological functions. A number of mechanisms permit or facilitate the uptake of macromolecules (Fig. 2–3). Both endocytotic uptake of macromolecules with or without use of specific receptors, and paracellular uptake have been described (17, 35). A particu-

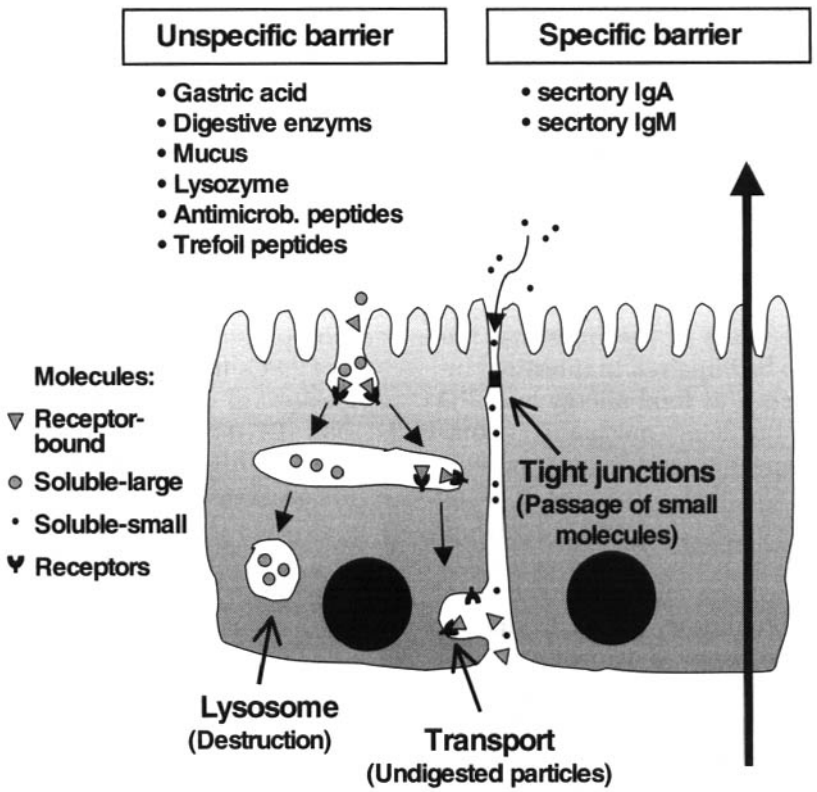

Figure 2–3. Structural and functional components of the intestinal barrier. The maintenance of the intestinal barrier depends on the integrity of the epithelial layer and on several protective factors that are secreted into the lumen. These factors stabilize the barrier (mucus, trefoil peptides), destroy the antigen structure (gastric acid, digestive enzymes, lysozyme, antimicrobial peptides), or neutralize antigens by specific binding (IgA, IgM). Large soluble and receptor-bound macromolecules are taken up by transcellular endocytosis and are then transported to the basolateral membrane or to lysosomes. Small soluble proteins are transported via a paracellular route by passing the tight junctions. Modified from (17).

lar type of epithelial cells named microfold cells or "M cells" covering Peyer's patches is primarily responsible for the normal uptake of macromolecules in the gut. M cells sample antigens and provide access to the underlying lymphoid tissue (36).

The amount of absorbed undigested protein depends on genetic factors and variables such as dietary intake, maturity of digestive processes, and presence of structural or functional abnormalities. Interestingly, some studies have shown that intestinal permeability is increased in patients suffering from food allergy, suggesting that the uptake of food antigens is elevated in food-allergic patients (37, 38). This may be caused, at least in part, by secondary events such as inflammation (39). This hypothesis is supported by the observation that, in animal models, allergen-dependent MC activation causes increased permeability of the intestinal mucosa (40). One study showed that intestinal permeability is increased in patients with bronchial asthma compared to healthy individuals, supporting the hypothesis that a general defect of the mucosal system may facilitate the development of allergic diseases such as asthma and food allergy (41).

Abnormal Immune Reaction in Food Allergy

Regulation of the Mucosal Immune Reaction in the Gut

Gut homeostasis is achieved not only by the regulation of mechanical and biochemical barrier functions limiting the exposure of immunocompe-

tent cells to potentially harmful antigens and toxins, but also by down-regulating the normal immune response to bacteria and other materials. This phenomenon was termed "oral tolerance" because it is induced following oral challenge with particular antigens. Interestingly, oral tolerance, which has been described primarily in the rodent system, covers not only a local but also a systemic tolerance against the orally administered antigen (42, 43).

The hyporesponsivness of the intestinal immune system seems to be impaired in intestinal inflammatory diseases such as food allergy and IBD (44, 45). Active down-regulatory mechanisms comprising different nonspecific (gastric acid, mucus, epithelium) and specific [IgA-producing B cells, immunosuppressive lymphocytes, and tolerogenic antigen-presenting cells (APCs)] immunological systems are required to raise tolerance toward dietary antigens. The breakdown of such mechanisms may increase the risk of sensitization to dietary proteins and, subsequently, of developing food allergy. Indeed, altered antigen uptake caused by barrier dysfunction or decreased specific antigen exclusion has been suggested as a relevant risk factor in the development of food allergy (34). Moreover, early introduction of solid food in babies increases IgE production and the risk of adverse immune reactions, suggesting that the immature GI barrier is of particular importance for the development of food allergy in early life (46). IgA deficiency or retarded IgA development in infants is associated with a somewhat higher risk of atopy (47). An inverse relationship between serum IgE and IgA-producing cells in the jejunal mucosa was found in food-allergic children (48).

The regulation of mucosal B cell switching to IgA- or IgE-producing cells depends on the antigen amount; the route of antigen presentation; the help of regulatory cells such as T cells and other cells including MCs, eosinophils, and basophils; the cytokine and chemokine milieu in tissue; and genetic susceptibility. In this respect, the subtypes of $CD4^+$ T helper (Th) cells are of particular interest (Fig. 2–4). The well-known Th1/Th2 concept has been extended by the characterization of TGF-β secreting Th3 cells and IL-10 expressing T regulatory cells (Tr1) (49, 50). TGF-β and IL-10 are cytokines that promote isotype switching from IgM to IgA in B cells (51). Th2-type $CD4^+$ cells producing IL-4, IL-5, and IL-13 are thought to play a critical role in the development of food allergy, because they promote development of IgE producing Bε cells. Indeed, increased numbers of Th2-type cells and elevated Th2-cytokine levels have been found in vivo in patients with food allergy (52, 53).

Antigen Presentation in the Gut

APCs in the gut are a heterogeneous group of cells consisting of professional APCs, such as dendritic cells (DCs), macrophages and B cells, and non-professional APCs such as epithelial cells, eosinophils, and MCs (54). A typical characteristic of APCs in the intestinal mucosa is the low expression of co-stimulatory molecules such as CD80 (B7–1) and CD86 (B7–2), interacting with CD28 and other counter-receptors on T cells (55). This observation provides one explanation for the hyporesponsivness of the GI immune system, since antigen presentation through major histocompatibility complex II (MHC II) class proteins without further co-stimulatory signals preferentially induces T cell anergy or deletion. In contrast, the up-regulation of co-stimulatory molecules, which is a characteristic feature in IBD, could drive an inappropriate immune response (55, 56). This kind of co-stimulation may also influence the immune response, because it was shown that a CD80/CD28 interaction favors a Th1 type response, whereas a CD86/CD28 interaction favors a Th2 response (57). The engagement of the alternative CD28-homologue B7 receptor, CTLA-4, which is expressed transiently on activated T cells, might provide inhibitory signals when B7 levels are low (58).

There is a remarkable heterogeneity of DCs in the gut. Different types of DCs have been shown to induce distinct immune responses (Fig. 2–4); this has led to the concept of DC1 and DC2 type cells exerting a polarizing signal that drives naive T cells toward a Th1 and Th2 type response, respectively (59). In the human system, plasmacytoid/lymphoid DCs (pDCs) and myeloid DCs (mDCs) have been defined based on phenotypic differences. The pDCs, thought to be equivalent to DC2, generate Th2 type responses, whereas mDCs equivalent to DC1 generate Th1 type responses (60, 61). However, the concept of "one cell type, one type of response" may oversimplify the functional properties of DCs, because the antigenic properties, cytoplasmic milieu, and maturation of DCs, rather than their ontogeny, also seem to influence the type of immune response (62). For example, Kapsenberg et al have shown that prostaglandin E_2 (PGE_2) induces the development of DC2 cells, whereas interferon-gamma (IFN-γ) induces IL-12 producing

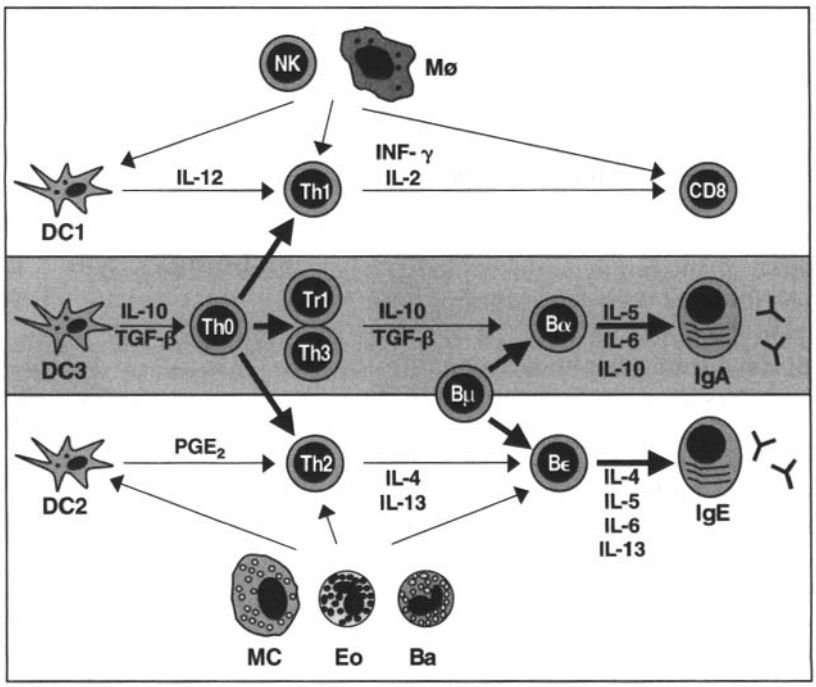

Figure 2–4. Regulation of the mucosal immune response of the gastrointestinal tract—the Th1/Th2/Th3 concept. Different T helper lymphocyte subtypes (Th1, Th2, Th3, and Tr1) drive the immune system toward a cytotoxic ($CD8^+$ cells), or a humoral (IgE- or IgA-dependent) response, respectively. The development of a particular Th cell type (Th1, Th2, or Th3) from naive Th0 cells is influenced by the characteristics of the antigen and by the mode of presentation (e.g., subtype of dendritic cell, cytokine milieu). A balance between Th1/Th2/Th3 and Tr1 immune responses is required for the maintenance of tolerance to harmless food antigens and commensal bacteria. Decreased immunosupressive Th3/Tr1 and enhanced Th2 type immune response is typical in food allergy. B, B cell; Eo, eosinophil; Ba, basophil; MØ, macrophage.

DC1 cells (59). IL-10, however, inhibited maturation of human DCs and converted immature DCs to tolerogenic APCs (also named DC3 cells) (63).

Moreover, the biochemical properties of allergens influence the kind of immune response mounted. In general, soluble proteins are more tolerogenic than particulate or globular antigens (20). Other biochemical characteristics of food allergens affect their absorption and their stability, and thus the amount of allergen with which the mucosal immune system is challenged. For example, the peanut protein Ara h 1 was recently shown to resist degradation because of the formation of stable homo-trimers (64). The observation that low doses of antigens activate regulatory T cells (Th3), whereas high doses of antigen rather induces anergy by apoptosis, further stresses the hypothesis that the dose of antigen influences the subsequent immunological response (20). Moreover, adjuvants may modulate the antigenic potential of food proteins (65).

Immunoglobulin E

IgE was discovered over 30 years ago (66). Since then its unique role in type I hypersensitivity has been recognized and extensively studied. IgE has the lowest plasma level (<100 μg/L in normal adults) and the shortest biological half-life (2.5 days or less) of all immunoglobulin classes. Its plasma level is up-regulated in atopic individuals and in the course of parasitic infections (67). Local production of IgE could be shown in the

nasal mucosa but has not been clearly demonstrated in the intestine, although it has been suggested repeatedly that it may exist (68). For example, IgE was found in the stool of two thirds of the children suffering from intestinal allergy. In contrast, fecal IgE was detected only in one third of the children with extraintestinal allergies and in 10% of healthy children (69). Furthermore, immediate hypersensitivity reactions and MC degranulation could be demonstrated following intramucosal administration of antigen by endoscopic means (70, 71). These findings strongly suggest a local IgE production in the gut, or at least the local presence of IgE derived from peripheral blood.

Enormous progress has been made in our understanding of the regulation of IgE synthesis in mice and humans. Sequential DNA rearrangements that allow the switch from an IgM-producing Bμ cell to an IgE-producing Bε cell depend on at least two signals (Fig. 2–5). CD40L, which binds CD40 on B cells, is necessary for the induction of class switching in B cells. Interestingly, in patients with "hyper-IgM syndrome" lacking IgG, IgA, and IgE, mutations within the genes coding for CD40L or CD40 were found, further confirming the role of the CD40/CD40L system for B cell regulation (67, 72). The second signal determining the immunoglobulin subclass expression in B cells is a soluble factor. For the induction of IgE and IgG_4 (IgG_1 in mice), which seems to be regulated simultaneously, IL-4 has been proposed as the most important factor (73). In humans, however, IL-13 seems to have at least partial functional homologies with IL-4, whereas murine B cells lack an IL-13 receptor (74). The functional relationship of IL-4 and IL-13 is emphasized by the fact that the receptors for both cytokines contain the same α-chain, and that both receptors transduce their signals via the transcription factor STAT 6 (75). IL-4 and STAT 6 knockout mice are unable to synthesize significant amounts of IgE under normal conditions and after infection with nematodes such as *Nippostrongylus brasiliensis* (76). However, minimal IgE production could be detected following infection of IL-4 knockout mice with *Leishmania major, Plasmodium chabaudi,* or the retrovirus that causes mouse immunodeficiency disease. This indicates that an IL-4 independent IgE production exists (67, 77). In contrast, high levels of IgE and allergic-like cutaneous lesions have been observed in IL-4 transgenic mice (78).

The classic model of the induction of IgE production in B cells is presented in Figure 2–5. B cells and other APCs present the particular antigen to primed Th2 cells, which then start producing IL-4. Furthermore, CD40L expressed on Th cells binds CD40 on B cells, thereby providing the second signal for the isotype switch in B cells. Data also show that other cell types, such as basophils, and possibly MCs and eosinophils, may express CD40L and IL-4 (79, 80). Indeed, these cells are capable of inducing IgE production in B cells in vitro (80, 81). The in vivo relevance of these findings is unclear. For example, MC-deficient mice show almost normal IgE levels (82). Therefore, it is unlikely that a single cell type is necessary for IgE induction. One might rather assume a

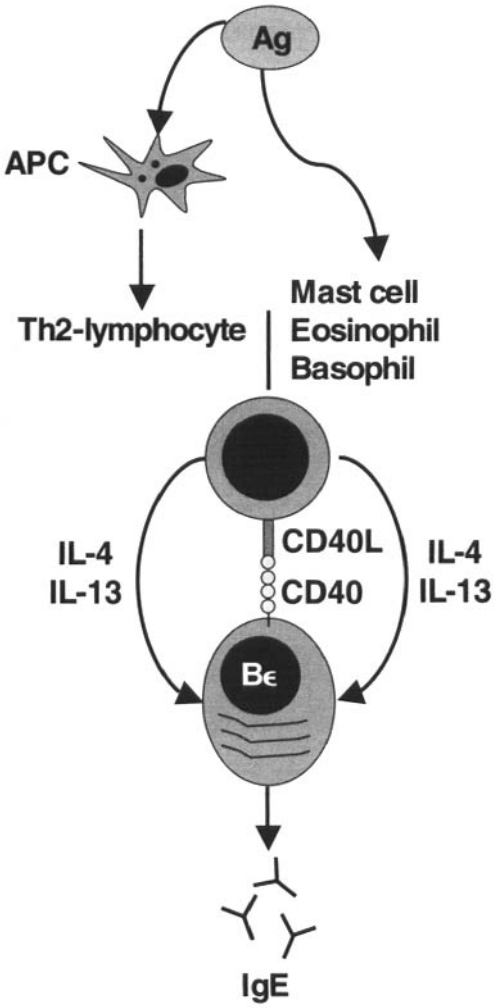

Figure 2–5. IgE switch in B cells. The switch from IgM-producing Bμ cells to IgE-producing Bε cells depends on two signals, CD40/CD40L interaction, and soluble IL-4 and/or IL-13. These signals might be provided by Th2 cells and basophils, possibly also MCs and eosinophils, although less efficiently. Ag, antigen.

redundant system, in which different cells contribute to the regulation of IgE production.

Because T helper lymphocytes only produce IL-4 following challenge with IL-4, this cytokine is a requirement for the formation of Th2 cells. There is ongoing discussion regarding the early source of IL-4, which may derive from MCs, eosinophils, basophils, and natural killer (NK) 1.1^+ $CD4^+$ cells (83). However, human MCs and eosinophils produce no, or only marginal amounts of, IL-4 (84, 85). Apart from IL-4, IL-13, and CD40L, many other factors modulate IgE production. Cytokines such as IFN-γ, IFN-α, TGF-β, IL-8, IL-10, IL-12, and PGE_2, reduce IgE synthesis, whereas IL-5, IL-6, IL-9, TNF-α, macrophage inflammatory protein-1α (MIP-1α), and CD23/CD21 interactions enhance it (67).

The Fc fragment of IgE binds with high affinity to type I IgE receptors (FcεRI) expressed in complete form on MCs and basophils in humans, and with low affinity to FcεRII found on other cell types including lymphocytes, monocytes/macrophages, DCs, Langerhans' cells, eosinophils, platelets, and some thymic epithelial cells (86). The function of FcεRII (also called CD23), which has been suggested to capture allergen and thus facilites allergen presentation (87), is a matter of debate (88).

FcεRI activation of MCs and basophils through the tetrameric $\alpha\beta\gamma_2$ complex is thought to be the primary event in antigen-driven allergic reactions. This ubiquitous activation of MCs and basophils causes the secretion of proinflammatory mediators, initiating a sequence of inflammatory response mechanisms (89–91). In vivo findings and in vitro experiments revealed that IgE itself up-regulates FcεRI on basophils (92) and MCs (93). In accord with this finding, FcεRI expression on MCs and basophils is reduced by 80% in IgE-deficient mice (93). In addition, IL-4, IL-13, and MIP-1α have been shown to up-regulate FcεRI on human MCs (67, 81, 94, 95). During the last several years, IgE receptor signaling has been studied in detail. However, most studies were performed in basophil cell lines or rodent basophils and MCs. Discussion of the details of this complex issue is beyond the scope of this chapter. Therefore, the reader is referred to some recent reviews on this topic (89, 90, 96).

Phasic Induction of Allergic Inflammation

The allergic immune response can be divided into three phases: the sensitization phase, the effector phase consisting of an acute and a facultative late-phase reaction, and a chronic phase that may be the result of repetitive late-phase reactions (Fig. 2–6). As already discussed, the first step in the development of an allergic disease is sensitization to a particular antigen. This process depends on the uptake and processing of the antigen by APCs such as dendritic cells, macrophages or B cells, and the subsequent presentation of antigenic peptides to naive $CD4^+$ T cells (Fig. 2–6). Under the influence of cytokines such as IL-4 and IL- 13, the naive Th0 cells are transformed to Th2 type lymphocytes required for B cells to become plasma cells producing specific IgE directed against the antigen (97, 98). Recurrent antigen exposure may then induce the effector phase, also named acute phase of allergic inflammation, after high-affinity FcεR-bearing MCs and basophils have bound antigen-specific IgE molecules on their surfaces. Cross-linking of these IgE molecules causes activation of MCs and basophils (of particular importance during the late phase), resulting in the release of histamine, leukotrienes, and other mediators. This "immediate reaction" is the basis for the well-known wheal and flare reaction occurring in the skin and at various mucosal sites, including the eyes, nose, lung, and GI tract (Fig. 2–6).

Immediate reactions occurring within seconds to minutes may be followed by late-phase reactions occurring within 2–24 hours; these reactions are characterized by a cellular infiltration with granulocytes (basophils and eosinophils) and lymphocytes (Th2 cells) (99). These reactions have been characterized primarily in the airways and skin. In many individuals, these reactions are elicited by allergen provocation tests. In the GI tract, these phases have been studied less extensively, but there is some evidence suggesting that they occur in a similar fashion (71, 100). A particular characteristic of GI allergy triggered by food antigen is that it may be delayed in time because of the passage time of dietary antigens through the gut. The repetitive occurrence of late-phase inflammatory reactions triggered by ongoing allergen exposure may lead to a chronic inflammatory response in sensitized individuals. The pathology of such a chronic inflammation consists of a mixture of immediate and late-phase reactions accompanied by arteriolar dilatation, increased vascular permeability, stimulation of sensory nerves, and impaired GI function. The proinflammatory mediators and cytokines induce the up-regulation of adhesion molecules and the release of chemotactic factors such as chemokines, causing a persistent infiltration of eosinophils, basophils, and

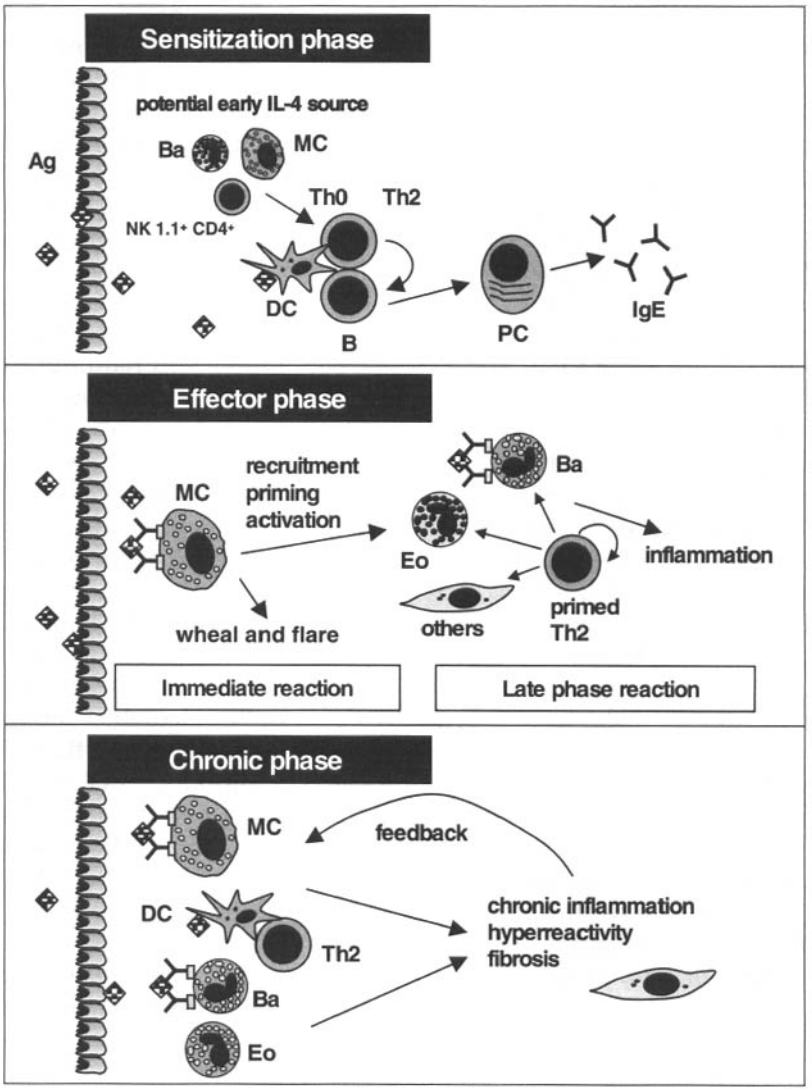

Figure 2–6. Phases of allergic inflammation. For explanation see text. Ag, antigen; DC, dendritic cell; Th2, Th2 lymphocyte; B, B cell; PC, plasma cell; MC, mast cell; Eo, eosinophil; Ba, basophil; Ly, lymphocyte.

allergen-specific lymphocytes; and subsequent chronic structural changes such as fibrosis and organ dysfunction (Fig. 2–6). It has been suggested that a persistent inflammation may elicit a kind of positive feedback loop that results in an ongoing inflammatory response even without further allergen contact (67).

Allergic Inflammation: Role of Mast Cells, Eosinophils, and Basophils

General Remarks

The role of MCs, eosinophils, and basophils in allergic inflammation is well established. MCs reside at mucosal surfaces and, therefore, have been suggested to initiate allergic reactions at mucosal sites, such as when food antigens enter the GI mucosa and bind to specific IgE on MC surfaces (71). In contrast, eosinophils and basophils are blood leukocytes that need to be recruited to sites of allergic inflammation before they exert their effector functions (101). Evidence for this scenario is based mainly on animal studies, in vitro experiments, histological findings in humans, and measurements of specific inflammatory mediators in body-derived fluids, stool, and tissue homogenates. For example, genetically modified mice that have defects in eosinophil (IL-5 and eotaxin knockout) or MC (*c-kit* mutant) physiology show a decreased immune response

in experimentally induced food allergy compared to normal mice (102, 103). In IL-9 transgenic mice that exhibit increased MC numbers, allergen exposure in sensitized animals caused an increase of specific IgE production and concomitant local edema in the small bowel (104). Such findings strongly suggest that MCs and eosinophils play a role in the pathophysiology of food allergies.

In patients suffering from food allergy, MC, eosinophil, and basophil numbers are elevated in peripheral blood and/or afflicted tissue sites. Inflammatory mediators produced by MCs, eosinophils, and basophils, such as histamine and its metabolite methylhistamine, tryptase, eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), IL-5, and TNF-α have been measured in serum, urine, and stool and have been shown to be increased in patients with food allergy (105, 106). The notion that MCs, eosinophils and basophils become activated after food allergen exposure to the skin, lung (107), or intestine (71, 108, 109) is further emphasized by histological studies showing degranulation and cytokine production in these cell types, and by the measurement of enhanced levels of proinflammatory mediators after allergen provocation tests.

However, one should consider MCs, eosinophils, and basophils not only as effector cells of allergic inflammation, but also as immunoregulatory cells involved in host defense against microbes and other pathogens (90, 110, 111). For example, these cells have a well-established role in defense against parasitic infections that are typically accompanied by MC hyperplasia, eosinophilia, basophila, elevated IgE levels, and a Th2-type immune response (27, 112, 113). Recent studies also indicate that these cells have the capacity to modulate the host's innate immune response to several bacteria and viruses (110, 114, 115). MCs and eosinophils can phagocytose bacteria, process and present bacterial antigens to T cells, and release proinflammatory mediators upon challenge with selected bacteria, leading to neutrophil accumulation (116). Some bacterial components such as lipopolysaccharide (LPS), peptidoglycan, activating CpG-oligonucleotides, and the fimbriae protein FimH have been identified as MC or eosinophil agonists, respectively, acting via so-called "pattern-recognition receptors" such as CD48 or Toll-like receptors (117–120). The responses are not restricted to bacteria, since Ig-binding superantigens like protein Fv, released in the intestine of patients affected by viral hepatitis, as well as HIV-1 glycoprotein gp 120, stimulate MCs and basophils though IgE crosslinking (121, 122).

Eosinophila and MC hyperplasia are also found in many non-allergic, non-infectious diseases such as IBD and other chronic inflammatory diseases, fibrotic disorders, and neoplasia. For many years, it has been thought that MCs are important in tissue remodeling, fibrinolysis, angiogenesis, and induction of fibrosis (91, 123, 124). Taken together, MCs, eosinophils, and basophils play a role not only in food allergy but also in a number of other infectious and inflammatory diseases, suggesting that such inflammatory cell types are of general physiological relevance for host defense and tissue transformation.

Morphology and Phenotype

MCs are round or oval cells with unlobed nuclei that are found in many tissues, such as the skin and mucosa, where they preferentially locate around blood vessels and nerves. They show typical staining characteristics of their proteoglycan and protease-rich cytoplasmic granules that led to the recognition of MCs by Paul Ehrlich in 1877 (125). Two MC subtypes have been described in rodents, the connective tissue MCs (CTMC), the dominant subtype in the skin and peritoneal cavity, and the mucosal MCs (MMC) located predominantly in intestinal lamina propria and in lung mucosa. The two subtypes show remarkable differences in size, histamine content, and proteoglycan and neutral protease composition, as well as in functional responsiveness to various secretagogues and inhibitory drugs (91, 126). Human MCs are commonly classified according to their protease content. MCs containing tryptase only (MC_T) predominate in the lung and intestinal mucosa. Tryptase- and chymase-positive MCs (MC_{TC}) are located mainly in the skin and the intestinal submucosa (127, 128). Transmission electron microscopic studies of human MCs indicated that MC_T have cytoplasmic granules with scroll-like configurations. MC_{TC} lack scroll-containing granules but contain granules with crystalline or "grating/lattice" substructures. However, occasional human MCs exhibit mixtures of these substructural patterns (129, 130). Human skin MCs (99% MC_{TC}) respond to IgE-independent agonists such as the anaphylatoxin C5a or substance P, whereas lung MCs (93% MC_T) are largely unaffected by these substances. Surprisingly, MCs isolated from other organs (tonsils or intestine) containing both subtypes do not respond to C5a or substance P. Thus,

the functional significance of this classification and the stability of the phenotypic characteristics is not yet established (131, 132).

Eosinophil is a term suggested by Paul Ehrlich for the granular leukocyte type that he described in 1879 (133) and that has striking affinity to the acid dye eosin. He explained this staining feature by the high content of cationic proteins within the cytoplasmic granules (133). Eosinophils, which are similar in size to neutrophils but have bilobed nuclei, normally account for only 1%–3% of peripheral blood leukocytes, and their presence in tissue is limited primarily to the GI mucosa, which forms the largest eosinophil reservoir of the body. In the course of diseases such as allergy, eosinophils can selectively accumulate in the peripheral blood or any tissue (134). Ultrastructural analysis shows that eosinophils contain different types of granules, the terminology of which varies in the literature (135). Primary granules are core-less, Charcot-Leyden crystal (CLC) protein-containing granules. Most granular proteins, except CLC and several preformed cytokines, are stored in the core-containing specific granules, also called secondary granules. Small-type granules may represent an activated compartment derived from specific granules that are characterized by enzymatically active arylsufate B. Eosinophils also have a large number of secretory vesicles called microgranules or tubulovesicular structures, which may be compartments holding membrane-bound receptors and secretory proteins that can be rapidly mobilized upon cellular activation. Eosinophils also contain non-membrane bound lipid bodies also found in MCs (135, 136). Hypodense eosinophils with a specific gravity of less than 1.085 g/mL can be distinguished from normodense eosinophils. Hypodense eosinophils are found after exposure to activating cytokines in vitro. Elevated numbers of hypodense eosinophils are also present in vivo in many hypereosinophilic disorders, indicating that they may represent an activated state. These cells show enhanced mediator release, adhesion and migration responses, and prolonged survival (137, 138).

Basophils, also described by Paul Ehrlich in 1879 (133), have often been considered as the circulating progenitor of tissue MCs because of their similar morphology and staining characteristics due to the basophilic granule contents, and their overlapping functional properties such as the IgE-dependent release of proinflammatory mediators. However, it is generally accepted now that MCs and basophils originate from separate lineages (90). However, it may be that basophils with some features of MCs (presence of tryptase, for example) can be found in patients with atopic disease (139). Basophils form a small population in the peripheral blood (0.5%–1% of total leukocytes) and reside there under normal conditions. They enter the tissue at sites of inflammation. Basophils have been detected particularly in allergic late phase reactions within the skin and the lung (99, 140), whereas their involvement in GI pathologies is largely unknown. Basophils are smaller than MCs and have a polylobed nucleus. They lack scroll-containing granules but do have CLCs and granules with particulate and crystalline substructures (129, 141).

Development and Tissue Recruitment

Eosinophils and basophils mature fully within the bone marrow and are subsequently released to the peripheral blood. Their migration from peripheral blood to the target organ is a stepwise process and depends on binding to the endothelium and subsequent transmigration into the tissue. MCs leave the bone marrow and enter the tissue as immature progenitors (Fig. 2–7). Studies in mice and humans demonstrate that a committed MC progenitor may exist in the peripheral blood (142, 143). Electron-microscopic studies revealed that MC progenitors are found not only in peripheral blood but also in tissue (144). These data indicate that MC migration from blood to parenchymal organs occurs at an early state of maturation. The development and recruitment of these cells is controlled by several growth factors, chemoattractants, and adhesion molecules on the surfaces of MCs, eosinophils, and basophils that recognize a complementary counter-receptor on the surface of the endothelium (101, 145, 146) (Table 2–1, Table 2–2, and Fig. 2–7). These are controlled by cytokines such as TNF-α, IL-1β, and IL-4 (101, 138). Selectins, and their binding partners the mucins, are thought to play a role in the early rolling, or "tethering," of the cells on the endothelium, although this interaction is too weak to promote extravasation. Further binding involving integrins and their counter-receptors, such as intercellular adhesion molecules (ICAMs) and vascular adhesion molecules (VCAMs), on the endothelial surface causes tight adhesion, followed by diapedesis of the cells. Subsequent migration of MC, eosinophils, and basophils into the tissue follows a gradient of chemotactic factors such as chemokines

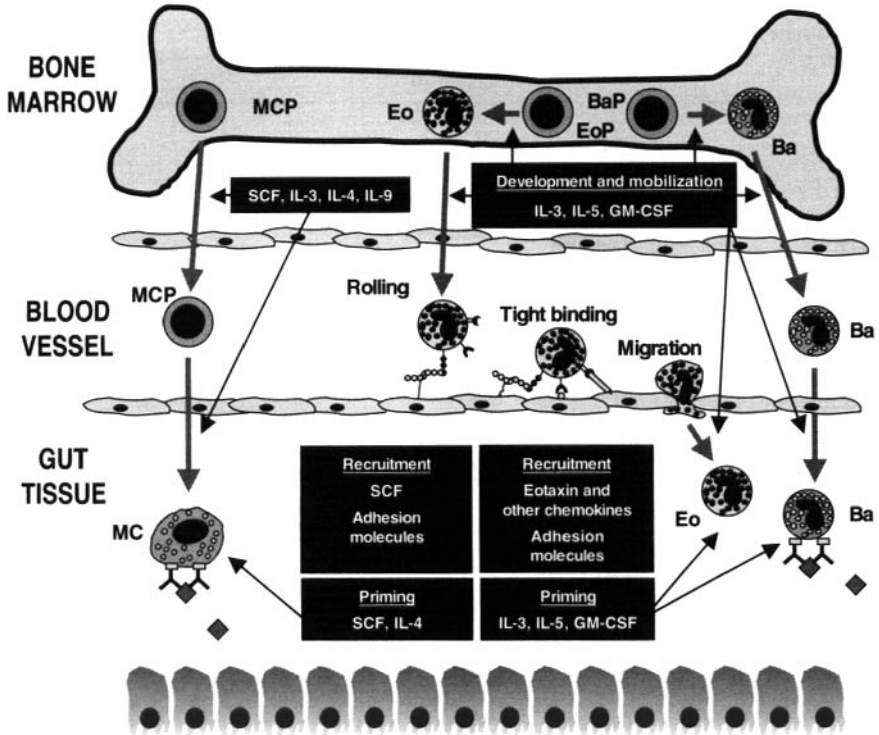

Figure 2–7. Development and recruitment of effector cells of allergic inflammation. MCs, basophils and eosinophils derive from myeloid progenitors of the bone marrow, where basophils and eosinophils but not MCs mature. MC progenitors (maturing in tissue), basophils and eosinophils are found in peripheral blood from where they move to the site of allergic inflammation. The major growth factors, chemokines and adhesion molecules involved in the different steps of cell development, maturation and priming for mediator release are indicated. For detailed explanation see text. MCP, mast cell progenitor; EoP, eosinophil progenitor; BaP, basophil progenitor.

produced by endothelial cells, fibroblasts, and inflammatory cells (147).

MC maturation is regulated by particular cytokines and other factors, such as PGE_2 and interaction with adhesion molecules (148, 149). Stem cell factor (SCF), provided either in a soluble form or membrane-bound on fibroblasts, endothelial cells, or stromal cells, is an essential factor for both MC maturation and growth in humans and mice (91, 150, 151). The importance of SCF and its receptor KIT is stressed by the fact that SCF- or *c-kit*-deficient mice are MC deficient (152). Furthermore, intradermal SCF administration in rodents and humans induces MC hyperplasia (153), and activating *c-kit* mutations have been found in many mastocytosis patients (154). IL-3, IL-5, IL-6, IL-9, nerve growth factor (NGF), and thrombopoietin (TPO) have been shown to promote MC development (Table 2–3 and Fig. 2–7), although, some data indicate that granulocyte macrophage-colony-stimulating factor (GM-CSF), IFN-α, IFN-γ, and TGF-β suppress MC growth. In humans, all factors are dependent on the presence of SCF, whereas in rodents, IL-3 alone induces partial MC development in vitro (155). IL-4 promotes MC maturation as indicated by enhanced FcεRI expression, cytokine production, and chymase synthesis. Interestingly, IL-4 diminishes the number of MCs developed from progenitor cells, but strongly enhances growth of tissue MCs, particularly the MC_T subtype derived from the human intestine and lung in the presence of SCF (94, 156). The importance of T lymphocyte-derived factors such as IL-4 in MC tissue homeostasis in vivo is confirmed by the observation that the number of MC_T MCs is substantially decreased in patients with congenital and acquired immunodeficiency diseases affecting T lymphocytes (157).

Table 2–1.
Adhesion Molecules on Human Mast Cells (MC), Eosinophils (E), and Basophils (B)

Type (name, CD)	*Primary ligands*	*Expression*
β1-Integrins (VLA)		
α2β1/VLA-2 (CD49b/CD29)	Collagen, laminin	MC
α3β1/VLA-3 (CD49c/CD29)	Fibronectin, collagen, laminin	MC
α4β1/VLA-4 (CD49d/CD29)	VCAM-1, MAdCAM-1, fibronectin	MC, E, B
α5β1/VLA-5 (CD49e/CD29)	Fibronectin	MC, B
α6β1/VLA-6 (CD49f/CD29)	Laminin	E
β2-Integrins		
LFA-1 (CD11a/CD18)	ICAM-1, -2, -3	MC, E, B
MAC-1 (CD11b/CD18)	C3bi, ICAM-1, -2, fibrinogen	E, B
p150,95 (CD11c/Cd18)	C3bi, fibrinogen	MC, E, B
αd (αd/CD18)	ICAM-3, VCAM-1	E, B
Other integrins		
αvβ3 (CD51/CD61)	PECAM-1, vitronectin	MC
α4β7 (CD107a)	MAdCAM-1, VCAM-1, fibronectin	MC, E, B
Ig gene superfamily		
ICAM-1 (CD54)	LFA-1, Mac-1	MC, E, B
ICAM-2 (CD102)	LFA-1, Mac-1	MC, B
ICAM-3 (CD50)	LFA-1, αd-integrin	MC, E, B
PECAM-1 (CD31)	PECAM-1, αvβ3-integrin	E, B
CD33	Sialoconjugates	MC
Selectins		
L-selectin (CD62 L)	GlyCAM-1, CD34, MAdCAM-1	MC, E, B
Mucins		
PSGL-1 (CD162)	P-selectin	MC, E, B
Lewis x (CD15)	P-selectin	E
Sialyl Lewis x (CD15s)	E-, P-selectin	MC, E, B
Sialyl-dimeric-Lewis x	E-selectin	E, B
Leucosialin (CD43)	ICAM-1	MC
Others		
Pgp-1 (CD44)	Hyaluronic acid	MC, E, B
Siglec-8	Sialic acid	MC, E, B

Table 2–2.
Chemotactic Factor Receptors on Human Mast Cells (MC), Eosinophils (E), and Basophils (B)

Type/Name	*Primary ligands*	*Expression*
Chemokine receptors		
CCR1	MIP-1α, RANTES	E, B
CCR2	MCP-1	B
CCR3	eotaxin-1, -2, -3, MCP-3, -4, RANTES, MEC	MC, E, B
CCR6	MIP-3α	B
CXCR1	IL-8	MC, E
CXCR2	IL-8, GRO-α	MC, B
CXCR4	SDF-1	E, B
Complement receptors		
C3aR	C3a	MC, E, B
C5aR	C5a	E, B
Others		
fMLPR	fMLP	E, B
PAFR	PAF	E, B

IL-3, IL-5, and GM-CSF are particularly effective in regulating eosinophil growth and maturation in mice and humans (Table 2–3 and Fig. 2–7). The receptors of these cytokines have different α chains and a common β chain, which is the signal transducing chain (158). However, in vivo and in vitro studies reveal that IL-5 is the most effective factor for the eosinophil lineage (159). However, mice lacking the IL-5 receptor α chain, as well as the α chains for the IL-3 and GM-CSF receptor, still produce morphologically normal peripheral blood eosinophils, albeit in reduced numbers (160). In contrast, IL-5 transgenic animals possess elevated eosinophil numbers (161). During infection experiments, IL-5 receptor knockout mice showed an impaired helminth clearance, and the IL-5 transgenic mice, an improved helminth clearance (102). In humans, treatment with an anti-IL5-mAb significantly reduced blood eosinophils and sputum eosinophils (162).

Table 2–3.
Mast Cell, Eosinophil, and Basophil Agonists

Function	*Agonists*
Mast cells	
Growth factors	SCF, IL-3, IL-4, IL-5, IL-6, IL-9, NGF, TPO (early progenitors), PGE_2
Mediator release	FcεRI-cross-linking, FcγRI-cross-linking, SCF, IL-4, NGF, LTB_4, bacterial components, substance P, somatostatin, VIP, C3a, C5a
Chemotaxis	SCF, RANTES, eotaxin-1, IL-8, TGF-β, NGF
Eosinophils	
Growth factors	IL-5, IL-3, GM-CSF, NO (prevents apoptosis)
Mediator release	C3a, C5a, fMLP, PAF, IL-3, IL-5, GM-CSF, INF-γ, TNF-α, eotaxin, MCP-3, MCP-4, RANTES, FcαRII-cross-linking, FcγRII-cross-linking, bacterial components
Chemotaxis	Eotaxin-1, -2, -3, MCP-2, -3, -4, MIP-1α, RANTES, MEC, IL-8, C3a, C5a, IL-5, PAF, LTB_4, substance P, somatostatin, VIP
Basophils	
Growth factors	IL-3, IL-5, GM-CSF, TGF-β, NGF
Mediator release	FcεRI-cross-linking, C3a, C5a, fMLP, PAF, IL-3, IL-5, GM-CSF, IL-1, INF-γ, NGF, MCP-1, -3, eotaxin-1, RANTES, MIP-1α, IL-8
Chemotaxis	Eotaxin-1, -2, -3, MCP-1, -3, MIP-1α, RANTES, SDF-1α, C3a, C5a, IL-3, IL-5, GM-CSF

Animal studies have revealed that eosinophil migration out of the bone marrow into the circulation is primarily regulated by IL-5, whereas eotaxin-1 is particularly important and rather selective for eosinophil recruitment from the peripheral blood into the gut (102) or the lung (163). Other chemoattractants have been studied in in vitro transmigration experiments or in knockout mouse models, and seem to be important in eosinophil recruitment (Table 2–3). These chemoattractants include the CCR3 ligands eotaxin-1, -2, and -3, the macrophage chemotactic factor-3 (MCP-3) and MCP-4, the chemokines RANTES and MEC, as well as the anaphylatoxins C3a and C5a (102, 163–165). Apart from cytokines and chemokines, a number of adhesion proteins are involved in the process of eosinophil recruitment into tissue (Table 2–1 and Fig. 2–7).

Basophils, like eosinophils and MCs, arise from $CD34^+$ progenitor cells and develop under the influence of adhesion factors and cytokines (Table 2-1). Numerous in vitro studies show development and maturation of morphological and functional basophil-like cells in the presence of IL-3 (141, 166). Further, IL-3 can maintain the viability of mature basophils in culture for several weeks (167). Infusion of recombinant IL-3 into non-human primates and IL-3 administration to humans after chemotherapy increases basophil counts (168). In addition to IL-3, other growth factors, such as IL-5, GM-CSF, NGF, and TGF-β have been identified (83, 169). The array of growth factors largely overlaps with the factors promoting eosinophil development (Table 2–3 and Fig. 2–7), which may explain the combined involvement of both cell types in many diseases. Only TGF-β may regulate selective basophil development, because it suppresses eosinophil growth and promotes that of basophils (169).

Mediators and Their Effector Functions

MCs, eosinophils, and basophils exert their effector functions mainly by releasing humoral factors such as proinflammatory mediators and cytokines (170). The mediators of all three cell types can be categorized into three groups: 1) preformed secretory granule-associated mediators; 2) de novo synthesized mediators; and 3) cytokines and chemokines that are synthesize mainly de novo but are also stored within secretory granules (Table 2–4).

Regulated degranulation is a crucial event in the activation of MCs, eosinophils, and basophils. Different types of degranulation have been studied in eosinophils and MCs (136). Ultrastructural analyses have shown that these cells undergo

Table 2–4.
Mediators of Mast Cells, Eosinophils, and Basophils

Mast cells	
Granule-associated	Histamine, tryptase, chymase, carboxypeptidase A, heparin, chondroitin sulfate E, many acid hydrolases, cathepsin G
De novo synthesized	$LTC_4/D_4/E_4$, LTB_4, PGD_2, PAF
Cytokines/chemokines	IL-1β, IL-3, IL-5, IL-6, IL-9, IL-10, IL-13, IL-16, IL-18, TNF-α, TGF-β, GM-CSF, IL-8, MIP-1α, bFGF, VPF/VEGF
Eosinophils	
Granule-associated	ECP, EDN (formerly called EPX), MBP, EPO, CLC
De novo synthesized	$LTC_4/D_4/E_4$, LTB_4, PAF, 15-HETE, PGE_1/E_2, TxB_2, oxygen metabolites (H_2O_2, O_2^-)
Cytokines/chemokines	IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-16, TNF-α, TGF-α, TGF-β, GM-CSF, IL-8, RANTES, MIP-1α, MCP-1, eotaxin-1, VPF/VEGF, PDGF-B
Basophils	
Granule-associated	Histamine, tryptase (minor amounts), chondroitin sulfate A, neutral protease with bradykinin-generating activity, β-glucoronidase, elastase, cathepsin G-like enzyme, MBP, CLC
De novo synthesized	$LTC_4/D_4/E_4$
Cytokines/chemokines	IL-4, IL-13, MIP-1α, IL-8 (mRNA)

compound exocytosis, which is characterized by fusion of granules and formation of intracytoplasmatic degranulation channels; piecemeal degranulation, which is a more restricted and selective release of substances in response to particular agonists (171); and necrotic degranulation typically found in allergic lesions (Fig. 2–8).

MC-derived preformed secretory granule-associated mediators are mainly histamine, the neutral proteases tryptase and chymase, and proteoglycans (Table 2–4). Histamine exerts its wide-ranging biological activities via binding to four histamine receptors (H1-H4). With regard to allergic inflammation, H1 seems to be of particular importance, because its activation affects the function of blood vessels (dilatation and increased permeability), smooth muscles (contraction), and epithelial cells (mucus production) (172). The H2 receptor regulates acid secretion in the stomach (173) and mediates some effects on lymphocytes and granulocytes (174). The H3 receptor has been proposed to be important for the regulation of neurons (175), whereas the role of the recently discovered H4 receptor is largely unknown (176). The neutral proteases tryptase and chymase are the major protein components of mast secretory granules. Tryptase degrades fibrinogen and kininogen, and generates C3a (177). It stimulates fibroblasts (178), endothelial (179), epithelial (180), and smooth muscle cells (181), and plays a role in MC-neuron interactions (182). Chymase converts angiotensin I into angiotensin II (183), inactivates thrombin (124), and degrades basement membranes (177). MC-derived heparin is thought to act as a cofactor for anti-thrombin III and tissue-type plasminogen activator, which is also produced by MCs. In vitro studies have shown that MC supernatants induce fibrin clot lysis and, therefore, MCs have been considered to play a role in endogenous fibrinolysis, which seems to be important for several tissue repair processes (124). Upon stimulation, MCs release lipid mediators, in particular cyclo-oxygenase and lipoxygenase metabolites of

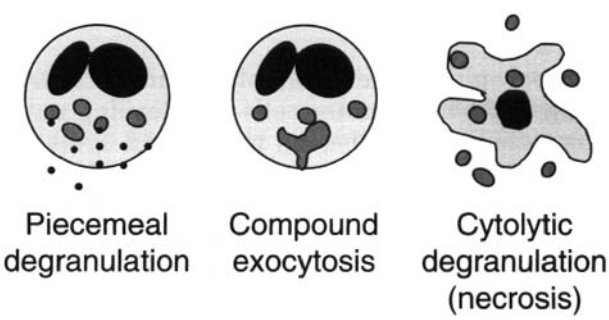

Figure 2–8. Principles of degranulation. Modified from (135, 136).

arachidonic acid. The major cyclo-oxygenase product is prostaglandin D_2 (PGD_2), and the major lipoxygenase products are leukotriene C_4 (LTC_4) and its peptidolytic derivatives LTD_4 and LTE_4. Platelet-activating factor (PAF) and minor amounts of LTB_4 are also produced by human MCs. These eicosanoids exert various proinflammatory and immunoregulatory effects (91, 184).

More recent studies indicate that human MCs produce numerous cytokines upon stimulation, both under normal conditions and during particular states of disease (185). The production of proinflammatory cytokines such as TNF-α and IL-6, and Th2 type cytokines such as IL-3, IL-5, and IL-13, has been studied in detail (84, 186). Conflicting results have been published concerning the release of IL-4 in human MCs, whereas IL-4 production in rodent MCs has been clearly demonstrated (187). In vitro studies with human MCs failed to show substantial IL-4 production upon IgE receptor cross-linking (84), but histological studies demonstrate MC-derived IL-4 in tissues of allergic patients (81, 188).

Eosinophils contain at least five characteristic proteins that are present in large quantities: ECP, EDN (also called eosinophil protein X, or EPX), major basic protein (MBP), eosinophilic peroxidase (EPO), and CLC protein (135). CLC protein and low amounts of EDN are also present in basophils (189), and traces of EDN and ECP are found in neutrophils (190). These proteins exert their effector functions when released into the extracellular compartment or in phagosomes that may contain internalized microbes. ECP, EDN, EPO, and MBP are cytotoxic proteins that cause tissue damage in eosinophil-associated inflammation and mediate direct antimicrobial effects. Furthermore, they alter basophil (ECP, EPO), MC (EPO, MBP), neutrophil (EPO, MBP), T cell (ECP), platelet (MBP), and smooth muscle (MBP) functions, indicating wide-ranging activities. Eosinophils also produce lipid mediators such as $LTC_4/D_4/E_4$, PAF, and thromboxane B_2 (TxB_2) and oxygen metabolites upon stimulation. Eighteen different cytokines and chemokines have been found in eosinophils (102, 110, 135, 191) (see also Table 2–4).

In basophils, histamine is synthesized and stored (about 1 pg per cell) complexed with the highly charged proteoglycan chondroitin sulfate, as opposed to heparin in MCs. It is released from the cytoplasmic granules after cell activation within inflamed tissue. Like MCs and eosinophils, basophils generate large amounts of $LTC_4/D_4/E_4$ but no PGD_2 (192), which is specifically MC-derived. Basophils are a major source of IL-4 and IL-13 (193, 194) which can be released upon IgE-dependent and IgE-independent stimulation (193, 195). In whole blood cultures, the IL-4 content correlates with the presence of basophils (196). On a per-cell basis, activated basophils produce more IL-4 and IL-13 than any other cell type. Because IL-4 and IL-13 are particularly important in initiating a Th2-type response and IgE production in B cells, such findings may be of crucial importance in relation to allergy (97, 98). The cytokine pattern produced by basophils is much more limited than that expressed by MCs and eosinophils. However, the specific expression of IL-4, which is produced in very small amounts by MCs and eosinophils, suggests a particular role for basophils, perhaps also in the early effector phase of allergic inflammation (Table 2–4).

Agonists and Antagonists

The release of so many potentially harmful mediators mandates a sophisticated regulation of effector functions in MCs, eosinophils, and basophils by different agonists and antagonists. MCs and basophils respond rapidly to the binding of antigen to IgE on the cell surface via the high-affinity IgE receptor (FcεRI). An array of mediators is released within minutes (Table 2–3). In vitro experiments showed detectable cytokine mRNA production within 15–30 minutes, whereas proteins were measurable after 2–10 hours, depending on the particular cytokine (84, 197). In vitro studies performed in rodents have shown that TNF-α is stored in the secretory granules of MCs and can be released within 20 minutes of FcεR cross-linking (198). In basophils, histamine and eicosanoid release is nearly complete by 20 minutes, whereas IL-4 and IL-13 production follows a time course, with a maximal response after 4 and 20 hours, respectively. Small amounts of IL-4 (<10 pg/10^6 basophils) become detectable within 5–10 minutes of stimulation, suggesting that pre-formed IL-4 is released (195).

Several IgE-independent MC and basophil triggers have been described (Table 2–3). SCF induces weak histamine and LTC_4 release in MCs, but substantially enhances the FcεR-dependent activation (199, 200). Whereas C5a, substance P, morphine, and compound 48/80 activate human skin MCs, these secretagogues are basically ineffective in human MCs derived from lung and in-

testine, further emphasizing MC heterogeneity (131). Apart from SCF, several cytokines (IL-1, IL-3, GM-CSF) and chemokines (IL-8, MIP-1α) were reported to induce MC histamine release in rodents (91). Many of these findings could not be confirmed in humans. Long-term IL-4 administration to cultured human intestinal MCs induces IL-5 production. Furthermore, IL-4-treated MCs release enhanced amounts of Th2-type cytokines (IL-3, IL-5, IL-9, IL-13) but reduce pro-inflammatory cytokine production upon FcεR cross-linking (94, 201). Also for basophils, a number of IgE-independent secretagogues have been described, such as the anaphylatoxins C3a and C5a, bacteria-derived formyl-methionyl-leucyl-phenylalanine (fMLP), PAF, eosinophil-derived MBP, cytokines (IL-3, IL-5, GM-CSF), and chemokines (MCP-1, MCP-3, eotaxin, RANTES, MIP-1α, IL-8). Of particular interest is the observation that cytokines such as IL-3, IL-5, and GM-CSF induce only small amounts of mediator release, but substantially enhance the effects of almost all IgE-dependent and IgE-independent agonists. The latter effect seems to be of greater importance, particularly in the late phase characterized by lymphocyte infiltration and enhanced cytokine production, and has been named "basophil priming." Similar observations could be made for other inflammatory cells such as eosinophils, suggesting a general principle that governs inflammatory cell regulation (90, 195, 202–208).

FcγR expression has been demonstrated in mice and human MCs as well as in human basophils (Table 2–5). Mouse MCs express low-affinity FcγRIIB and FcγRIII. Cross-linking of FcγRIII induces similar but weaker responses than FcεRI cross-linking (209). In vivo studies with MC-deficient mice revealed that the FcγRIII-dependent MC stimulation is important in the inflammatory response to multivalent IgG immune complexes (210). FcγRIIB, by involving so-called immunoreceptor tyrosin-based inhibition motifs (ITIMs), inhibits mediator and cytokine release triggered by FcεRI aggregation (211–213). Human MCs express FcγRI, which becomes up-regulated by INF-γ. Crosslinking of the high-affinity FcγRI leads to histamine and cytokine release. In addition, flow cytometry analyses demonstrated the expression of FcγRII, and RT-PCR analysis revealed RNA expression of FcγRII A, B1, B2, but not C. FcγRIII appears expressed on human MCs to only a small extent. The function of FcγRII and FcγRIII in human MCs has to be elucidated (214, 215). Human basophils also express the FcγRII (Table 2–5). It has been suggested that, like rodent MCs, the activation of the FcγRII opposes the FcεRI-mediated responses (212).

Several eosinophil secretagogues are known (Table 2–3), such as C5a, C3a, fMLP, and PAF, that cause degranulation directly, whereas other stimuli, such as IL-3, IL-5, GM-CSF, and complement factors have weak or no direct effects. However,

Table 2–5.
Fc Receptors on Mast Cells (MC), Eosinophils (E), and Basophils (B)

Receptor (CD)	*Chains*	*Binding affinity*	*Ligands*	*Expression MC, E, B*	*Other cells*
FcγRI (CD64)	α, γ	Ig1: $10^8\ M^{-1}$	1) IgG1 = IgG3, 2) IgG4, 3) IgG2	MC*, E†	M, N†, DC
FcγRIIA (CD32)	α	Ig1: $2\times10^6\ M^{-1}$	1) IgG1, 2) IgG2‡ = IgG3, 3) IgG4	MC, E,B§	M, N, LC, P
FcγRIIB (CD32)	α‖	Ig1: $2\times10^6\ M^{-1}$	1) IgG1 = IgG3, 2) IgG4, 3) IgG2	MC, E, B§	M,N,B
FcγRIII (CD16)	α, β, γ	Ig1: $5\times10^5\ M^{-1}$	1) IgG1 = IgG3, 2) IgG4, 3) IgG2	MC#, E	M,N,B
FcαRI (CD89)	α, γ	IgA1, IgA2: $10^7\ M^{-1}$	IgA1 = IgA2	E	M,N
FcεRI	α, β, γ	IgE: $10^{10}\ M^{-1}$	IgE	MC,B,E†,**	M**, DC**, LC**
FcεRII†† (CD23)	single	IgE: $10^8\ M^{-1}$,	IgE, others‡‡	E	B, T, M, LC

M, monocytes; N, neutophils; B, B cells; T, T cells; LC, Langerhans' cells; P, platelets. *Only human MCs. †Inducible. ‡Only some allotypes of FcγRII-A bind IgG2. §CD32 expression has been shown, but to date it is not clear whether FcγRIIA or FcγRIIB is expressed. ‖Contains an ITIM motif (inhibitory). #Expression in rodent MCs; in human MCs only minimal expression is inducible. **β-chain is not expressed. ††Two isoforms (a and b) exist. FcεRIIa is mainly expressed on B cells FcεRIIb also on other cells. ‡‡Binds also to CD21 (C3bR and Epstein-Barr virus R), and β_2-integrins (CD11b and CD11c). Modified from (227).

this set of cytokines sensitizes or "primes" eosinophils for enhanced mediator release to other stimulants, including otherwise ineffective agonists (159, 216). Interestingly, PAF produced by eosinophils itself has been considered an autocrine secretagogue, since PAF antagonists inhibit both IgG- and IL-5-induced eosinophil superoxide production and degranulation (217). Furthermore, chemokines such as the CCR3 ligands MCP-3, MCP-4, RANTES, and eotaxin-1 induce degranulation in eosinophils (218). Eosinophil activation by cytokines and immunoglobulins is critically dependent on β2-integrins, especially on Mac-1 (CD11b/CD18). The binding to the counter-receptor of Mac-1, ICAM-1, promotes eosinophil stimulation by PAF, GM-CSF, secretory IgA, and IgG (219).

Fc-receptors have been considered to be involved also in eosinophil activation. FcαRI, FcεRI (without β chain), and FcγRsII and III are expressed on eosinophils (Fig. 2–5 and Table 2–5) . A minor population of resting eosinophils showed surface expression of FcγRI upon stimulation. Secretory IgA and also IgG are strong signals for degranulation in eosinophils (135, 220–222). Compared to basophils and MCs, the role of IgE-cross-linking in eosinophils is less clear. It has been suggested that eosinophil IgE-dependent pathways play a role in allergic disease. However, this is still a matter of controversy, because FcεRI is produced in eosinophils, but not necessarily expressed on the cell surface in a functionally active manner (223). Selective EDN release has been found after stimulation with complexed IgE in one study (224), whereas another study failed to show EDN, LTC4, or superoxide anion production upon FcεRI cross-linking (225).

Conclusion

Food allergy is defined as adverse reactions toward food mediated by aberrant immune mechanisms. The clinical symptoms of food allergy vary considerably among patients depending on the kind of food allergen, the afflicted body site (skin, respiratory tract, gut, other), and the phase of disease (immediate versus late phase, mild versus severe, etc.). This variability indicates that it is unlikely that one distinct immune mechanism underlies food allergy. Possibly not only the clinical presentation but also the pathophysiology is variable, and many of the involved mechanisms are not well understood. This is particularly true in the GI tract, where food allergy is initiated but may not become symptomatic. These circumstances complicate the clinical management of afflicted patients and explain why diagnostic means and therapeutic strategies are not satisfactory to date. However, it also illustrates that improvement of our knowledge of the mechanisms of food allergy would certainly improve the clinical concepts.

As for other types of allergic diseases, the best-defined mechanism is the IgE-mediated allergic reaction. It is generally accepted that many, but not all, forms of food allergy are IgE-mediated. In particular, the IgE dependency has been confirmed for the oral allergy syndrome and other reactions occurring a short time after food ingestion. The mechanisms are much less clear for some delayed reactions involving the lower GI tract or extraintestinal sites. Moreover, in children and adolescents, the percentage of IgE-mediated food reactions is higher than in adults.

The key for understanding the pathophysiology of food allergy lies in the GI tract, the place of food antigen uptake and primary immunological recognition. Therefore, we focused our overview on the GI barrier and its mucosal immune system, as well as on the inflammatory cells such as MCs and eosinophils found in high numbers in normal and particularly in inflamed mucosa. A large section is related to IgE-dependent food allergy. However, we reviewed IgE-independent mechanisms as well. It is clear that immune-mediated reactions and non-immune mediated mechanisms are involved in the pathophysiology of food allergy. For example, antigen uptake must precede any immunological interaction. Therefore, all events facilitating the penetration of food allergen into the mucosal tissue (loss of innate immune functions, disruption of the epithelial barrier, concurrent infections, disturbance of gut flora, etc.) may be important in the development of food allergy. Only during a second phase, when a sufficient amount of antigen has crossed the intestinal barrier, does the specific immune system becomes relevant. We learned that the normal GI immune system is privileged to develop tolerance toward most luminal antigens such as bacterial or food proteins. Although we still do not understand the mechanisms of this tolerance in all its detail, it is likely that food allergy (as well as other immune-mediated diseases such as IBD) is a consequence of loss of oral tolerance against luminal antigens. It is still a matter of debate as to why only 1%–5% of all individuals are afflicted, why only particular antigens induce symptoms, and why in some patients the GI tract is the shock organ but in others, extra-

intestinal sites. These and many other questions stress the necessity of continued research. Our knowledge has improved substantially within the last decade. For example, we have learned much about the functional properties of the central effector cells of allergic inflammation—namely MCs, eosinophils and basophils—and their mediators. We have begun to characterize what modulates sensitization to particular food antigens, such as environmental factors (hygiene, microbial burden, numbers of siblings, etc.), genetic factors, and factors that affect GI barrier function. Hopefully, as this knowledge becomes better specified, new diagnostic and therapeutic strategies will arise to improve the clinical management of patients and to enable new preventive concepts.

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3

Food Antigens:
Structure and Function

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Summary

This chapter provides a summary of molecules that are involved in IgE-mediated food allergies. The application of recombinant DNA technology to the characterization of food allergens has greatly enhanced our knowledge about their structure and function. Based on the recent progress made in the field of food allergen characterization we describe disease-eliciting allergenic molecules according to their occurrence in various foods and discuss possible patho-mechanisms of IgE-mediated food allergies. Furthermore we develop scenarios how the use of recombinant food allergens may improve the diagnosis of IgE-mediated food allergies. Finally concepts for antigen-specific therapy of food allergy based on recombinant DNA technology and synthetic peptide chemistry are presented.

Introduction

The term "adverse reactions to foods" summarizes a wide range of symptoms that can occur after ingestion of certain foods and their ingredients. These symptoms may be caused by a variety of different mechanisms. A position paper by the European Academy of Allergy and Clinical Immunology (EAACI) classifies the reactions according to the underlying patho-mechanisms in toxic and non-toxic reactions (1). If non-toxic adverse reactions to foods are mediated immunologically, the term "food allergy" may be used. The most common forms of food allergy are either connected to or mediated by the production of IgE antibodies to otherwise harmless food antigens and hence may be summarized under the term "IgE-mediated food allergy."

Under normal circumstances, the mucosal immune system is able to distinguish between harmful and harmless antigens. This is essential for mounting protective immune responses and at the same time preventing exaggerated immune reactions against harmless food antigens that would cause mucosal pathology (2). The quality and magnitude of the resulting immune response (tolerance versus priming) seem to be dictated by the type of antigen-presenting cells (APCs) expressing

differential surface molecules and transmitting signals to the lymphocyte and by the local cytokine milieu (3).

On the basis of the clinical appearance, the nature of the disease-eliciting allergens, and the underlying immunological mechanisms, two forms of IgE-mediated food allergy can be distinguished. The first form of IgE-mediated food allergy occurs shortly after birth and during early childhood. The sensitization/priming process appears to occur via the gastrointestinal tract, and in affected children food allergy represents the first manifestation of the atopic syndrome. The most important allergen sources involved in early food allergy are milk, eggs, legumes (e.g., peanut, soybean), meat, fish, and cereals (4). This early manifestation of food allergy is frequently associated with atopic dermatitis (AD). A substantial proportion of the affected children "outgrow" the manifestations of food allergy and AD but, unfortunately, often develop respiratory allergy thereafter (5).

The second type of food allergy develops later in life and is seen mainly in adults with respiratory allergy. This form of IgE-mediated food allergy is believed to be based on allergic cross-sensitization to inhalant allergens (6–8). Patients suffering from allergy to cross-reactive respiratory allergens may develop symptoms of food allergy toward food sources containing cross-reactive allergens. These food allergens can cause symptoms ranging from mild and local forms such as oral allergy syndrome (OAS), to life-threatening anaphylactic shock, often seen in the mugwort-birch-celery syndrome (9, 10). Many of the cross-reactive allergens implicated in the second form of food allergy have been characterized thoroughly and have been produced as recombinant allergens (reviewed in [11, 12]). These molecules can be used as reagents to study the patho-mechanisms underlying food allergies but also for diagnostic and therapeutic purposes.

The fact that children suffering from the first form of food allergy frequently grow out of the disease may be interpreted as so-called "oral tolerance" (13). This phenomenon may suggest therapeutic strategies to utilize the gut-associated lymphoid tissue (GALT) for the induction of tolerance against food allergens (2).

Classification of Food Allergens

A summary of plant-derived food allergens is given in Table 3–1 (14–39). Because of the rapid progress made in the molecular characterization of allergens, it has become possible to assign to the various food allergen sources their disease-eliciting allergen molecules and to categorize them by their names, biological functions, and molecular weights. In this context it is important to discriminate between the terms "allergen source" (e.g., apple, peanut), "allergen extract" (i.e., extract prepared from an allergen source that contains allergenic and non-allergenic molecules as a mixture, e.g., peanut allergen extract), and the term "allergen," which should be reserved for a defined allergenic molecule (e.g., major apple allergen [Mal d 1]) (40). The plant-derived food allergens listed in Table 3–1 are categorized either according to the allergen nomenclature system or by a specific name or designation related to their functions or characteristics. It is clear that certain classes of proteins occur as allergens in many different allergen sources, a fact that explains the frequently observed sensitization to different allergen sources as being due to immunological cross-reactivities. One example of extensive cross-reactivity is sensitization to profilin, a cytoskeletal actin-binding protein that is a cross-reactive allergen in pollen, fruits, vegetables, and even humans (41–45). Other prominent plant-derived food allergens belong to the family of pathogenesis-related (PR) plant proteins (e.g., major birch pollen allergen Bet v 1 and homologous allergens) (12). Although the biological function of the PR proteins has not yet been fully elucidated, it is well established that these proteins may be up-regulated after plants have been subjected to stressful conditions (e.g., pathogen attack). Members of the PR protein group (e.g., β-1,3 glucanases, class I chitinases, thaumatin-like proteins, allergens homologous to the major birch pollen allergen Bet v 1, lipid transfer proteins) have been described in fruits, vegetables, pollen, and other plant-derived products (e.g., latex) (11, 12). Sensitization to cross-reactive members of this allergen family may therefore result in clinical sensitivity to various allergen sources containing homologous allergens. Other plant-food allergens that can be found in a variety of different plant species are proteases, amylase inhibitors, and several seed storage proteins (reviewed in [11]). The fact that certain of these allergens belong to proteins that are predominantly expressed in seeds (e.g., seed storage proteins) but cannot be detected in relevant amounts in other plant tissues (e.g., pollen) that become airborne, suggests that patients reacting with these proteins were

Table 3–1.
Plant-Derived Food Allergens

Allergen Source	*Allergen Name*	*Function/Homology/Information*	*Molecular Weight/ Number of Amino Acids*	*(References)/ Accession Numbers*
Apple	Mal d 1	Homologous to birch pollen, Bet v 1	159 aa	(14, 15)
	Mal d 2	Thaumatin family	245 aa	AAC36740
	Mal d 3	Lipid transfer protein	115 aa	AAF26450
	Profilin	Actin-binding protein	14 kDa	(16)
Apricot	Pru ar 1	Homologous to birch pollen, Bet v 1	160 aa	AAB97141
	Pru ar 3	Lipid transfer protein	91 aa	P81651
Avocado	Per a 1	Endochitinase	326 aa	CAB01591
		β-1,3 glucanase	30 kDa	(17)
Banana		Class I chitinase	>30 kDa	(18, 19)
		β-1,3 glucanase	30 kDa	(20)
Barley	Hor v 15	Trypsin alpha amylase inhibitor	146 aa	P16968
		Peroxidase	36 kDa	(21)
Bell pepper	Cap a 2	Profilin, actin-binding	131 aa	CAD10376
Brazil nut	Ber e 1	2 S albumin	154 aa	CAA38363
Carrot	Dau c 1	Homologous to birch pollen, Bet v 1	154 aa	(22)
		Cyclophilin	20 kDa	(23)
Cashew	Ana o 1	Vicilin-like	50 kDa	(24)
Castor bean	Ric c 1	2 S albumin	258 aa	P01089
Celery	Api g 1	Homologous to birch pollen, Bet v 1	154 aa	(25, 26)
	Api g 3	Chlorophyll a/b binding protein	264 aa	CAA99993
	Api g 4	Profilin, actin-binding	134 aa	AAD29409
Chestnut		β-1,3 glucanase	30 kDa	(17)
Cherry	Pru av 1	Homologous to birch pollen, Bet v 1	160 aa	AAC02632
	Pru av 2	Thaumatin-like protein	245 aa	AAB38064
	Pru av 4	Profilin, actin-binding	131 aa	AAD29411
Hazelnut	Cor a 1.0401	Homologous to birch pollen, Bet v 1	161 aa	AAD48405
Kidney bean	PR-1	Pathogenesis-related protein 1	156 aa	CAA43637
	PR-2	Pathogenesis-related protein 2	155 aa	CAA43636
Kiwi	Act c 1	Cysteine protease	380 aa	P00785
		β-1,3 glucanase	30 kDa	(70)
Leaf mustard	Bra j 1	2 S albumin	129 aa	P80207
Maize		Pectate lyase	438 aa	S43335
	Zea m 14	Lipid transfer protein	120 aa	P19656
Papaya		Papain	345 aa	AAB02650
Pea		Pollen allergen-like	258 aa	CAA59470
Pear	Pyr c 1	Homologous to birch pollen, Bet v 1	17 kDa	(27)
	Pyr c 4	Profilin, actin-binding	14 kDa	(28)
	Pyr c 5	Phenylcoumaran benzylic ether reductase		(29)
Peach	Pru p 3	Lipid transfer protein	91 aa	P81402

(*continued*)

Table 3–1. (Continued)
Plant-Derived Food Allergens

Allergen Source	*Allergen Name*	*Function/Homology/Information*	*Molecular Weight/ Number of Amino Acids*	*(References)/ Accession Numbers*
Peanut	Ara h 1	Vicilin	>600 aa	P4337, P4338
	Ara h 2	Conglutin	156 aa	AAK96887
	Ara h 3	Glycinin	507 aa	AAC63045
	Ara h 4	Glycinin	530 aa	AAD47382
	Ara h 5	Profilin, actin-binding	131 aa	AAD55587
	Ara h 6	Conglutin-like	129 aa	AAD56337
	Ara h 7	Conglutin-like	160 aa	AAD56719
		Lectin, phytohemagglutinin	236 aa	S14765
		Oleosin	16–18 kDa	(30)
Pepper		Homologous to birch pollen, Bet v 1	17 kDa	(31)
		Profilin	14 kDa	(31)
Pineapple		Profilin, actin-binding	14 kDa	(32)
Plum	Pru d 1	Lipid transfer protein	91 aa	P82534
Potato	Sol t 1	Patatin, storage protein	377 aa	P15476
Rape seed	BNIII	2 S albumin	79 aa	P24565
Rice	RAG2	Trypsin alpha amylase inhibitor	166 aa	Q01885
	RA5	Trypsin alpha amylase inhibitor	157 aa	S31078
	RA14B	Trypsin alpha amylase inhibitor	166 aa	S59922
	RA16	Trypsin alpha amylase inhibitor	157 aa	S59924
	RA17	Trypsin alpha amylase inhibitor	162 aa	S21157
Sesame seed				
Soybean	Gly m 1.0101	Soybean hull allergen	42 aa	AAB34755
	Gly m 2	Hull allergen	20 aa	A57106
	Gly m 3	Profilin, actin-binding	131 aa	O65809
		Lipoxygenase	839 aa	DASYL2
		Alpha or beta-conglycinin	605 aa	CAA35691
		A1aBx subunit of glycinin	495 aa	CAA26723
		A5A4B3 subunit of glycinin	562 aa	CAA26478
	G1 subunit of glycinin	495 aa	CAA33215	
		Lectin Le1	285 aa	AAA33983
		Kunitz trypsin inhibitor	208 aa	CAA56343
		Trypsin inhibitor	217 aa	CAA45777
		G1 glycinin	>50 kDa	(33)
		G2 glycinin	22 kDa	(34, 35)
Tomato		Profilin	14 kDa	(36)
		β-1,3 glucanase	30 kDa	(37)
Walnut	Jug r 1	2 S albumin	139 aa	AAB41308
	Jug r 2	Vicilin-like protein	593 aa	AAF18269
White mustard	Sin a 1	Seed storage protein	145 aa	PC1246
Wheat		Profilin, actin-binding	14 kDa	(38)
	Tri a 19	Omega 5 gliadin		(39)
		Peroxidase	36 kDa	(21)

probably sensitized primarily via the gastrointestinal tract. However, considerable evidence exists that patients reacting with many other plant food allergens (e.g., Bet v 1- or profilin-homologous plant food allergens) were sensitized initially to pollen-derived allergens most likely via the respiratory route, and they consecutively mount IgE cross-reactions with foods containing homologous allergens (6–8). It thus appears that food allergy can be induced via either the respiratory or the gastrointestinal tract. It has, however, not been definitively determined what routes and

mechanisms of allergen exposure are involved in the boosting of established food allergy.

Meanwhile, a considerable number of plant-food allergens have been characterized down to the molecular level, but it has not been possible to define criteria that would allow an unambiguous prediction of the allergenic character of a given protein (46). This problem has received increasing attention with regard to whether genetically modified plants represent potent allergens sources, and how the allergenic potential of the engineered plant should be assessed. Clearly, criteria would be desirable that would allow predictions of whether a protein that has been inserted into a transgenic plant can become a new allergen or may induce the expression of other allergens. The common assumption that a protein with a high degree of sequence similarity to a known allergen will also behave as an allergen has been contradicted by the finding that isovariants of allergens exist that differ from an active variant by only a few amino acids but completely lack allergenic activity (47). Likewise, it has turned out that other characteristics (e.g., stability against digestion) cannot be used to definitively predict whether a protein can act as food allergen (48). For example, members of the profilin or Bet v 1 food allergen family can be digested easily under conditions where other food allergens remain stable (49). Finally, the contribution of carbohydrates to the allergenicity of allergens has remained a controversial issue, especially in light of recent studies suggesting poor biological activity of carbohydrate-containing allergens (50).

Assumptions similar to those made for plant-derived food allergens seem to be applicable to animal-derived food allergens (Table 3–2) (51–57). The fact that proteins with highly divergent biological functions (e.g., albumin, a serum protein; parvalbumin, a calcium-binding muscle protein) can act as allergens contradicts the hypothesis that allergenicity may be strictly related to a certain intrinsic biological property of a given protein (e.g., enzymatic nature). As in plants, several cross-reactive allergens can be identified in animal-derived food that also occur in respiratory allergen sources (e.g., tropomyosin and arginine kinase in invertebrates and animal-derived food) (55, 58). Other food allergens are expressed in a tissue-specific manner (e.g., muscle) or only in certain animals (e.g., fish parvalbumins).

It can thus be stated that the coexistence of many forms of allergy (e.g., OAS) can be attributed to immunological cross-reactivity of allergens from different sources with homologous sequence, structure, and function. However, it has not yet been possible to establish unambiguously the allergenic potential of a given protein only on the basis of biological function, sequence, or structure. Therefore, it is necessary to establish the allergenic potential of a given antigen individually by classical immunological in vitro and in vivo testing. The question of whether a given antigen represents an important food allergen must be evaluated by IgE reactivity, in vitro cellular stimulation (e.g., basophil histamine release, T cell proliferation), and provocation testing in patients. Whether a certain antigen can induce de novo an allergic immune response can be analyzed in experimental animal models.

Possible Mechanisms of Food Allergen-Induced Allergic Reactions

Food allergy that is associated with the recognition of food antigens by IgE antibodies may be characterized by a large variety of disease manifestations, including local inflammation (e.g., edema, swelling in the mouth) immediately after ingestion of allergen-containing material, diarrhea, reactions in other organs (skin, lung), and systemic anaphylactic reactions. Immediate reactions at the site of allergen exposure (e.g., mucosa of the oral cavity or gastrointestinal tract) most likely result from allergen-induced cross-linking of IgE antibodies on mast cells and release of biologically active mediators (e.g., histamine, leukotrienes) (Fig. 3–1). Mast cell degranulation requires contact with an intact allergen containing several IgE epitopes capable of cross-linking mast cell-bound IgE antibodies. However, proteolytic digestion of food allergens will yield non-IgE reactive peptides or IgE-reactive haptenic structures with poor cross-linking capacity. It may therefore be assumed that IgE-mediated effector cell activation plays a major role in the elicitation of local mucosal reactions, but contributes to systemic reactions only if an intact allergen is taken up systemically.

The second type of possible food-allergy reaction that requires at least partly preserved IgE epitopes is depicted in Figure 3–2. For respiratory allergens, IgE-mediated presentation of allergens by APCs (B cells, monocytes, dendritic cells) via the low- (FcεRII) and high-affinity (FcεRI) IgE receptor leads to strong induction of T cell proliferation and release of proinflammatory cytokines

Table 3–2.
Animal-Derived Food Allergens

Allergen Source	*Allergen Name*	*Function/Homology/Information*	*Molecular Weight/ Number of Amino Acids*	*(References)/ Accession Numbers*
Cattle	Bos d 4	Alpha-lactalbumin	142 aa	AAA30367
	Bos d 5	Beta-lactoglobulin	178 aa	CAA32835
	Bos d 6	Albumin, serum protein	607 aa	AAA51411
	Bos d 8	Alpha-s1 casein	214 aa	AAA30428
	Bos d 8	Beta casein	224 aa	AAA30430
	Bos d 8	Kappa casein, B2 variant	190 aa	AAA30433
		IgG	160 kDa	(51)
Chicken	Gal d 1	Ovomucoid	210 aa	P01005
	Gal d 2	Ovalbumin	387 aa	P01012
	Gal d 3	Ovotransferrin	705 aa	P02789
	Gal d 4	Lysozyme C	147 aa	P00698
	Gal d 5	Albumin	615 aa	P19121
		Ovalbumin Y gene	388 aa	AAA68882
		Vitellogenin II	1852 aa	VJCH2
Clam		Tropomyosin	284 aa	AAG08989
Crab	Cha f 1	Tropomyosin	264 aa	AAF35431
Fish				
Carp	Cyp c 1	Parvalbumin	12 kDa	(52)
Cod	Gad c 1	Parvalbumin	113 aa	P02622
Salmon	Sal s 1	Parvalbumin	109 aa	Q91482
		Type I collagen, gelatin		(53)
Gastropod	Tur c 1	Tropomyosin	146 aa	JE0229
	Tod p 1	Tropomyosin (Squid)	38 kDa	(54)
King Prawn		Arginine kinase	40 kDa	(55)
Lobster	Pan s 1	Tropomyosin	274 aa	AAC38996
		Arginine kinase	40 kDa	(55)
Mollusk	Hal d 1	Tropomyosin	284 aa	AAG08987
Mussel	Per v 1	Tropomyosin	284 aa	AAG08988
	Arginine kinase	40 kDa	(55)	
Oyster		Tropomyosin	160 aa	AAC61869
	Cra g 1	Tropomyosin	233 aa	(56)
Shrimp	Pen a 1	Tropomyosin		AAB31957
	Met e 1	Tropomyosin	274 aa	AAA60330
Snail		Tropomyosin		(57)

(59–61). It is thus possible that intact food allergens and food allergen fragments containing IgE-binding sites may also be picked up and presented by APCs containing IgE receptors, and that this mechanism could be important for certain forms of delayed food allergy.

A third way that food allergens could induce specific T cell activation without involvement of IgE is displayed in Figure 3–3. Recently it was shown that injection with T cell epitope-containing peptides of the major cat allergen, Fel d 1, which lacked IgE epitopes, led to systemic reactions in cat-allergic patients in an MHC-class II-restricted manner (62). These results indicate that short allergen-derived peptides without IgE epitopes can induce strong, harmful T cell-mediated inflammatory responses. Many food allergens are digested in the gastrointestinal tract into non-IgE-reactive peptides that can be easily absorbed. It is therefore possible that such allergen-derived peptides could induce severe systemic reactions by a mechanism similar to that described for the cat allergen-derived peptides.

However, reports that evaluate the T cell re-

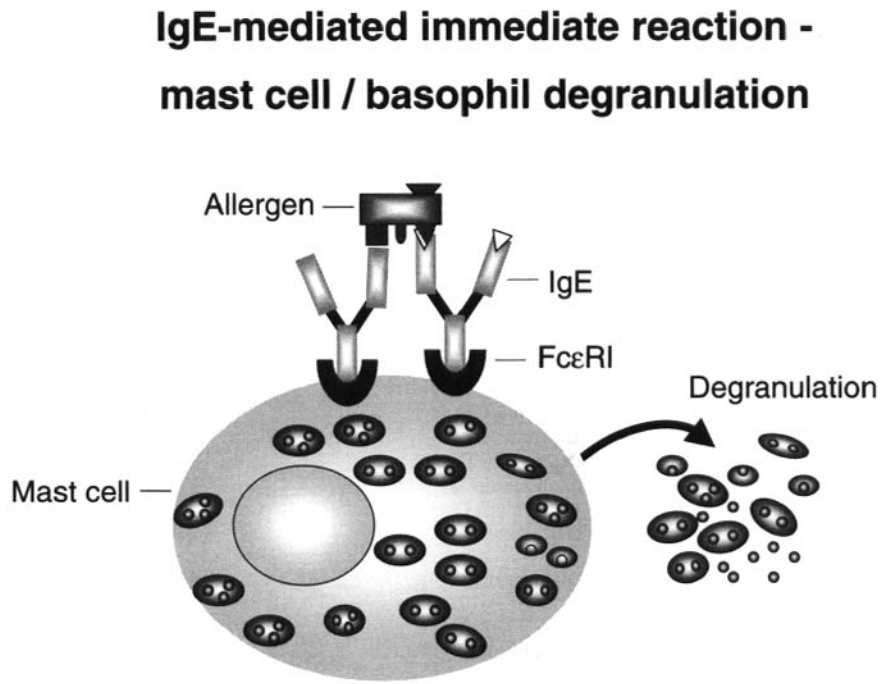

Figure 3–1. Allergen-induced cross-linking of mast cell-bound IgE antibodies and mast cell degranulation. Cross-linking of mast cell-bound IgE antibodies by intact food allergens containing several IgE epitopes induces immediate release of mast cell-derived mediators and acute inflammation.

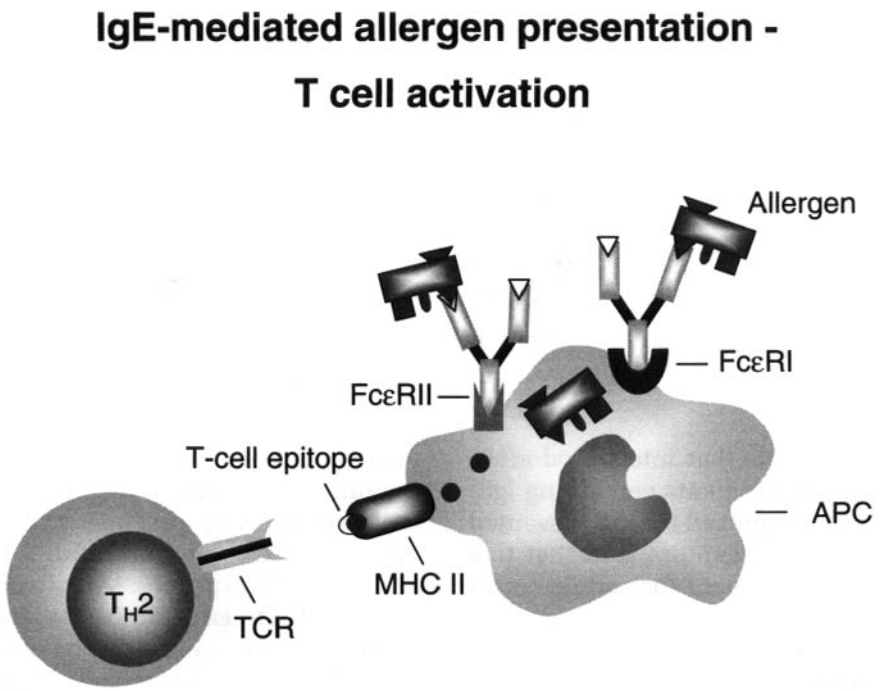

Figure 3–2. Presentation of allergens via receptor-bound IgE induces T cell activation. IgE-mediated presentation of allergens or allergen fragments containing IgE epitopes via high- (FcεRI) or low- (FcεRII) affinity T cell receptors (TCR) present on various APCs can lead to strong T cell activation and release of proinflammatory cytokines.

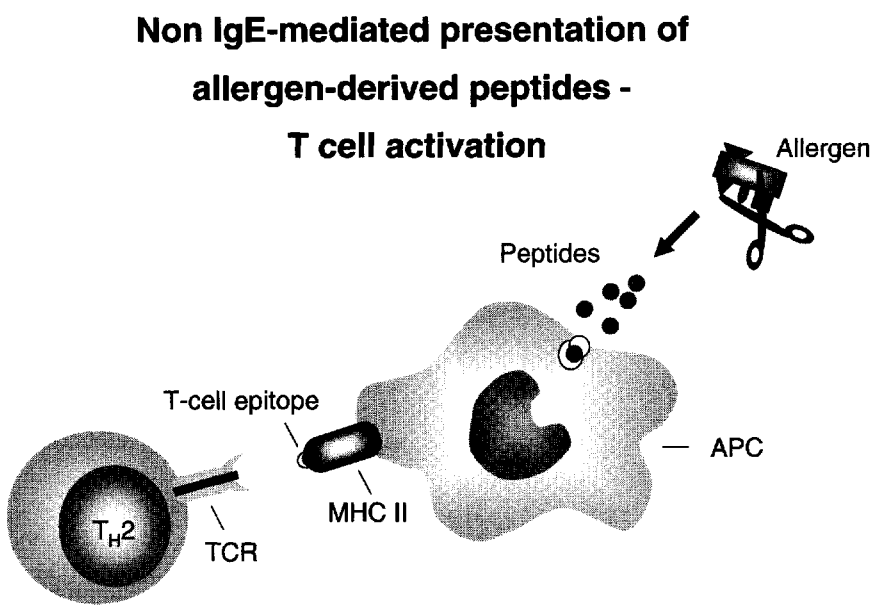

Figure 3–3. Presentation of allergen-derived T cell epitope-containing peptides activates specific T cells. Allergen processing by APCs or digestion of allergens may yield allergen-derived peptides that lack IgE epitopes and that can be presented to specific T cells, leading to T cell activation.

sponse to food allergens are rare. T cells specific for allergens from peanut, hen's egg, or cow's milk have been cultured from the peripheral blood of food-allergic patients and have been investigated (63–71). Like T lymphocytes specific for inhaled allergens, the majority of the food allergen-specific T cell clones (TCC) revealed a Th2-like pattern of cytokine production. Approximately 35%–40% of children with refractory, moderate to severe AD displayed IgE-mediated clinical reactivity to cow's milk allergens (4, 72). In these patients, ingestion of food allergens leads to acute cutaneous symptoms and aggravation of the eczema. Thus it is possible that immunologically active food allergens can enter the blood circulation and activate T lymphocytes of both Th1 and Th2 subsets, which then home to the skin and cause typical inflammatory ("late-type") reactions (73–75). Interestingly, allergen exposure of milk-allergic patients expanded a population of T cells homing to the site of allergen sensitization, i.e. the gut-associated immune system (76).

Plant food allergy in adults occurs mainly as a consequence of sensitization to respiratory, pollen-derived allergens, e.g., the birch-apple syndrome. The clinical association between birch pollen and food allergy is based on the presence of cross-reactive allergens in pollen, fruits, vegetables, and spices (16, 29, 49, 77, 78). However, until now the information about cross-reactivity between pollen and food allergens at the level of allergen-specific T lymphocytes has been limited. Birch pollen allergic patients suffering from AD reacted with a worsening of eczema after oral challenge with pollen-related food (79). In the skin lesions of these patients a birch pollen-specific T cell response was found, indicating the existence of cross-reactivity at the T cell level. A study analyzing the cellular cross-reactivity between Bet v 1 and its homologous protein in apple, Mal d 1, showed several cross-reactive T cell epitopes between Mal d 1 and Bet v 1 (80). In addition, the majority of T cell clones that were generated with recombinant Mal d 1 reacted with Bet v 1, supporting the concept that Bet v 1 is indeed the major sensitizing agent in this cross-reactivity syndrome. In patients suffering from food allergy and AD it was found that food allergen-activated Bet v 1-specific T cells homed to the skin and exacerbated skin lesions (81). Because it is known that Bet v 1-related proteins in food are degraded within seconds under physiological gastric conditions and consequently have lost their IgE-binding capacities (82), IgE-independent T cell activation may be considered as a underlying mechanism (Fig. 3–3).

However, many questions about the basic immunological mechanisms underlying the pathogenesis of food allergy remain open. In this context, the generation of food-allergen-specific T cell

cultures using defined (i.e., recombinant) food allergens will be useful to characterize cytokine secretion, proliferative responses, T cell receptor usage, and immunodominant T cell epitopes.

Improvement of Food Allergy Diagnosis by Recombinant Allergens

Diagnosis of IgE-mediated food allergy is based on in vitro and in vivo testing with allergen extracts that are prepared from the respective allergen sources. The results are in many cases unsatisfying, because of several problems related to the test substances (i.e., allergen extracts) and methods of testing (in vitro, in vivo). Allergen extracts are prepared from allergen sources, and therefore contain varying amounts of allergenic and non-allergenic components (40). In some allergen sources it is very difficult to obtain extracts containing sufficient amounts of allergens, because these sources (e.g., fruits) either contain large amounts of non-allergenic moieties or allergens are degraded during extraction. Also, different kinds of a given fruit (e.g., different apples) contain widely varying amounts of allergens, and many other factors (e.g., degree of maturation, ripening) strongly influence allergen content (83, 84). Another possible problem with allergen extracts from fruits is contamination with fungi or allergen-containing material of other origin, which may lead to false-positive test results. Finally, it is impossible to control or determine the amounts of the individual major and minor allergens in a given allergen extract; furthermore, the allergenic activity of the different allergens in an extract can vary widely (85).

It is thus very difficult to obtain reliable test results with many food allergen extracts. Furthermore, testing with allergen extracts can only determine allergen-containing sources but not the specific disease-eliciting allergen molecules.

The second major problem in the diagnosis of allergy is that results obtained by serological measurement of allergen-specific IgE antibodies do not always correlate with the biological sensitivity (85). Many patients with high allergen-specific IgE antibody levels failed to mount significant symptoms upon allergen exposure, whereas in other patients symptoms of allergy were observed despite low levels of allergen-specific IgE. Although high levels of food allergen-specific IgE are generally correlated with clinical food allergy (86), it still is widely believed that in vivo provocation testing, preferably by double-blind placebo-controlled food challenge, is necessary to unambiguously establish a diagnosis of food allergy.

We believe that the use of recombinant allergens will substantially improve the diagnosis of food allergy for many reasons. Recombinant allergens are defined molecules that can be manufactured under consistent quality criteria in defined molecular mass units. Using recombinant allergen molecules it is possible to determine precisely the sensitivity profile of the patient, and thus to identify the disease-eliciting allergen molecules (40). The biological activity and degree of cross-reactivity of many recombinant allergens is well established, so these molecules can be used as diagnostic gatekeepers for better selection of therapies because they help establish patients' sensitivity profiles (87). Using recombinant allergens it will be possible to discriminate allergens with strong allergenic activity from those with low biological activity and thus to identify the relevant disease-eliciting molecules.

Because of the efforts of several laboratories it will be possible to obtain complete panels of recombinant food allergens that recreate the epitope repertoire of natural allergen extracts. The introduction of sophisticated expression methods facilitates production of recombinant allergens as biologically active molecules that initially were difficult to produce in prokaryotic expression systems. It is thus likely that recombinant allergens will soon replace natural allergen extracts for the diagnosis of food allergy. Recombinant allergens will be superior to allergen extracts for in vitro as well as in vivo testing also for several practical reasons. In vitro tests with recombinant allergens will have a better test performance because of the lack of non-allergenic components that interfere with the coupling chemistry to solid supports or labeling in liquid phase. Biological testing will be also greatly facilitated because recombinant allergen-based test solutions will be free of characteristic tastes or smells that otherwise would bias double-blind testing. Furthermore, the presence of defined amounts of allergens in the test solution will allow to determine precisely the biological sensitivity of a patient.

Chips containing microarrayed recombinant allergens were developed recently for in vitro allergy diagnosis (88, 89). Microarrayed allergens make it possible to determine a patient's complex IgE reactivity profile to a great number of allergens with a single blood test in a short time. In the future, therefore, allergy diagnosis may be achieved

by an initial serological analysis with a multiallergen test (e.g., allergen microarray) followed by biological testing to identify specifically the most harmful allergen molecules.

Therapeutic Concepts for the Treatment of Food Allergy

Detailed, specific diagnosis of food allergy with recombinant allergen molecules will allow unambiguous identification of the disease-eliciting allergen molecules (40). On the basis of this information and knowledge about the occurrence of allergens in various allergen sources and their cross-reactivities, it will be possible to establish precise measures of allergen avoidance for prophylactic purposes. The availability of defined allergen molecules will help establish assays for the specific detection and quantification of allergens in complex allergen sources and thus help to discriminate allergen-rich foods from foods of low allergen content.

Patients suffering from manifestations of food allergy due to cross-reactivity of pollen and plant-food allergens may benefit from specific immunotherapy with cross-reactive pollen allergens. For example, immunotherapy with birch pollen allergens led to improvement of apple allergy (90). Using recombinant allergens it will be possible to identify patients who are sensitized to cross-reactive allergens in pollen and plant food. Controlled clinical immunotherapy studies will then tell us whether immunotherapy with pollen allergens does have beneficial effects on plant food allergy.

Specific immunotherapy with food allergens is rarely conducted because of difficulties in preparing allergen extracts of sufficient quality, and the risk of inducing severe anaphylactic side effects during immunotherapy (91). It is, however, likely that recombinant DNA technology will advance considerably the progress in the field of immunotherapy of food allergy for several reasons that are similar to those described above for diagnosis.

First, it will be possible to produce defined recombinant food allergens for immunotherapy that could be used to prepare good-quality formulations (e.g., adjuvant-bound allergens, sublingual formulations), avoiding the problems associated with the low quality of allergen extracts (40).

Second, recombinant allergens can be used for treatment tailored to the sensitization profile of the patient. Component-resolved immunotherapy would thus minimize the risk that patients may develop new sensitivities toward allergens in extracts to which they were not sensitized before treatment (92, 93).

The risk of anaphylactic side effects during immunotherapy may be considerably reduced by administration of recombinant or synthetic food allergen derivatives that have been engineered to reduce their allergenic activity. The hypoallergenic allergen derivatives can be engineered according to the intended treatment strategy (e.g., preservation of immunogenic structures and/or T cell epitopes) and hence will facilitate evaluation of alternative treatment strategies in controlled experiments and studies (94).

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Databases for Allergens

Farrp (Food Allergy Research and Resource Program) Allergen Database: http://www.allergenonline.com

Structural Database of Allergenic Proteins, The University of Texas Medical Branch: http://129.109.73.75/SDAP/

4

Food Biotechnology and Genetic Engineering

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Introduction

The population of the world is expected to increase by 2.5 billion people in the next 25 years. Food requirements for this growing population are expected to double by the year 2025. In contrast, the annual rate of increase in cereal yield has declined such that it is below the rate of population increase (1). In order to feed this growing population, crop yield will have to be increased and some of the increase in yield will be due to genetic engineering of foods. In addition, the incidence of food allergies appears to be on the rise, particularly in developed countries (2, 3). Genetic engineering of food crops should have little practical consequence for the occurrence, frequency, and natural history of food allergy if simple precautions are observed. Essential aspects of the health safety assessment for products derived from this technology are discussed in this chapter, and the accepted strategy for addressing any of its potential impact on food allergy will be reviewed in detail. It should be noted that no single, predictive assay appears to be capable of assessing the allergenic potential of all proteins introduced into food crops (4). However, through the use of in vivo and in vitro immunological assays in combination with a comparative evaluation of the characteristics of known food allergens, a sound scientific basis for allergenicity assessment has evolved. The biochemical properties of common food allergens have been described in this book and elsewhere (5, 6): allergens tend to be stable to proteolysis, may be glycosylated, tend to be abundant, and tend to be resistant to heat (cooking or processing). Thus, these factors have been used to discriminate potentially harmful allergens from safe proteins entering the food supply.

Plant Biotechnology

Twenty years ago the improvement of crop productivity was a sophisticated process, albeit dependent on trial and error. Many years of meticulous observations were required to determine whether desired traits were stable in the new varieties and cultivars of food crops created by this process. Crop improvement and the science of plant breeding depended on existing intraspecies genetic variation of plants, interspecies introgression of traits from "wild" or taxonomically similar plants, and on the creation of new genetic variability by chemical or irradiation mutagenesis. Although there are limitations to these approaches, crop scientists and geneticists were nevertheless able to improve crop yield and food production per unit area of agricultural land severalfold by creating new and more productive crops, and by improving agronomic practices.

With the advent of molecular biology and biotechnology it became possible not only to identify a desirable phenotypic trait but also to identify the

precise genetic material responsible for that genetic trait. Recombinant DNA and plant transformation techniques have made it possible to alter the composition of individual plant components (lipids, carbohydrates, proteins) beyond what is easily possible through traditional breeding practices. Direct and stable gene transfer into plants was first reported in 1984 (7, 8). Since then, at least 88 different plant species and many economically important crops have been genetically engineered (9), usually via *Agrobacterium* (10, 11) or particle gun technologies (12, 13).

The thrust of most first generation biotech crops has been to improve resistance to insect predation, increase resistance to pesticides for easier weed control, confer immunity to viral pathogens, and improve ripening characteristics of fresh fruit and vegetables. These crops are essentially unchanged from the nontransformed parental crops and have no significant changes in key nutrients. To a lesser extent, products with enhanced functional or nutritional properties have appeared as a result of intended alteration of specific metabolites such as oil (lipid) profiles, amino acid composition, and starch (carbohydrate) content. However, the majority of current products have had their biggest impact on agricultural practices of producers (i.e., by reducing pesticide use, improving soil conservation practices, and reducing energy inputs on farms). The availability of these so-called "agronomic" traits has driven the adoption of biotech crops since the introduction of the first product, the Flavr Savr tomato, in 1994 (Fig. 4–1). Today, over 90% of the worldwide acreage of biotech crops are agronomic traits, as shown in Table 4–1 (14). Over the next 5–10 years the proportion of food biotechnology products that have been developed for significant nutritional and functional benefits are expected to increase significantly (15).

Below we describe the development of Roundup Ready soybeans to illustrate the applica-

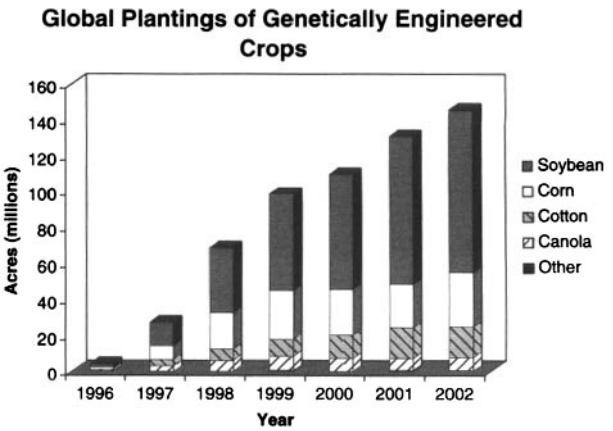

Figure 4–1. Worldwide acreage of biotech crops since introduction in 1995. Based on data reported in James (14) and literature cited therein. Of the 465 million acres of soybean, corn, cotton, and canola planted in the crop year 2002, a total of 145 million acres were planted with biotech seed.

Table 4–1.
Current Biotechnology Food Crops Approved for Food and/or Animal Feed Use by the Food and Drug Administration

Crop	*Introduced Gene(s)*	*Source of Gene(s)*	*Trait*
Corn	Cry 3Bb1	***Bacillus thuringiensis***	Resistance to coleopteran insects, in cluding corn root worm
	Cry 1F/Phosphinothricin acetyl transferase (PAT)	*B. thuringiensis/Streptomyces viridochromogenes*	Resistance to certain lepidopteran insects/tolerance to the herbicide glufosinate
	EPSPS	***Agrobacterium sp.***	Tolerance to the herbicide glyphosate
	Barnase	*B. amyloliquefaciens*	Male sterility
	Modified EPSPS	Corn	Tolerance to the herbicide glyphosate
	Cry9C protein/Phosphinothricin acetyl transferase (PAT)	***B. thuringiensis/S. hygroscopicus***	Resistance to several lepidopteran insects/tolerance to the herbicide glufosinate
	DNA adenine methylase (DAM)/ Phosphinothricin acetyl transferase (PAT)	*E. coli/S. viridochromogenes*	Male sterility/tolerance to glufosinate
	CryIAc	***B. thuringiensis***	Resistance to European corn borer
	CryIAb/EPSPS	*B. thuringiensis/Agrobacterium sp.*	Resistance to European corn borer; tolerance to the herbicide glyphosate
	CryIAb	*B. thuringiensis*	Resistance to European corn borer
	Barnase/phosphinothricin acetyl transferase (PAT)	*B. amyloliquefaciens/S. hygroscopicus*	Male sterility/tolerance to glufosinate
	Phosphinothricin acetyltransferase (PAT)	*S. hygroscopicus*	Tolerance to glufosinate

(*continued*)

Table 4–1.
Current Biotechnology Food Crops Approved for Food and/or Animal Feed Use by the Food and Drug Administration

Crop	*Introduced Gene(s)*	*Source of Gene(s)*	*Trait*
Canola	**Nitrilase**	***Klebsiella ozaenae***	Tolerance to the herbicide bromoxynil
	Phytase	***Aspergillus niger***	Degradation of phytate in animal feed
	Barnase/phosphinothricin acetyl transferase (PAT)	*B. amyloliquefaciens/S. hygroscopicus*	Male sterility/tolerance to glufosinate
	Barstar/phosphinothricin acetyl transferase (PAT)	*B. amyloliquefaciens/ S. hygroscopicus*	Fertility restorer/tolerance to glufosinate
	Phosphinothricin acetyltransferase (PAT)	*S. hygroscopicus*	Tolerance to glufosinate
	12:0 acyl carrier protein thioesterase	*Umbellularia californica*	**High laurate canola oil**
	EPSPS/Glyphosate oxidoreductase (GOX)	*Agrobacterium sp.* strain CP4, *Achromobacter*	Tolerance to the herbicide glyphosate
Soybean	Phosphinothricin acetyltransferase (PAT)	*S. hygroscopicus*	Tolerance to glufosinate
	GmFad2–1 gene	**Soybean**	High oleic acid soybean oil
	EPSPS	*Agrobacterium sp.* strain CP4	Tolerance to the herbicide glyphosate
Cotton	**Nitrilase/Cry1Ac protein**	*Klebsiella pneumoniae/ B. thuringiensis*	Tolerance to bromoxynil/resistance to certain lepidopteran insects
	Acetolactate synthase (ALS)	***Nicotiana tabacum***	Tolerance to the herbicide sulfonylurea
	EPSPS	*Agrobacterium sp.* strain CP4	Tolerance to the herbicide glyphosate
	CryIAc protein	***B. thuringiensis***	Resistance to cotton ballworm, pink bollworm, and tobacco budworm
	Nitrilase	***K. ozaenae***	Tolerance to the herbicide bromoxynil
Sugarbeet	**EPSPS**	*Agrobacterium sp.* strain CP4	Tolerance to the herbicide glyphosate
	Phosphinothricin acetyltransferase (PAT)	*S. hygroscopicus*	Tolerance to glufosinate
Tomato	**CryIAc protein**	*B. thuringiensis*	Resistance to certain lepidopteran insects
	S-adenosylmethionine hydrolase	*E. coli* bacteriophage T3	Delayed fruit ripening due to reduced ethylene synthesis
	ACCS gene fragment	**Tomato**	Delayed ripening due to reduced ethylene synthesis
	Polygalacturonase (PG)	**Tomato**	Delayed softening due to reduced pectin degradation
	1-aminocyclopropane-1-carboxylic acid deaminase (ACCD)	*Pseudomonas chloraphis*	Delayed softening due to reduced ethylene synthesis
	Polygalacturonase (PG) antisense gene	**Tomato**	Delayed softening due to reduced pectin degradation
Potato	CryIIIA/PVY coat protein	*B. thuringiensis*/Potato virus Y (PVY)	Resistance to Colorado potato beetle and PVY
	CryIIIA/PLRV replicase	*B. thuringiensis*/Potato Leafroll virus	Resistance to Colorado potato beetle and PLRV
	CryIIIA	*B. thuringiensis*	Resistance to Colorado potato beetle
Rice	Phosphinothricin acetyltransferase (PAT)	*S. hygroscopicus*	Tolerance to glufosinate
Cantaloupe	S-adenosylmethionine hydrolase	*E. coli* bacteriophage T3	Delayed fruit ripening due to reduced ethylene synthesis
Radicchio	Barnase/phosphinothricin acetyl transferase (PAT)	*B. amyloliquefaciens/ S. hygroscopicus*	Male sterility/tolerance to glufosinate
Squash	Coat proteins from CMV, ZYMV, and WMV2	Cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus 2 (WMV2)	Resistance to the viruses CMV, ZYMV, and WMV2
	ZYMV and WMV2 coat proteins	Zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus 2 (WMV2)	Resistance to the viruses ZYMV and WMV2
Papaya	**PRV coat protein**	Papaya ringspot virus (PRSV)	**Resistance to PRSV**
Flax	Acetolactate synthase (csr-1)	*Arabidopsis*	Tolerance to the herbicide sulfonylurea

Data were compiled from the FDA web site (www.cfsan.fda.gov) and represent all completed consultations from 1994 to 2001.

tion of agricultural biotechnology. We then briefly summarize the safety assessment procedures for food biotechnology, illustrated by reference to the data developed for Roundup Ready soybeans. Following this general discussion, we provide a detailed account of current approaches and issues in allergy assessment for these products, also illustrated by the data developed for Roundup Ready soybeans.

Roundup Ready Soybeans—A Case Study in Food Safety Assessment

Soybean (*Glycine max*) ranks fifth in world production of major crops after wheat, maize, rice, and potato. In the US, soybeans represent $5.6 billion in farm gate receipts (14, 16). Soybeans represent approximately one third of all crops grown in the US. The major food use of soybeans is the oil, whereas 96% of soybean meal is used for animal feed. Approximately 75% of vegetable food-grade oil used in foods such as shortenings, margarines, and salad/cooking oils is from soybeans. Soybean flour (meal) is used in foods such as soups, stews, beverages, desserts, bakery goods, cereals, and meat products and extenders (17). New varieties of soybeans were the most common transgenic crop planted, representing 63% of the total acres planted with biotech traits in 2001 (14). The most common biotechnology trait was herbicide tolerance, followed by insect protection (14).

Development and Benefits of Roundup Ready Soybeans

The genetically engineered soybean line GTS 40–3-2 was developed to allow the use of glyphosate, the active ingredient in the wide-spectrum herbicide Roundup, as a weed-control option for soybean. This genetically engineered soybean line contains a glyphosate-tolerant form of the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) isolated from the common soil bacterium, *Agrobacterium tumefaciens* strain CP4 (CP4 EPSPS). The EPSPS enzyme is part of the shikimate pathway that is involved in the production of aromatic amino acids and other aromatic compounds in plants (18). When conventional plants are treated with glyphosate, the plants cannot produce the aromatic amino acids needed to survive. GTS 40–3-2 was developed by introducing the CP4 EPSPS coding sequence into the soybean variety A5403, a commercial soybean variety of Asgrow Seed Company, using particle-acceleration (biolistic) transformation. A5403 is a maturity group V cultivar that combines consistently high yield potential with resistance to races 3 and 4 of the soybean cyst nematode (SCN). It also possesses good standability, excellent seedling emergence, and tolerance to many leaf and stem diseases.

Weed control in soybeans represents a major financial and labor input by growers. Grassy weeds (monocots) are controlled by one class of herbicides, and broadleaf (dicot) weeds are controlled by a different class of herbicides. Because soybeans are broadleaf plants, their physiology and biochemistry are similar to that of broadleaf weeds. Therefore, in conventional soybeans, it is technically challenging to control both grassy and broadleaf weeds without harming the soybean plants themselves (16).

Glyphosate is used as a foliar-applied, non-selective herbicide and is effective against the majority of grasses and broad-leaf weeds. Glyphosate has no pre-emergence or residual soil activity (18). Furthermore, glyphosate degrades rapidly in soil, is not prone to leaching, and is essentially non-toxic to mammals, birds, and fish (19–21).

Roundup Ready soybeans offer growers an additional tool for improved weed control. Control of weeds in the soybean crop is essential, as weeds compete with the crop for sunlight, water, and nutrients. Failure to control weeds within the crop results in decreased yields and reduced crop quality. In addition, weeds reduce the efficiency of the mechanical harvest of the crop.

Roundup Ready soybeans have been produced commercially in the US, Argentina, and Canada beginning in 1996 and provide the following environmental and economic benefits:

- Improved efficacy in weed control compared to herbicide programs used in conventional soybeans, as specific pre-emergent herbicides that are used as prevention are replaced by a broad-spectrum post-emergent herbicide that can be used on an as-needed basis (22). The introduction of Roundup Ready soybeans in the US has resulted in a 12% reduction in the number of herbicide applications from 1996 to 1999, even though the total soybean acres increased by 18% (16). This decrease in herbicide applications means that growers make fewer trips over the field to apply herbicides.
- A reduction in herbicide costs for the farmer. It's been estimated that US soybean growers

spent $216 million less in 1999 for weed control (including a technology fee for Roundup Ready soybean), compared to 1995, the year before Roundup Ready soybeans were introduced (16).

- Less labor required due to elimination of hand weeding and of high cost, early post-directed sprays, which require special equipment.
- High compatibility with Integrated Pest Management and soil conservation techniques (23), resulting in a number of important environmental benefits, including reduced soil erosion and improved water quality (24–26), improved soil structure with higher organic matter (27, 28), improved wildlife habitat, and improved carbon sequestration (29, 30) and reduced CO_2 emissions (27, 31).

Safety Assessment of Roundup Ready Soybeans

Safety Assessment Principles

In 1996, a joint report from an expert consultation sponsored by the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) of the United Nations concluded that "biotechnology provides new and powerful tools for research and for accelerating the development of new and better foods" (32). The FAO/WHO expert consultation also concluded that it is vitally important to develop and apply appropriate strategies and safety assessment criteria for food biotechnology to ensure the long-term safety and wholesomeness of the food supply.

Following these criteria, foods derived from biotechnology have been extensively assessed to ensure that they are as safe and nutritious as traditional foods. All foods, regardless of whether they are derived from biotech crops or traditionally bred plants, must meet the same rigorous food safety standards. Numerous national and international organizations have considered the safety of foods derived from biotech crops. They have concluded that the food safety considerations are basically the same for food derived from biotech crops as for those foods derived using other methods like traditional breeding.

This concept of comparing the safety of the food from a biotech crop to that of a food with an established history of safe use is referred to as "substantial equivalence" (33, 34). The process of substantial equivalence involves comparing the characteristics, including the levels of key nutrients and other components, of the food derived from a biotech crop to the food derived from conventional plant breeding. When a food is shown to be substantially equivalent to a food with a history of safe use, "the food is regarded to be as safe as its conventional counterpart" (32). An FAO/WHO expert consultation in 1995 concluded that "this approach provides equal or greater assurance of the safety of food derived from genetically modified organisms as compared to foods or food components derived by conventional methods" (32). As a practical matter, this method brings together an evaluation of the introduced proteins, and it accounts for unexpected effects due either to the protein per se or to pleiotropic effects created by gene insertion as assessed at the level of phenotype: the agronomic and compositional parameters of the biotech crop in comparison to traditional counterparts (35).

CP4 EPSPS Protein Safety

Usually when a gene is chosen for transformation into a crop, the encoded protein has been well characterized in terms of function (mechanism of action, evolutionary heritage, physicochemical properties, etc.). This information has been extensively evaluated during the development of biotech crops such as NewLeaf potato (36), RoundupReady soybeans (37), and YieldGard corn (38). An important consideration in protein safety is whether or not the protein can be established to have been used or eaten previously—is there a history of safe use?

The CP4 EPSPS protein produced in Roundup Ready soybeans is functionally similar to a diverse family of EPSPS proteins typically present in food and feed derived from plant and microbial sources (39). The EPSPS proteins are required for the production of aromatic amino acids in plants and microbes. The enzymology and known function of EPSPS proteins generally, and CP4 EPSPS specifically, indicate that this class of enzymes perform a well-described and -understood biochemical role in plants. From the perspective of safety, this characterization indicates that metabolic effects owing to the expression of the CP4 EPSPS gene are limited to conferring the Roundup Ready trait alone. Part of this evaluation includes the known structural relationship between CP4 EPSPS and other EPSPS proteins found in food, demonstrated by comparison of the amino acid sequences with conserved identity of the active site residues, and

the expected conserved three-dimensional structure based on similarity of the amino acid sequence. With respect to amino acid sequence, there is considerable divergence among known EPSPSs. For instance, the amino acid sequence of CP4 EPSPS is 41% identical at the amino acid level to *Bacillus subtilis* EPSPS, whereas the soybean EPSPS is 30% identical to *B. subtilis* EPSPS. Thus, the divergence of the CP4 EPSPS amino acid sequence from typical food EPSPS sequences is on the same order as the divergence among food EPSPSs themselves (37).

The detailed enzymology (37) and subsequent biochemical composition evaluations (40, 41), confirm and demonstrate that CP4 EPSPS, as expressed in line 40–3-2, has the predicted and expected metabolic effects on soybeans: the production of aromatic amino acids via the shikimic acid biosynthetic pathway.

Another tool used in the assessment of potential toxic effects of proteins introduced into plants is a comparison of the amino acid sequence of the protein to that of known toxic proteins. Homologous proteins derived from a common ancestor have similar amino acid sequences and are structurally similar, and they often share common function. Therefore, it is undesirable to introduce DNA into a food crop that encodes a protein homologous to a protein that is toxic to animals and people. Homology is determined by comparing the degree of amino acid similarity between proteins using published criteria (42). The CP4 EPSPS protein does not show meaningful amino acid sequence similarity when compared to known protein toxins.

Lack of protein toxicity is confirmed by evaluating acute oral toxicity in mice or rats (43). This study is typically a 2-week program in which the pure protein is fed to animals at doses that should be 100 to 1000 times higher than the highest anticipated exposure via consumption of the whole food product containing that protein. Table 4–2 summarizes the data from several acute oral toxicity studies. Although these studies were designed to obtain LD_{50}s, in fact no lethal dose has been achieved for these proteins (39, 43–46). For CP4 EPSPS, there were no treatment-related adverse effects in mice administered CP4 EPSPS protein by oral gavage at doses up to 572 mg/kg, the highest tested. This dose represents a significant (about 1300-fold) safety margin relative to the highest potential human consumption of CP4 EPSPS and assumes that the protein is expressed in multiple crops in addition to soybeans (39).

Table 4–2.
Summary of the Data from Standardized Acute Oral Toxicity LD_{50} Studies in Mice

Protein	*Crop*	*NOEL (mg/kg)**
Cry1Ac	Cotton, tomato	4200
Cry1Ab	Corn	4000
Cry2Aa	Cotton	3000
Cry2Ab	Corn, cotton	3700
Cry3A	Potato	5200
Cry3Bb1	Corn	3780
CP4 EPSPS	Soybean, cotton, canola, sugarbeet	572
NPTII	Cotton, potato, tomato	5000
GUS	Soybean, sugarbeet	100
GOX	Canola, cotton, corn, sugarbeet	100

The NOEL (no observable effect level) was the highest dose tested for each protein. When accounting for the level of these proteins in the crops in which they are found (Table 4–1), these doses represent between 10^4 and 10^6 times the levels typically consumed as food. Abbreviations: Cry1Ac, Cry1Ab, Cry2Aa, Cry2Ab, Cry3A, Cry3Bb1 are all "crystal" proteins from *Bacillus thuringiensis;* NPTII, neomycyn phosphotransferase II; GUS, β-glucuronidase; GOX, glyphosate oxidoreductase.

Phenotype Evaluation (Substantial Equivalence)

Compositional analyses are a critical component of the safety assessment process that integrates with the evaluation of the trait (e.g., CP4 EPSP synthase) described above. Each of the measured parameters assesses the cumulative result of numerous biochemical pathways and hence assesses a wide range of metabolic pathways. Comparisons of various nutrients and anti-nutrients are made both to a closely related traditional counterpart as well as to the established published range for the specific component within that crop, to compare the observed levels to the natural variation of that component in current plant varieties. The composition of Roundup Ready soybeans has been thoroughly characterized, and the results of these studies have been published (40, 41). Over 1400 individual analyses have been conducted, and they establish that the composition of Roundup Ready soybeans is substantially equivalent to the non-transgenic parental soybean variety and other commercial soybean varieties. Table 4–3 summarizes the composition of Roundup Ready soybeans and traditional soybeans, which included:

- *Proximate analysis:* protein, fat, fiber, ash, carbohydrates, and moisture
- *Anti-nutrients:* trypsin inhibitors, lectins, phytoestrogens (genistein and daidzein), stacchyose, raffinose, and phytate
- *Fatty acid profile:* percentage of individual fatty acids

Table 4–3.
Summary of Historical and Literature Ranges for the Nutritional Composition of Roundup Ready Soybeans

Component	*Historical Roundup Ready Soybean Range*[a]	*Literature Soybean Range*[b]
Proximates (% dw)		
Moisture (% fw)	5.32–8.85	5.30–11 (47–49)
Protein	37.0–45.0	36.9–46.4 (48)
Fat	13.27–23.31	13.2–22.5 (48, 50)
Ash	4.45–5.87	4.29–5.88 (47)
Carbohydrates	27.6–40.74	29.3–41.3 (47)
Fiber (% dw)		
Acid detergent fiber	9.76–12.46	Not Available
Neutral detergent fiber	11.02–11.81	Not Available
Crude fiber	5.45–9.82	5.74–8.10 (47, 53)
Amino acid (g/100g dw)		
Alanine	1.48–1.88	1.49–1.87 (51, 52)
Arginine	2.20–3.57	2.45–3.49 (51, 52)
Aspartic Acid	3.85–5.25	3.87–4.98 (51, 52)
Cystine	0.54–0.69	0.50–0.66 (47, 53)
Glutamic Acid	6.00–8.34	6.10–8.72 (51, 52)
Glycine	1.48–1.90	1.60–2.02 (47, 51, 52)
Histidine	0.91–1.18	0.89–1.16 (1, 51, 52)
Isoleucine	1.51–1.95	1.46–2.12 (51, 52)
Leucine	2.60–3.37	2.71–3.37 (51, 52)
Lysine	2.30–2.88	2.35–2.86 (51, 52)
Amino acid (% total aa)		
Methionine	0.50–0.62	0.49–0.66 (51, 52)
Phenylalanine	1.64–2.20	1.70–2.19 (47, 51, 52)
Proline	1.76–2.30	1.88–2.61 (51, 52)
Serine	1.80–2.60	1.81–2.32 (51, 52)
Threonine	1.39–1.74	1.33–1.79 (51, 52)
Tryptophan	0.42–0.64	0.48–0.63 (47, 53)
Tyrosine	1.23–1.58	1.12–1.62 (51, 52)
Valine	1.58–2.02	1.52–2.24 (51, 52)
Alanine	4.29–4.42	Not Available
Arginine	7.31–8.16	Not Available
Aspartic Acid	11.46–11.98	Not Available
Cystine	1.48–1.67	Not Available
Glutamic Acid	18.53–19.02	Not Available
Glycine	4.34–4.41	Not Available
Histidine	2.66–2.72	Not Available
Isoleucine	4.29–4.43	Not Available
Leucine	7.63–7.87	Not Available
Lysine	6.46–6.66	Not Available
Methionine	1.36–1.46	Not Available
Phenylalanine	4.89–5.04	Not Available
Proline	5.20–5.27	Not Available
Serine	5.76–6.08	Not Available
Threonine	3.37–3.50	Not Available
Tryptophan	1.05–1.15	Not Available
Tyrosine	3.50–3.66	Not Available
Valine	4.50–4.66	Not Available
Fatty acids (% of total FA)[b]		
12:0 Lauric Acid	<0.01% fw–0.40	Not Available
14:0 Myristic Acid	<0.01 fw–0.17	Not Available
16:0 Palmitic Acid	10.63–12.75	7–12 (54) 9.63–13.09 (55)
16:1 Palmitoleic Acid	0.11–0.17	Not Available
17:0 Heptadecanoic Acid	0.10–0.17	0.11–0.14 (47)
17:1 Heptadecenoic Acid	<0.01% fw	Not Available
18:0 Stearic Acid	4.01–5.93	2–5.5 (54) 2.69–4.40 (55)
18:1 Oleic Acid	15.56–32.52	20–50 (54) 19.63–36.58 (55)
18:2 Linoleic Acid	42.41–54.48	35–60 (54) 42.61–58.16 (55)
18:3 Linolenic Acid	4.99–10.37	2–13 (54) 5.66–8.58 (55)

(*continued*)

Table 4–3. (Continued)
Summary of Historical and Literature Ranges for the Nutritional Composition of Roundup Ready Soybeans

Component	*Historical Roundup Ready Soybean Range*[a]	*Literature Soybean Range*[b]
Fatty Acids (% of Total FA)[c]		
20:0 Arachidic Acid	0.30–0.51	0.31–0.43 (47)
20:1 Eicosenoic Acid	0.14–0.28	0.14–0.26 (47)
22:0 Behenic Acid	0.49–0.62	0.46–0.59 (47)
Isoflavones (Total as aglycones)		
Daidzein (ug/g dw)	90.5–1260	161–1190 (47, 53)
Genistein (ug/g dw)	106–1243	230–1380 (47, 53)
Glycitein (ug/g dw)	<10.8–184	Not Available
Miscellaneous		
Vitamin E mg/100g dw	1.85–4.26	1.95 (56)
Trypsin inhibitor (TIU/mg DW)	35.5–59.5	26.4–93.2 (57)
Lectin (HU/mg fw)	0.5–1.6	0.8–2.4 (47)

[a]Range of values from Roundup Ready soybean event 40–3-2 (40,41).
[b]Commercial/non-transgenic control values: [1](40); [2](47); [3](48); [4](49); [5](50); [6](51); [7](41); [8](52); [9](53); [10](54): units in mg/100g edible portion; [11](55)
[c]"<0.01% fw" is below the lower limit of quantitation.

- *Amino acid composition:* levels of individual amino acids

In addition to a demonstration of substantially equivalent composition, further agronomic evaluation of the biotech crop is necessary to establish that there are no unexpected biological effects of the introduced trait. Although compositional assessments provide good assurance that no untoward metabolic, nutritional, or antinutritional effects have been introduced, an additional and very sensitive measure is to compare a wide variety of biological characteristics at the whole plant level. The basic question asked is: Does the biotech crop fit within the usual definition of that crop? For example, do Roundup Ready soybeans still possess the expected plant performance of traditional soybeans? Agronomic and yield characteristics are very sensitive to untoward perturbations in metabolism and in genetic pleiotropy.

Wholesomeness (Nutrition) of Roundup Ready Soybeans

Farm animal nutrition studies have provided supplementary confirmation of the substantial equivalence and safety in crop biotechnology. Currently there are many options for crop studies in animals, the choice of which depends on the crop being engineered and its intended use. In over 65 farm animal studies completed to date, the factors evaluated include feed intake, body weight, carcass yield, feed conversion, milk yield, milk composition, digestibility, and nutrient composition of the resulting animal-derived foods (56).

A series of animal feeding studies have been completed using diets incorporating raw or processed Roundup Ready soybeans. The animal feeding studies included two separate 4-week studies in rats (one with unprocessed soybean meal and one with processed soybean meal), a 4-week dairy cow study, a 6-week chicken study, a 10-week catfish study, and a 5-day quail study. Animals were fed either raw soybean, or unprocessed or processed soybean meal (dehulled, defatted, toasted). Included in these studies were control groups fed a non-modified parental soybean line from which both events were derived. Results from all groups were compared using conventional statistical methods to detect differences between groups in measured parameters.

All soybean samples tested provided similar growth and feed efficiency for rats, chickens, catfish, and quail (57). Milk production, composition and rumen fermentation parameters for dairy cows were also comparable across all groups (57). Results for other parameters measured in each feeding study were also similar across all groups. When compared to the US population as a whole, the levels of soybean consumption (in mg/kg of body weight) in these animal feeding studies were 100-fold or more higher than the average human daily consumption of soybean-derived foods in the US. These studies all confirmed the food and feed safety and nutritional equivalence of diets from Roundup Ready soybeans.

General Assessment Strategy for Food Allergy

The consumer marketplace reflects widespread interest in and concern about adverse reactions to certain foods and food additives. A consumer survey indicated 30% of the people interviewed reported that they or some family member had an allergy to a food product (58). This survey also found that 22% avoided particular foods on the mere possibility that the food may contain an allergen. In reality, food-allergic reactions affect only 6%–8% of children and 1%–2% of the adult population (59–61). The most common food allergies known to affect children are IgE-mediated reactions to cow's milk, eggs, peanuts, soybeans, wheat, fish, and tree nuts. Approximately 80% of all reported food allergy in children are due to peanuts, milk, or eggs. Although most childhood food allergies are outgrown, allergies to peanuts, tree nuts, and fish are rarely resolved in adulthood. In adults the most common food allergies are to peanuts, tree nuts, fish, and shellfish. The incidence of IgE-mediated reactions to specific food crops is increasing, particularly in developed countries, likely due to increased levels of protein consumption. Allergic reactions are typically elicited by a defined subset of proteins that are abundant in the food.

Identification and purification of allergens is essential for the structural and immunological studies that are necessary to understand how these molecules stimulate IgE antibody formation (62). In the past several years a number of allergens have been identified that stimulate IgE production and cause IgE-mediated disease in humans. Significant information now exists on the identification and purification of allergens from a wide variety of sources, including foods, pollens, dust mites, animal danders, insects, and fungi (62). However, despite increasing knowledge of the structure and amino acid sequences of the identified allergens, specific features associated with IgE antibody formation have not been fully determined (62).

Because potential allergens cannot at present be accurately identified based on a single characteristic, the allergy assessment testing strategy as originally proposed by the US Food and Drug Administration (FDA) (63), and further modified by FAO/WHO scientific panels (64, 65), proposes that all proteins introduced into crops be assessed for their similarity to a variety of structural and biochemical characteristics of known allergens. On the other hand, because the primary method of disease management for food-allergic people is avoidance, a core principle of these recommended strategies is to experimentally determine whether candidate proteins for genetic engineering into foods represent currently known food allergens, and this question can be tested directly. Prevention of unwanted exposures to food allergens is addressed by accurate labeling of food ingredients; labeling is seen as a central tool in food protection policy in the US. For biotechnology, this public health imperative is achieved by excluding known allergens from consideration for transgenesis.

This hazard identification strategy assesses the introduced protein with respect to origin (e.g., is it from a known allergenic source?), sequence homology to known allergens, stability in an in vitro pepsin digestion assay, and IgE binding capacity in in vitro and in vivo clinical tests when appropriate.

Analyzing the Sources of Introduced Genes

The source of the introduced gene is the first variable to consider in the allergy assessment process. If a gene transferred into a food crop is obtained from a source known to be allergenic, the assessment process calls for in vitro diagnostic tests to determine whether the target protein binds IgE from patients allergic to the source of the protein. In addition, in vivo diagnostic tests such as skin prick tests (SPTs) and double-blind-placebo-controlled-food challenge (DBPCFC) may be required if the protein is to be introduced into a commodity crop. The US FDA recognizes this need and realizes that such risks to consumers can be avoided (63). In addition to tests to determine potential allergenicity, the use of labels that clearly indicate the presence of ingredients that may cause harmful effects, such as allergies, gives consumers the opportunity to avoid these foods or food ingredients. For example, to assist people who suffer from celiac disease, the FDA has determined that products containing gluten should be identified as to the source—i.e., wheat versus corn gluten (wheat gluten cannot be safely consumed by these patients, unlike corn gluten). In the case of food allergy, voluntary labeling already occurs for certain snack foods that do not ordinarily contain peanuts, but that may come into contact with peanuts during preparation. This type of

labeling provides protection for peanut allergy sufferers and helps prevent accidental and unwanted exposure. The FDA has also stated that, if known allergens are genetically engineered into food crops, the resulting foods must be labeled disclosing the source of the introduced genes (63, 66). Moreover, proteins derived from known allergenic sources should be treated as allergens until demonstrated otherwise. The methodology to assess whether the transferred protein is allergenic is described below.

Different approaches can be taken to assess the potential allergenicity of a protein that originates from a non-allergenic source. As described below, a search for amino acid sequence homology of the introduced protein with all known allergens can be performed. In addition, the physicochemical properties of the introduced protein can be compared with the biochemical properties of known food allergens. From biochemical analysis of a limited number of allergens, certain characteristics shared by most, but not necessarily all, allergens can be identified. For example, food allergens are typically low-molecular weight glycosylated proteins that are relatively abundant in that food source. In addition, many have acidic isoelectric points; multiple, linear IgE binding epitopes and resistance to denaturation and digestion (70). These characteristics are purported to be important to the allergenicity of a protein for various reasons. The low molecular weight and glycosylation of food allergens was believed to facilitate movement of the allergen across the gut mucosa, allowing it to gain access to the immune system and stimulate a Th2-type (IgE-producing) response. The observation that most food allergens are relatively abundant in the food source was explained by the idea that the immune system was more likely to encounter these proteins than those present as a small percentage of the total protein ingested. The acidic isoelectric point of some food allergens may lead to longer transit times in the gastrointestinal (GI) tract because they precipitate at the low pH encountered in the stomach. Resistance to denaturation and digestion of an allergen is thought to be important because the longer the significant portion of the protein remains intact, the more likely it is to trigger an immune response. Finally, most food allergens have multiple, linear binding epitopes so that even when they are partially digested or denatured, they are still capable of interacting with IgE and causing an allergic reaction (68).

Amino Acid Sequence Comparisons to Known Allergens

The proteins introduced into all genetically engineered plants that have been put into commercial use in the US have been screened by comparing their amino acid sequence to those of known allergens and gliadins, as one of many assessments performed to evaluate product safety (4, 69). Additionally, the amino acid sequence of the introduced protein is screened against all known proteins in publicly available sequence databases to identify proteins that could have other potential safety concerns. The extent of sequence similarities between the introduced protein and database sequences of allergens, gliadins, and other proteins can be efficiently assessed using the FASTA sequence alignment tool (70). Although the FASTA program directly compares amino acid sequences (i.e., primary protein structure), the alignment data may be used to infer higher order structure (i.e., secondary and tertiary protein structures). Proteins that share a high degree of similarity throughout their entire length are often homologous, sharing secondary structure and common three-dimensional folds (71). Homologous proteins are more likely to share allergenic cross-reactive conformational and linear epitopes than unrelated proteins; however, the degree of similarity between homologs varies widely and homologous allergens do not always share epitopes (72). Aalberse (73) has noted that proteins sharing less than 50% identity across the full length of the protein sequence are unlikely to be cross-reactive, and immunological cross-reactivity may not occur unless the proteins share at least 70% identity.

There is some concern that the FASTA search might miss short sequence regions that are identical or highly similar to an existing allergen and that have the potential to bind IgE. Because IgE-binding epitopes have been identified for only a few allergens, it is not yet possible to construct a comprehensive IgE-binding epitope database for a more accurate search. Further, most of the epitopes that have been identified are known through in vitro mapping studies without regard to antibody affinity and using sera from only a few allergic individuals. Although some IgE epitopes may be as short as five amino acids (74, 75), the majority of characterized IgE-linear epitopes are eight amino acids or longer (76–78). Although many of these reports have demonstrated IgE binding, few have tested the affinity (avidity) of the binding, or the

allergic significance of the in vitro binding, and it is clear from some reports that high-affinity binding requires eight or more amino acids (74, 79)

In the absence of a complete description of IgE epitopes for all known allergens, a theoretical database of all potential epitopes for these same allergens can effectively be screened by scanning all overlapping peptides (in this case, eight or more amino acids in length) of all the allergens in the database and comparing them in pairwise fashion to all same-size potential peptides of the test protein, using computer software or by scanning manually. This approach can be viewed as highly conservative and all-inclusive, because most of the theoretical peptides compared with the query sequence do not represent bona fide epitopes. One FAO/WHO scientific panel recommended using a six-amino acid window for this type of analysis (65). However, Hileman et al (80) subsequently demonstrated that an amino acid window size of less than eight residues resulted in the identification of many irrelevant sequences. Therefore, the use of an eight-amino acid window represents a compromise to identify most of the potentially cross-reactive single epitopes while reducing the probability of identifying a large number of irrelevant similarities (false positives) that would be identified using a smaller window. It should also be recognized that two IgE binding epitopes on the same molecule are required to cross-link high affinity IgE receptors on mast cells and induce an intracellular signal. If sufficient numbers of receptors are stimulated, the mast cell will degranulate, releasing histamine and leukotrienes. Therefore, a single match in this analysis may or may not be clinically significant and must be assessed by a second tier of studies such as in vitro and in vivo IgE assays discussed below. Likewise, to reduce the possibility of missing a known epitope of smaller than eight contiguous identical amino acids, we recommend an additional sequence comparison to a database of known IgE epitopes that fit into this size range. By combining these two steps, it is possible to reduce both the number of false negatives and the number of false positives in sequence comparison analyses.

Pepsin Digestibility

Models of digestion are commonly used to assess the stability of dietary proteins (81–83). A digestion model using simulated gastric fluid (SGF) was adapted to evaluate the allergenic potential of dietary proteins (81). In this model, resistance to digestion by pepsin has been used as criterion for distinguishing food allergens from safe, non-allergenic dietary proteins. Although these digestibility models are representative of human digestion, they are not designed to predict the half-life of a protein in vivo.

The digestive stability of the major allergens found in the most common allergenic foods were the first to be studied. The stability of some of the major allergens of peanut, soybean, egg, and milk relative to that of common non-allergen food proteins were determined in the standard pepsin digestion assay (81). Under the conditions described for SGF in this study, all food allergens were more resistant to pepsin hydrolysis than were common plant proteins. For example, the Ara h 2 allergen of peanut was stable for at least 60 minutes in the pepsin digestion assay, whereas other non-allergen plant proteins such as rubisco (spinach leaf) or acid phosphatase (potato) were digested in less than 15 seconds. However, not all allergens from the most common allergenic foods were stable in the pepsin digestion assay for 60 minutes. Stability of the whole protein or fragments from the allergens tested ranged from 8 minutes to 60 minutes, whereas all of the non-allergen plant proteins tested did not survive in the pepsin digestion assay for more than 15 seconds.

Since this initial report, numerous studies have repeated the pepsin digestion assay on these major food allergens (84). In general, the original findings that these allergens were stable to pepsin digestion relative to non-allergen proteins were confirmed, but the length of time that either the whole protein or fragments of the allergen were stable did not always agree. The most likely explanation for this quantitative difference is the subtle changes in the pepsin digestibility assay or in the method by which the proteins of interest were detected. For example, changes to enzyme concentration, pH, protein purity, and method of detection could have large effects on the interpretation of any in vitro assay. For this reason, the International Life Sciences Institute (ILSI) has proposed a standardization process for the assay that will attempt to assess these variables so that results from different laboratories can be directly compared. Federal, academic, and industry labs from Europe, North America, and Japan will participate in this test in which pH, pepsin concentration, allergen purity, and method of detection have all been standardized (85).

Allergens from less-common foods have also been studied using the pepsin digestion assay. For

example, allergens from fruit and vegetable foods have been tested for their ability to survive pepsin digestion. Allergens from these sources are typically classified as cross-reactive allergens because they share significant structural homology with another allergen. Good examples of this are the allergens of fruit that share significant sequence homology with aeroallergens. In these cases it is believed that the individual is sensitized to an allergen via the respiratory route and then exhibits clinical symptoms after ingestion of a food that contains a protein of sufficient sequence homology to the sensitizing allergen. For example, the Bet v 1 homologous allergens of apple (Mal d 1), pear (Pyr c 1), apricot (Pru ar 1), and cherry (Pru av 1) are, in general, labile to enzyme digestion (84). The pepsin-sensitive cross-reactive proteins typically cause localized symptoms of the oropharynx, including tingling or swelling of the lips, tongue, or glottis, and uncommonly cause systemic or GI symptoms. The lack of stability of this class of food allergens in the pepsin digestion assay suggests that pepsin stability may not be a useful predictor of sensitization. More likely is that the characteristics of this class of allergen simply reflect the discrete sensitization and elicitation processes unique to patients exhibiting oral allergy syndrome (OAS).

In Vitro Immunoassays of Allergenicity

In vitro assays such as radioallergosorbent tests (RAST) (86, 87), enzyme-linked immunosorbent assays (ELISA) (88), or immunoblotting assays should be undertaken to determine whether an allergen has been transferred to the target plant. These assays use IgE fractions of serum from appropriately sensitized individuals who are allergic to the food from which the transferred gene was derived. Serum donors should meet clinically relevant criteria, including a convincing history (89) or positive responses in DBPCFCs (86, 90). An FAO/WHO scientific panel (65) has recommended that, in addition to using serum IgE from individuals who are allergic to the food from which the transferred gene was derived, serum IgE from patients allergic to plants in the same botanical family also be used in these assays. Positive results from one or more of these in vitro assays provide strong evidence that an allergen or a cross-reactive allergen has been transferred. A clear positive result from the in vitro tests would require that any food that contained the transferred gene be labeled as containing a gene from that source.

In Vivo Assays of Allergenicity

For transgenic proteins from allergenic sources or with significant sequence homology with known allergens, further evaluation is required to determine whether the introduced protein could precipitate IgE-mediated reactions. In vivo SPT may be required for some proteins. SPT is an excellent negative predictor of allergenicity but is only 50%–60% predictive if a positive result is obtained (91). The best in vivo test of allergenicity is the DBPCFC. This procedure involves testing with sensitive and non-sensitive patients under controlled clinical conditions. Patients who are known to be allergic to proteins from the source would be tested directly for hypersensitivity to food containing the protein encoded by the gene from the allergenic source. The ethical considerations of this type of assessment would include factors such as the likelihood of inducing anaphylactic shock in test subjects, potential value to test subjects, availability of appropriate safety precautions, and approval of local institutional review boards. If sensitive patients underwent a reaction in these tests, food derived from crops containing the protein would require labeling. In practice, however, such a discovery has lead to the discontinuation of product development for brazil-nut allergen containing soybeans.

Changes in Endogenous Allergens (Substantial Equivalence)

In the context of substantial equivalence, it is important to establish that the expression of new genes, or effects due to the insertion of genes into plant genomes, does not alter the levels of endogenous (existing) allergens in food crops. This is likely to be especially true for crops that are commonly allergenic, such as soybeans, wheat, rice, or tree nuts. From the perspective of human health risk, it is generally agreed that a substantive change in the allergenicity of allergenic foods that leads to increased incidence or severity of food allergy should be evaluated and considered in safety assessment (38). To date, evaluations of endogenous allergens have typically been performed for crops that fall into the top eight "commonly" allergenic food groups. Experimentally, these evaluations involve in vitro IgE immunoassays employing western blot, ELISA, ELISA inhibition, or a combination of these techniques. Examples of such approaches include the initial evaluation

of Roundup Ready soybeans by Western blot (92) and more recently using both Western blot and ELISA techniques (93). Both studies concluded that there were no meaningful differences between genetically modified and traditional soybeans.

Allergy Assessment Summary—Roundup Ready Soybeans

Source of CP4 EPSPS: The gene encoding CP4 EPSPS was isolated from the common soil bacterium *Agrobacterium tumefaciens* strain CP4. This enzyme is present in all plants, bacteria, and fungi. However, animals do not synthesize their own aromatic amino acids and therefore lack this enzyme. Because the aromatic amino acid biosynthetic pathway is not present in mammalian, avian, or aquatic life forms, glyphosate has little if any toxicity for these organisms. In addition, the EPSPS enzyme is normally present in food for human consumption derived from plant and microbial sources, indicating that the protein has a long history of safe use.

Bioinformatic Analysis of CP4 EPSPS: A search for amino acid sequence similarity between the CP4 EPSPS protein and known allergens was conducted according to the methods described in this chapter. The search revealed no significant amino acid sequence homologies with known allergens either by the FASTA alignment or the eight amino acid search. In addition, analysis of the amino acid sequence of the inserted CP4 EPSPS enzyme did not show homologies with known mammalian protein toxins and was not judged to have any potential for human toxicity.

In Vitro Digestibility of CP4 EPSPS: An in vitro pepsin digestion assay was performed as described in Astwood et al (81) using *E. coli*-produced CP4 EPSPS that had previously been shown to be biochemically identical to that produced in plants. The intact CP4 EPSPS protein was digested rapidly and no stable fragments were detected after only 15 seconds exposure to the enzyme. These results indicate that the CP4 EPSPS protein is unlikely to be an allergen.

These data taken together with the comprehensive characterization data for the CP4 EPSPS protein and very low expression level of the CP4 EPSPS gene (the protein accumulates to less than 0.05% of total soybean meal protein) suggest that CP4 EPSPS is neither currently a known food allergen nor likely to become a food allergen as consumed in Roundup Ready soybeans.

Trends in the Science of Risk Assessment

Animal Models for Predicting Allergenicity

There has been considerable interest in the development of animal models that would permit a more direct evaluation of the sensitizing potential of novel proteins. Attention has focused on the production of IgE in response to the novel protein and a wide variety of animals are being examined for this purpose including rodents (94–96), dogs (97), and swine (98). Several variables are being tested in the development of each model organism. Some of the variables being tested are route of sensitization, dose, use of adjuvant, age of organism, diet, and genetics. Unfortunately, no validated models are currently available for assessing the allergenic potential of specific proteins in naïve subjects. This is in part due to the extremely complex nature of the immune response to foods and proteins, and in part to the fact that most of the animal models of food allergy were originally developed to understand the mechanisms of allergenicity rather than to assess the allergenic potential of novel proteins. Although some progress is being made in certain models (99, 100) much work remains to be done before there is confidence that any one model will provide positive predictive value with regard to protein allergenicity.

Refinements of In Vitro Pepsin Digestion Assay

As described above, the pepsin digestion assay can be a reasonable contributor to an overall allergy assessment of specific proteins. However, even more enlightening information may be obtained if the underlying structural basis for an allergen's ability to resist pepsin digestion are known. It is with this in mind that the sequence specificity of the pepsin substrate and the minimum peptide size required for eliciting the clinical symptoms of allergy are discussed.

Pepsin is an aspartic endopeptidase obtained from the gastric mucosa of vertebrates. However, all mammalian pepsins have similar specificities. Pepsin preferentially cleaves the peptide bond be-

tween any large hydrophobic residue (leucine, phenylalanine, tryptophan, or tyrosine) and most other hydrophobic or neutral residues except proline (101). To cleave the peptide bond between two hydrophobic residues, the active site groove of pepsin binds to a segment of the protein containing the sessile peptide bond and four amino acids on either side of the cleavage site. A number of studies have evaluated the efficiency of pepsin cleavage and the effect of various amino acids around the sessile peptide bond. To facilitate discussion, the positions have been assigned identification labels such that the amino acid (aa) residues located on the amino-terminal side of the sessile bond are labeled P_1, P_2, P_3, or P_4, and on the carboxyl-side they are labeled P_1', P_2', etc. The bond between P_1 and P_1' is the sessile bond. The most efficiently cleaved peptides have aromatic or hydrophobic residues at both the P_1 and P_1' positions. The rate of pepsin cleavage is slowed if a proline is at amino acid position P_2' or if arginines are in the P_2, P_3, or P_4 positions (102, 103).

The resistance of a protein to pepsin digestion raises the possibility that it will be taken up by antigen processing cells at the mucosal surface of the small intestine and could sensitize susceptible individuals who have consumed the protein, leading to the production of antigen-specific IgE. In addition, it is possible that a pepsin-resistant peptide could provoke an IgE-mediated allergic response in those who are already sensitized. IgE plays a pivotal role during the induction of an allergic response by triggering effector cells such as the tissue mast cells (MCs) (and possibly blood basophils) to release histamine, leukotrienes, and inflammatory proteases. This response occurs when two or more IgE molecules are bound to a single peptide fragment while the antibody is bound to the high-affinity IgE receptors (FcεRI) on these effector cells. Studies of RBL (rat basophilic leukemia) cells indicate that it probably requires the cross-linking of well over 1000 of the 200,000 or so FcεRI receptors on a single cell to cause degranulation of that cell (106). IgE antibody cross-linking occurs through the binding of multivalent antigens by IgE molecules bound to the surface of mast cells. Although various IgE-antigen binding arrangements are possible, only certain ones will lead to productive signaling and degranulation of the mast cells (105, 106). The binding is only effective if it is maintained long enough (by a high affinity interaction), and if the spatial relationship and rigidity of the antigen are sufficient to cross-link and induce intracellular signaling. Baird, Holowka, and Kane et al (107, 108) used haptens with linkers of various sizes to determine the effective spacing for degranulation and to study intracellular signaling. Results demonstrated that oligomerization of the FcεRI-IgE-antigen molecules was effective at inducing degranulation. Further, minimum spatial distances were identified using artificial hapten-spacer constructs, indicating that, although tight IgE binding can occur with bivalent haptens spanning 30 angstroms (Å), the RBL cells were not induced to degranulate. Bivalent haptens of about 50 Å were required to obtain modest degranulation, and similar haptens spaced between 80 Å and 240 Å apart seemed to provide optimum degranulation (107, 108). These results may provide guidance on the sizes of peptides that might be required to cause an allergic reaction upon challenge.

To evaluate the minimum peptide size that might effectively cross-link receptors on mast cells, the maximum overall spacing (length) may be calculated, but various assumptions must be made regarding epitope size and peptide conformation. The first assumption regards the size of a typical IgE binding epitope observed in a food allergen. Most food allergen IgE binding epitopes are reported to range in size from six to 15 amino acids in length (109). Therefore, the absolute minimum size of a peptide would have to be 12–30 amino acids long and contain two IgE binding epitopes. However, this does not take into account the data of Kane et al (107, 108) that show the IgE binding epitopes must be at least 80–240 Å apart to provide optimum degranulation. Assuming the two IgE binding epitopes are separated by the minimum length of 80 Å, and that the diameter size for an amino acid such as alanine is 5 Å, the minimum size for a peptide that would be expected to elicit clinical symptoms of an allergic reaction would be 29 amino acids long or a peptide of about 3190 daltons (Da) [29 aa × 110 (average aa molecular weight)]. These calculations do not take into account the secondary structure of the peptide. For example, the peptide could be in an α helical arrangement, a β pleated sheet, or a random coil, depending on its amino acid sequence. Mast cell degranulation would only be possible if each end of the fragment represents a strong IgE binding epitope and if the peptide is in a β-strand conformation. Based on this rationale, it is improbable that the presence of a protease-resistant fragment of <3 kDa in the in vitro pepsin digestion assay would be able to degranulate mast cells; therefore, this fragment would not be likely to pose a risk to consumers.

Whereas the discussion above is theoretical, recent evidence shows that pepsin resistant allergen fragments produced in an in vitro pepsin digestion assay were >3 kDa and contained multiple IgE binding epitopes. The major peanut allergen Ara h 2 is a 17-kDa protein that has eight cysteine residues that could form up to four disulfide bonds. Upon treatment with pepsin, a 10-kDa fragment was produced that was resistant to further enzymatic digestion. The resistant Ara h 2 peptide fragment contained intact IgE-binding epitopes and several potential enzyme recognition sites that were protected from enzymatic activity by the compact structure of the protein. Amino acid sequence analysis of the resistant protein fragments indicated that these sites contained most of the immunodominant IgE-binding epitopes. These results provide a link between allergen structure and the immunodominant IgE-binding epitopes within a population of food-allergic individuals, and they lend additional biological relevance to the in vitro pepsin digestion assay (110).

The link between food allergenicity and protein stability appears to have been confirmed, at least for milk and wheat allergies. Buchanan and colleagues (111, 112) have shown that when stability of the major allergens from these foods is disrupted by reduction of disulfide bonds, the allergens were strikingly sensitive to pepsin digestion and lost their allergenicity as determined by their ability to provoke skin test and GI symptoms in previously sensitized dogs (111, 112). Other food allergens will have to be tested in the same manner to determine whether this is a general characteristic of food allergens.

In an attempt to assess the positive and negative predictive values (PPV and NPV, respectively) for the pepsin digestion assay in identifying potential food allergens, Bannon et al (113) compared the stability of 20 known food allergens and 10 non-food allergens, and calculated a PPV for these proteins of 0.95 and an NPV of 0.80. This analysis indicates that the pepsin digestion assay is a good positive and negative predictor of the potential of a protein to be an allergen. However, the results should be interpreted with some caution, because food allergens associated with OAS were not included in this analysis, and only 30 proteins were tested in this manner. In any event, assay standardization and the study of many proteins (allergens and non-allergens) will inform the allergy assessment strategy with respect to the robustness and predictive power of this physicochemical property of proteins.

Removing Allergens from Foods

Genetic engineering can be used to reduce the levels of known allergens by post-transcriptional gene silencing using an RNA antisense approach, or to reduce their allergenicity by reducing disulfide bonds that are critical for allergenicity by using thioredoxin or by directly modifying the genes encoding the allergen(s).

The RNA antisense approach has been successfully applied to reduce the allergenic potential of rice. Most rice allergens have been found in the globulin fraction of rice seed (113–116). The globulins and albumins have been estimated to constitute about 80%–90% of the total protein in rice seeds. From this fraction a 16 kDa α-amylase/trypsin inhibitor-like protein was identified as the major allergen involved in hypersensitivity reactions to rice (116–117). Using this antisense RNA approach, Nakamura and Matsuda (118) generated several rice lines that contained transgenes producing antisense RNA for the 16 kDa rice allergen. These authors successfully lowered the allergen content in rice by as much as 80% without a concomitant change in the amount of other major seed storage proteins (Fig. 4–2).

The concept of reducing disulfide bonds to reduce allergenicity has been tested on allergens

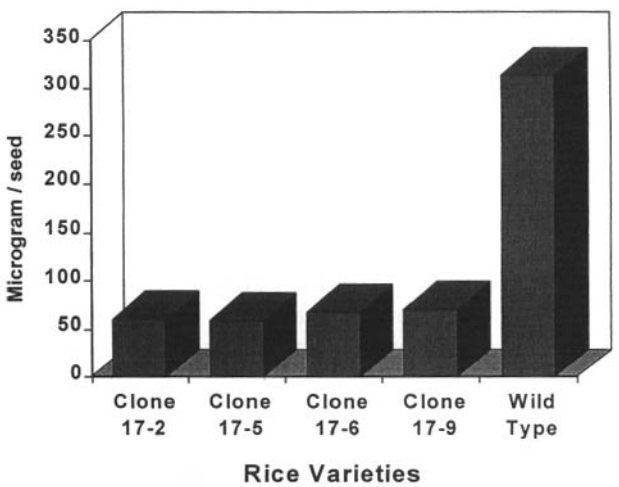

Figure 4–2. Suppression of a 16 kDa rice allergen using antisense technology. Rice allergen levels were quantified by ELISA from each genetically engineered rice variety (clones 17–2, 17–5, 17–6, and 17–9) and were compared with wild-type rice seeds. By permission from Matsuda T, Nakase M, Adachi T, et al. Allergenic proteins in rice: strategies for reduction and evaluation. Presented at the Symposium of Food Allergies and Intolerances, Bonn, Germany, May 10–13, 1995.

Figure 4–3. Thioredoxin mitigation of milk allergen reactivity in dogs sensitized to milk. Milk was incubated in physiologic buffered saline containing 5 μL of 100 mmol/L dithiothreitol (DTT) and boiled for 5 minutes prior to skin testing in milk allergic dogs. By permission from de Val et al (112).

in wheat and milk by Buchanan and colleagues and shown to significantly reduce the allergic symptoms elicited from sensitized dogs (111, 112, 119). The authors exposed either the purified allergens or an extract from the food source containing the allergens to thioredoxin purified from *E. coli,* and then performed skin tests and monitored GI symptoms in a sensitized-dog model. Allergens that had their disulfide bonds reduced by thioredoxin showed greatly reduced skin reactions and GI symptoms (Fig. 4–3). These results provide a critical proof of concept for this approach prior to constructing transgenic wheat lines that overproduce thioredoxin.

One of the more ambitious approaches to reducing allergenicity of food crops is by modifying the genes encoding the allergens so that they produce hypoallergenic forms of these proteins (120, 121). This approach is based on the observation that most food allergens have linear IgE binding epitopes that can be readily defined using overlapping peptides representing the entire amino acid sequence of the allergen and serum IgE from a population of individuals with hypersensitivity reactions to the food in question (67). Once the IgE binding epitopes are determined, critical amino acids can be identified that, when changed to another amino acid, result in loss of IgE binding to that epitope without modification of the function of that protein. Any changes that result in loss of IgE binding can then be introduced into the gene by site-directed mutagenesis.

Overlapping peptides and serum IgE from patients with documented peanut hypersensitivity were used to identify the IgE binding epitopes of the major peanut allergens Ara h 1, Ara h 2, and Ara h 3. At least 23 different linear IgE binding epitopes located throughout the length of the Ara h 1 molecule were identified (122). In a similar fashion, 10 IgE binding epitopes and four IgE binding epitopes were identified in Ara h 2 and Ara h 3, respectively (79, 123). Mutational analysis of each of the IgE binding epitopes revealed that single amino acid changes within these peptides had dramatic effects on IgE binding characteristics. Substitution of a single amino acid led to loss of IgE binding (68, 69, 122). Analysis of the type and position of amino acids within the IgE binding epitopes that had this effect suggested that substitution of hydrophobic residues in the center of the epitopes was more likely to lead to loss of IgE binding (77). Site-directed mutagenesis of the cDNA encoding each of these allergens was then used to change a single amino acid within each IgE binding epitope. The hypoallergenic versions of these allergens were produced in *E. coli* and tested for their ability to bind IgE from peanut-sensitive patients. The modified allergens demonstrated a greatly reduced IgE-binding capacity when individual patient serum IgE was compared to the binding capacity of the wild-type allergens (124, 125).

International Consensus—A Common Strategy

The development of national and international regulations, guidelines, and policies to assess the safety of food products derived from genetically engineered plants has led to broad discussions and a general consensus on the types of information that are appropriate to assess the potential allergenicity of such foods. Gaining international consensus on allergy assessment is critical because many genetically engineered plant products are commodity products (e.g., corn, soybean, wheat) grown and traded globally. A consensus approach provides producers, regulators, and consumers with the assurance that the risk of allergy to these products is appropriately addressed prior to their marketing, and that a consistent assessment approach is used around the world.

Conclusion and Future Considerations

The allergy assessment testing strategy, as it is presently formulated, is a tiered hazard identification approach that utilizes currently available scientific data on allergens and the allergic response. It is extremely important to emphasize that all aspects of the safety assessment testing strategy must be considered when assessing a novel protein, not just the results from a single arm of this strategy. Although the current hazard assessment approach has served the public interest well, it may not be adequate in the assessment of future products that might contain proteins with unknown or unpredictable mechanisms of action or that may be similar to food allergens while concomitantly providing significant nutritional and human health benefits. Considering the advances in the science of allergy assessment that are detailed in this chapter, the allergy assessment strategies proposed by Metcalfe et al (69), and the most recent recommendations by the scientific advisory panel of the FAO/WHO (65), we have described the current practices and issues in allergy assessment. This strategy takes advantage of the past assessments but, by its tiered design, attempts to place more importance on the "weight of evidence" from each test rather than relying too heavily on one test to determine whether a protein is likely to have allergenic potential

We conclude that the current testing strategy will need to be integrated into a risk assessment model where risk is defined as a function of the level of the hazard and the level of exposure to the hazard. This strategy consists of four steps: hazard assessment, dose-response evaluation, exposure assessment, and risk characterization (126). To apply risk assessment principles to the issue of the allergenicity of proteins and food biotechnology, new data must be collected for each step in this process. This review of progress on these issues indicates that the process of integration has already begun. For example, the issue of dose-response evaluation is being addressed by several investigators who are exploring threshold doses for traditional allergenic foods in clinically allergic patients (127). The issue of exposure assessment consists of three parts: the abundance of the protein in the food, the stability of the protein in the GI tract, and the amount of the genetically modified crop consumed in the diet. We believe that the protective value of current testing approaches and of future approaches that adopt sound risk assessment principles have provided and will provide robust assurances to risk managers and consumers alike.

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5

Safety Issues in Food Biotechnology

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Introduction

The advantages offered by foods derived from biotechnology are well documented. Genetically transformed crops promise improved pest and disease resistance; higher yields; superior flavor, appearance and nutrition; and reduced susceptibility to adverse environmental stresses such as temperature and drought (1–3). The US National Center for Food and Agricultural Policy has reported that genetically transformed corn was responsible for harvests that were significantly greater than those from non-transformed corn (3). Similarly, cotton biotechnology resulted in 5 million fewer acres of pesticide applications while increasing harvests by 85 million pounds. Additional benefits have included reductions in synthetic pesticides and production costs, conservation of arable forests and wetlands, and improved food safety via controlled levels of pathogenesis-related toxins (e.g., potential cancer-causing molecules such as fumonisin have been reduced in genetically transformed corn that is resistant to insect infestation [4, 5]). With estimates of the world population climbing above 7.5 billion by the year 2020 and 9 billion by 2050 (6), most, if not all, of these benefits will be critical to reaching necessary levels of worldwide nourishment. This is especially true in regions of Asia and Africa that are under marked pressures from population and food constraints. Despite these benefits, the potential health hazards of each genetically transformed food must be understood to ensure that no harm results from intended uses and consumption levels.

To date, no clearly substantiated and generally accepted adverse health reports (including food allergy) have been directly related to biotechnology of genetically modified foods. As with any other novel food variety or additive, however, new genetically transformed foods must be determined to be as safe as conventional foods using existing methodology before being introduced to consumers. With the exception of grains and domestic animals, food crops and animals may produce naturally occurring toxic substances that alone can make a food unsafe (reviewed by IFT [1]).

Genetically transformed foods present an unusual scenario, because they are essentially equivalent to conventionally bred crops that are already generally regarded as safe (GRAS) based on history of use. Thus, safety considerations for genetically transformed foods and conventional foods should be essentially the same. As such, safety assessment of food altered via biotechnology is often discussed in terms of substantial equivalence, which takes into consideration the hazards of the unmodified crop (i.e., Is the genetically modified food as safe as its conventional precursor, which is generally regarded as safe?). And whereas crop hybridization has resulted in new varieties of plants containing thousands of new gene combinations, current genetic transformations of crops involve only one to several genes per species, resulting in traits or hazards that should be more easily studied and identified. Thus, precise alteration of specific traits should actually improve the potential for safety evaluations of characterized foods.

Under these premises, consultations for organizations including the World Health Organization (WHO), the Food and Agriculture Organization (FAO), the Royal Society and the Organization for Economic Cooperation and Development (OECD) have generally taken the position that genetically modified crops are unlikely to be less safe than traditionally bred foods (7–9).

Perhaps the most prominent organism utilized in genetic transformation of crops, *Bacillus thuringiensis,* has 40 years of use and toxicology studies to suggest it is generally safe (10). Despite this, one report suggests that spraying with pesticides derived from *B. thuringiensis* may induce specific IgE and IgG production (11). This presents an interesting dichotomy between minimal health risks as perceived via safe usage and the identification of hazards such as allergenicity.

Safety Concerns

Gene Flow

One of the early concerns about human consumption of genetically transformed food dealt with the transfer of recombinant, genetic material to human cells and/or to microorganisms in the human gut. This particular concern stemmed from the traditional use of antibiotic resistance marker genes used to identify cells with incorporated DNA, as transformation frequencies occur once per million cells. In recent years, this concern has diminished for food derived from recombinant DNA technology. Technological advances in molecular biology make it possible to remove resistance markers after multiplication of genes in bacteria but before transformation of plant genome with those genes. Other markers, such as green fluorescent protein (GFP), can also be considered to determine stable transformations (12). DNA from any source is easily digested and is indistinguishable once degraded, and thus is generally considered safe (13). Because recombinant DNA would be less than 1/250,000 of the total amount of DNA consumed from a given food, the chances of recombinant DNA transfer to humans appears to be orders of magnitude less than for the "conventional" DNA (7). Consistent with this premise, when transgenic plants were fed to mice, coliform bacteria subsequently isolated from the murine feces did not contain antibiotic resistance genes (14). Outside of the laboratory, no transgenes have been detected in the cells of cows fed genetically transformed corn (15). Although the transfer of any genes into human cells requires a specific set of circumstances and thus appears unlikely (9, 16), genetically modified foods containing genes that confer resistance toward antibiotics of importance to humans may not be approved in the future.

Toxicity

Although food allergy is a prevalent concern in the arena of food biotechnology, the general toxicity potential of these products is also under scrutiny and must be considered alongside allergenicity potential when determining food safety. Many of the substantial equivalency concepts applied to evaluating potential toxicity of genetically transformed foods provide useful insight and appreciation into allergenicity testing.

Individuals clearly may experience both immunologic and non-immunologic adverse reactions to food. Toxic reactions may occur in a person that ingests enough of a food component. Adverse reactions to food may also be non-toxic, which thus relies on the physiological susceptibilities of individuals, and includes allergenicity (classifications detailed by Bruijnzel-Koomen et al [17]). The ability to identify such possibly adverse features of genetically transformed foods presents difficult challenges when compared to that of testing toxicity of "pure" chemicals (e.g., pesticides, medications). In an effort to identify potential hazard, it is relatively straightforward to administer pure xenobiotics to laboratory animals at concentrations that are even higher than those expected for human exposures. In contrast, foods consist of complicated mixtures, and have such bulk that only modest amounts of the agent in question can be administered to animals without altering their normal diet; thus, potential effects may be confounded with those observed due to conventional food exposure or nutritional imbalances. Because most agencies and producers recognize this problem, the role of substantial equivalence between the recombinant DNA plant and its conventional counterpart has been promoted as a logical starting point in safety assessment, while keeping in mind that a lack of equivalence does not imply that the food is unsafe. Potential cancer-causing molecules, such as fumonisin, have been shown to be reduced in genetically transformed corn that is resistant to insect infestation (4, 5). It has been suggested that animal feeding studies designed to address toxicity should thus not be considered unless characterization of the food suggests

that available information is inadequate to arrive at a reassuring safety assessment (18).

The concept of substantial equivalence has been largely attributed to the efforts of the OECD in 1993. As already mentioned, a recombinant DNA-containing plant's profile should be evaluated for both expected and unexpected changes that might alter such characteristics as structure, function, nutrients, toxins, and allergens from those of the equivalent conventional food. Demonstrating substantial equivalence has largely relied on agronomic traits (crop height, yield, disease resistance, climatic tolerance), nutrient composition (proteins, fats, carbohydrates, vitamins), and toxicants (glycoalkaloids, allergens). Key molecules will naturally change on a case by case, or food by food, basis. To date, no single analytical technique has been fully accepted or appears to be completely adequate. Those most often considered include genomic sequencing, DNA microarrays or mRNA differential display, proteomics, or metabolomics (7, 19).

DNA analysis may be used to compare the novel and conventional genomic sequences of plants. Unfortunately, knowledge of the DNA genome from conventional plants needs to be better understood before hazard and risk can be adequately assessed. Although advances have been made recently, as evidenced by the completed sequencing of the *Arabidopsis thaliana* genome (20), plant genomics is somewhat behind that of mammalian genomics.

Evaluating substantial equivalence in terms of genomic changes is also criticized in that it does not identify expressed products. Differential display of mRNA begins to address this issue by bridging DNA analysis with protein expression. This high-throughput approach, however, is generally considered to be laborious and technically difficult, demonstrates cDNA annealing inconsistencies, and has questionable sensitivity, thus leading to concerns regarding reproducibility.

DNA microarrays require small amounts of sample to evaluate a high number of genes simultaneously with good sensitivity. The procedure depends on mRNA hybridizing to cDNA libraries, and therefore possesses some of the disadvantages noted above. Microarrays also generally require known genomic sequences for cDNA libraries and do not provide actual expression patterns or levels. Evaluating mRNA has advantages of simplicity, automation, and high throughput, plus amplification, but frequently correlates less than 50% with protein levels. Because most food allergens are proteins, analytical approaches that do not evaluate protein expression are limited in the information they provide.

Proteomics and metabolomics move toward comparing the protein expression or compositional profiles of conventional and recombinant DNA plants. Proteomics can be used to evaluate quantities and localization of proteins in an organism, and extends beyond targeted analyses of individual traits. The predominant method in proteomics is two-dimensional (2-D) gel electrophoresis, followed by digestion of protein spots and peptide mass fingerprinting, to evaluate a host of proteins simultaneously (21). Although this approach visualizes thousands of proteins from single tissues, it is technically difficult and more complex than analyzing genome arrays. Cation exchange and reversed-phase liquid chromatography, used in combination with mass spectroscopy techniques, can provide greater sensitivity and separation of proteins from involved mixtures quickly, while demonstrating broad peptide profiles (22, 23). Analytical proteomics has been successfully used to evaluate peptide changes during seed and plant treatments, processing, or pathogen attack (24, 25). This work indirectly emphasizes that, to effectively utilize this technology, recombinant DNA plants must be handled and grown under the same conditions as those of conventional plants. Also, information must be available about conventional plants' genomes and expression profiles.

Metabolome analyses define relative changes in molecule expression via comparative experiments, and are essentially compositional or chemical fingerprint comparisons of plant molecules using mass spectroscopy methods (e.g., infrared [IR] or nuclear magnetic resonance [NMR]) (26). Differences are subsequently determined using cluster analyses. Although relatively poor in resolution, IR spectroscopy has been used to demonstrate a difference in nitrile levels among stressed and unstressed tomatoes (27). Again, the power of these techniques requires comparison of a genetically transformed crop with its conventional counterpart that has been grown side-by-side. Small environmental variances in the proteome and metabolome can easily be detected and thus confound data interpretations.

Carbohydrate profiling can serve as a more targeted approach to characterize posttranslational oligosaccharide linkages and glycosylation of proteins in plant tissue, thus identifying differences which could be informational in evaluating adverse traits such as allergenicity potential (28). As

with any new test method, an appropriate approach will require standardization, validation, and knowledge collected on genomes, profiles, and their natural variations. Currently, genetically transformed foods are being consumed without evidence of adverse, toxic reactions directly related to biotechnology. As such, allergenicity potential thus becomes a primary concern when assessing the safety of recombinant DNA plants and foods.

Allergenicity

To date, the majority of biotechnology applications have transfected genes for proteins that impart pest resistance into food crops. Because most allergens are proteins (although relatively few proteins are allergens), the allergenic potential of foods—especially those transformed with proteins novel to human exposure—is of concern. The prevalence and complexity of allergy adds to this concern, as does the fact that a clear and confident path forward, other than that proposed by the International Food Biotechnology Committee and the International Life Sciences Institute in 1996 (29), has not been widely accepted. IgE-mediated reactions are the primary basis for most allergies to food, and they are responsible for the most concern because such reactions may result in death via anaphylaxis (Table 5–1). These responses can follow the release of chemical mediators from mast cells and basophils due to interactions between food proteins and specific IgE on the surfaces of these effector cells.

Allergenic foods typically contain multiple allergens. These allergens are designated using the first three letters of the genus, the first letter of the species, and a number to indicate the order of designation. Major allergens are designated by the International Union of Immunological Societies and are, in the case of food, usually defined by the criterion that greater than 50% of patients allergic to that food react to a particular protein. Examples of allergen designations from the foods that appear to cause nearly 90% of reported food allergy reactions are listed in Table 5–2.

Table 5–1.
Immediate Allergic Reactions to Food Antigens

Allergic Reaction	*Site of Reaction*	*Immune Effector Cell(s)*
Rhinoconjunctivitis	Eyes, upper respiratory tract	Basophil/mast cell
Oral allergy syndrome	Mouth	Mast cell
Urticaria/angioedema	Skin	Basophil/mast cell
Atopic dermatitis	Skin	Basophil/mast cell, eosinophil
Asthma	Lower respiratory tract	Mast cell, eosinophil, lymphocyte
Gastrointestinal reactions	GI mucosa	Mast cell, eosinophil
Systemic anaphylaxis	Skin, respiratory tract, GI tract, cardiovascular system	Basophil/mast cell

GI, gastrointestinal.

Recent reports evaluating food allergy in randomly selected young adults from Australia and from the 1998 European Community Respiratory Health Survey demonstrated a probable IgE-mediated (via skin prick test [SPT]) food allergy prevalence of less than 1.5% (30, 31). This is in reasonable agreement with previous reports that approximated food allergies in the U.S. and the U.K. to be near 1.3% and 1.5%, respectively (reviewed by Ebo and Stevens [32]). The prevalence of food allergy in children 3 years of age or younger was estimated to be considerably higher (6%) by Bock (33). More recently, studies of European children suggest that IgE-mediated food allergy may range to 10% after 1 year of life, 7% at 2 years, and then decrease to 3% by 5 to 6 years (34–36). The loss of sensitivities to food allergens (particularly those from milk and egg) by older children is not uncommon, whereas patients with allergies to nuts or seafood appear less likely to lose their clinical reactivity (37). Patients that have been diagnosed as atopic are more likely to incur food allergies. For example, between 38% and 56% of children and young adults with atopic dermatitis have been shown to exhibit food allergies following double-blind placebo-controlled food challenge (DBPCFC) (38, 39). Similarly, sensitization to food (egg, milk, nut, potato, wheat, celery, soy) was identified in 21 out of 45 adult patients (47%) with severe atopic dermatitis (40).

The pathophysiologic mechanisms involved in the development of food hypersensitivity are incompletely understood. Under normal circumstances in most individuals, ingested food proteins are not allergenic and thus do not elicit allergic immune responses. This absence of reactivity may be due to suppression, tolerance, or anergic mechanisms of the immune system. However, a deficiency of such processes in some individuals, usually on a genetic basis, results in their immune system recognizing some foreign proteins as allergenic, leading to food-induced allergic reactions. Recent studies have provided novel insights into a possible mechanism involved in these

Table 5–2.
Major Allergenic Foods

Food	*Allergen Source*	*Allergen*	*IUIS Designation*
Milk	*Bos domesticus* (cattle/milk)	α-Lactalbumin	Bos d 4
		β-Lactoglobulin	Bos d 5
		Serum albumin	Bos d 6
		Immunoglobulin	Bos d 7
		Caseins	Bos d 8
Soy	*Glycine max* (soybean)	Glycoprotein (HPS)	Gly m1A
		Glycoprotein (HPS)	Gly m1B
		β-Conglycinin	
		Vicilin	
Peanuts	*Arachis hypogaea* (peanut)	Vicilin	Ara h 1
		Conglutinin	Ara h 2
Tree nuts	*Bertholletia excelsa* (Brazil nut)	2S albumin	
	Juglans regia (walnut)	2S albumin	
		Vicilin	
Crustaceans	*Penaeus indicus* (Indian shrimp)	Tropomyosin	Pen I 1
Fish	*Gadus callarias* (cod)	Allergen M	Gad c 1
	Salmo salar (salmon)	Parvalbumin	Sal s 1
Egg	*Gallus domesticus* (hen)	Ovomucoid	Gal d 1
		Ovalbumin	Gal d 2
		Conalbumin	Gal d 3
		Lysozyme	Gal d 4

tolerance processes (41, 42). T lymphocytes (Th2 cells in the context of IgE-mediated allergy) are critical in the development of specific immune responses and subsequent allergic reactions. Th3 cells in the gastrointestinal mucosa produce transforming growth factor β (TGF-β) upon antigen stimulation and have been shown to inhibit the activation of corresponding antigen-specific Th1 and Th2 cells, thus suppressing the immune response to potential antigen (or allergens) in nonallergic persons (41, 42).

Although hundreds of proteins have been identified as allergens, and their amino acid sequences have been sufficiently characterized to be incorporated into comprehensive allergen databases (43), the precise common features of a protein that make it allergenic remain elusive. The more common food allergens are generally water-soluble proteins that have a reasonable degree of glycosylation and range in size from 10 kDa to 70 kDa (reviewed in [44]). Many allergens appear to be storage proteins and as such are present in large amounts. But although exposure to a protein is a necessary part of allergic sensitization, many food allergens are present in much smaller amounts, demonstrating the importance of potency versus exposure dose. Many food allergens have homology to, and thus can be classified as, pathogenesis-related proteins (45, 46). These classes of proteins play roles in plant defense mechanisms and may increase in situations of environmental stress, physical damage, or infestation. A number of food allergens are stable and resistant to heat (i.e., cooking) and digestive processes (47, 48). Conversely, some food allergens are unstable. For example, known allergens from apples and milk have been shown to be degraded by heat or enzymes (44, 49). Furthermore, denaturation of proteins via digestion or heat processing may actually enhance the allergic properties of foods, as has been suggested for peanuts (50) and milk β-lactoglobulin (51). Despite these general similarities among known allergens, the exceptions are sufficient enough that predicting allergenicity potential of novel proteins based solely on these characteristics is inadequate.

Identification of Food Allergens

While evaluating the potential toxicities of foods transformed by biotechnology is no small task, the approach to determining substantial equivalency is identifiable and techniques are available. In the case of predicting allergenicity

potential, current approaches possess deficiencies that make evaluations of novel proteins problematic. The decision tree advanced in 1996 relies on gene source and an amino acid sequence homology comparison to known allergens, serum IgE reactivity from known allergic patients, and protein stability in the presence of enzyme(s) (29). This strategy would have appropriately identified methionine-rich 2S albumin as an allergen. This protein, transfected into soybeans to improve methionine deficiency, was later determined to be a major Brazil nut allergen (52). Because the Brazil nut is a known allergenic food (i.e., source of transfected gene is allergenic), IgE reactivity was eventually tested and demonstrated via radioallergosorbent test (RAST), immunoblots, and SPT, and thus would have been identified through this decision tree approach.

However, the ability to obtain clinically verified serum from allergic patients in sufficient numbers to be used in risk assessment is a limitation of this approach. Also, most transferred genes will presumably encode proteins that are not from allergenic source(s). Furthermore, measuring IgE reactivity in vivo via SPT or DBPCFC raises ethical concerns.

As mentioned, a number of known food allergens appear to be relatively resistant to heat and protease degradation (47, 48). Thus, heat stability and enzyme digestibility (e.g., by pepsin or trypsin) have been utilized to help identify potential allergens. Caution should be used to prevent overinterpretation of results from these approaches, because 1) protein stability may be an indication of integrity of proteins passing through the stomach or may reflect structure, and thus is not a direct assessment of allergenic potential; 2) several stable non-allergens and labile allergens exist; and 3) limited data are available that demonstrate relative enzyme or heat stability among known allergens versus proteins with weak to no allergenic potential. Stability characteristics of proteins following treatment with digestive proteases may also reflect a protein's behavior during enzymatic alteration by antigen processing cells.

Another limitation of an enzyme digestibility assay is a lack of standardization. Ratios of protein to enzyme, thresholds of "stability" in terms of time until degradation, and techniques to detect digestion have large implications on digestibility interpretations. Investigators at the US FDA National Center for Food Safety and Technology have compared allergens and non-allergens from different protein classes (e.g., storage and contractile proteins, enzymes and lectins). Digestion data failed to demonstrate that food allergens were more stable than non-allergens, or than proteins of unknown allergenicity (53).

In 2001 a revised decision tree approach was set forth by the FAO/WHO, which drew from academia, industry, and government to convene a group of experts (Consultancy) in protein chemistry, animal models of safety assessment, immunology, food processing, and food labeling to advance the strategy for allergy safety assessment of genetically transformed foods (54). The result was a modified decision tree that added features, such as IgE reactivity with foods that are broadly related to the gene source of the transferred DNA, and an increased emphasis on animal models and expression levels of the novel protein, to push the boundaries of safety assessment.

The 2001 decision tree also increased the rigor in evaluation of amino acid sequence homology from eight amino acids down to six, along with a 35% identity match over any 80 or more contiguous amino acids throughout the sequence of the protein. A six-amino acid homology screen has since been evaluated via a sequence analysis of corn (*Zea mays*) (43). Analyzing 50 randomly selected corn protein sequences (out of 4116 sequences total), 84% (42/50) demonstrated homology within a 658-allergen database. When an eight-amino acid criteria was utilized, only 6 out of 50 were identified as having allergenic potential. Hileman et al (43) further suggested that a 35% structural identity screen, combined with the eight amino acid "epitope homology screen," demonstrates the best selectivity while still maintaining a conservative approach. Such research allows further modification of the parameters of the FAO/WHO decision tree approach, as suggested would be needed by the Consultancy.

Although reliable animal models have yet to be generally accepted, the 2001 FAO/WHO report on allergenicity assessment of foods derived from biotechnology encouraged the use of animals to help evaluate allergenicity potential. This has prompted subsequent consultations and workshops organized to advance the science of animal allergenicity models (e.g., ILSI Protein Allergenicity Technical Committee, Workshop on Animal Models to Detect Allergenicity to Foods and Genetically Modified Products [Health Canada], and Assessment of the Allergenic Potential of Genetically Modified Foods [US EPA, FDA, and NIH]). One view stemming from these meetings is that an appropriate animal model designed for hazard identification of allergenicity potential should function in the context of specific IgE production. How-

ever, given the differences in immune responses between various animal species and humans, this may not be necessary. Further, the model need not necessarily mimic human exposure (i.e., oral) or clinical response(s), nor should it be required to demonstrate IgE production following exposure to non-allergens. As a hazard identification model, it should accurately reflect allergen potency of known allergens in an order that is similar to that reported for humans (e.g., peanut > egg > milk > potato) as measured by some parameter of response. A number of laboratory species are being evaluated but two—the Brown Norway (BN) rat and the BALB/c mouse—appear to best accommodate a rapid hazard identification approach.

The BN rat is a high IgE-responding rat strain that is thought to mimic the genetic phenotype of atopic humans. BN rats administered food allergens (e.g., ovalbumin, cow's milk proteins, soy proteins) via daily oral gavage demonstrated specific IgE production by 4–5 weeks as measured by a passive cutaneous anaphylaxis assay (55, 56). Although the BN model demonstrates relative allergen potencies similar to those in human data, the response is accurate only after at least two generations of BN rats with a diet free of the protein under investigation (57). This requirement may present a problem when evaluating novel, unknown proteins and increase study time to over 6 months.

The BALB/c mouse is another IgE-responding rodent that is being investigated as a potential allergenicity-screening model. BALB/c mice exposed systemically (i.e., by intraperitoneal injection) over 3–4 weeks produce IgE specific for allergens from foods such as peanut, egg, milk, and potato (58–60). In mice maintained on a normal laboratory diet, the order of relative potencies of allergens appears to be consistent with human reports. Other mouse strains are also being evaluated for use as models, as are mice genetically engineered to have enhanced allergic responses.

In addition to these two rodent models, allergic models using "atopic" dogs and neonatal swine have been considered. Wheal and flare, and gastroscopic sensitivity, to several common food allergens have been demonstrated in an inbred colony of high-IgE producing dogs (61). Following live virus vaccinations, these young dogs demonstrated allergic immune responses between 1 and 9 months of subcutaneous injections of food extracts (e.g., peanut, milk, soy) in alum. Newborn piglets maintained on an allergen-free diet can also be induced to present dermal and gastrointestinal allergic responses as early as 5 weeks after systemic injection with peanut extracts mixed with cholera toxin (62). These dog and swine models bring with them additional costs and resources, and animal welfare concerns.

Other approaches to assurance of food safety, and that have been discussed in support of allergenicity assessment, include determination of protein expression levels, and postmarket surveillance. Although allergenicity of a protein is likely more important than exposure dose, the upper limit of transfected gene product(s) would be an informative piece of evidence in determining sensitization potential. Unfortunately, insufficient data exist to support threshold levels of allergens required for sensitization and elicitation of allergic responses. Furthermore, expression levels might be useful only to help determine risk once a protein is deemed allergenic. However, it appears that doses ranging from hundreds of micrograms to tens of milligrams are necessary to elicit a clinical allergic reaction from more common allergens (peanut, milk, egg) (63). The amount of allergen exposure required to induce sensitization remains unknown.

Although it is not an a priori evaluation of allergenicity potential, postmarket surveillance may be able to provide safety information regarding long-term consumption and exposure. The most commonly proposed strategy involves adverse event reporting, such as that done under the Center for Food Safety and Applied Nutrition (CFSAN) Adverse Events Reporting System (CAERS) (www.cfsan.fda.gov). A cluster of adverse reports would subsequently need to be confirmed via IgE reactivity to validate causation between the offending food ingredient and self-reported effect(s). Assessment of this approach suggests that adverse event reporting may not detect a modest allergic outbreak against the background of allergic reactions due to convention foods. Furthermore, postmarket surveillance may be useful only if it can be combined with the daunting task of strict "traceability" of genetically transformed food.

Because of the limitations of the several approaches outlined above, the Codex Alimentarius Commission (i.e., CODEX), the FAO/WHO joint organization that devises and standardizes international food codes, has advanced an approach to safety assessment of genetically modified foods that incorporates simultaneous evaluations using a variable number of the methodologies reviewed above, to be modified as the science for each makes them more trustworthy predictors of allergenicity (18).

Table 5–3.
Potential Scientific Approaches to Evaluate Allergenicity of Genetically Modified (GM) Foods

Questions	*Bioinformatics (Sequence Homology)*	*Stability*	*Biochemical Profile*	*Patient IgE Reactivity*	*Animal Model*
Does the gene encode a protein similar to a known allergen?	X				
Is protein "foreign"? Can it elicit immune recognition?				X	X
Does protein demonstrate cross-reactivity with known allergen?	X			X	X
Is GM food substantially equivalent to conventional counterpart?			X		X
What is the potential for exposure?		X	X		X

Because no single approach to evaluating allergenicity potential determines absolutely the allergenic potential of a food protein, the best current approach to accurate safety assessment is to use a combination of techniques in an organized and consistent manner (Table 5–3). Animal models may eventually be determined the most informative tool for identification of the allergenic potential of genetically modified food, and efforts to refine these models should continue. Because of the potential utility of hazard identification by in vivo exposure of laboratory animals, refinement of animal models deserves the full attention of biotechnology stakeholders in advancing safety assessment of genetically modified foods. For now, IgE reactivity in human patients remains an available direct approach in the evaluation of direct immune recognition, but only if the protein has homologous characteristics to another allergen. In practical terms, allergen-homologous proteins will more often than not be excluded from research and development efforts after bioinformatic analyses. An additional problem with testing reactivity of patient IgE is the availability of appropriate and clinically well-defined sera samples. While an all-inclusive serum bank is often discussed, the collection, characterization, and maintenance of such a bank represents a substantial commitment of resources.

The usefulness of information provided by amino acid sequence comparisons indicates that this approach should also receive attention. Investigators have effectively designed and used custom-made databases and algorithms to screen novel protein sequences against known allergens. To advance a consistent process of allergenicity assessment, however, a standardized bioinformatics approach must be agreed on. Amino acid homology screening is conducted early in product development and it should remain a primary step in allergenicity assessment. It should not be used as the sole approach, however, because appreciation of sequence homology as it relates to allergen characteristics remains to be better understood in the context of hazard as well as risk. And although information on stability of proteins has been used in combination with bioinformatics in evaluating allergenicity potential, the utility of this technique lies in the tenet that protein stability is necessary for mucosal exposure to the immune system. As such, this approach may continue to be useful in the assessment of protein exposure, once the hazard potential for allergenicity potential has been elucidated, and thus should be standardized to provide consistent—and comparable—results among investigators. Proteomics and metabolomics provide equally useful data in regard to exposure, and are useful with regard to overall compositional equivalency. Currently, an organized, consistent, methodical approach that incorporates multiple techniques appears to provide the most effective safety assessment of allergenic potential of genetically modified foods.

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6

Development of Immunological Tolerance to Food Antigens

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Introduction

The prevalence of allergic diseases has increased progressively since the 1960s, especially in First World countries, and the increases appear to be continuing in many countries. The most prominent increases have occurred in allergy to inhaled antigens. However, there is evidence to suggest that at least some forms of food allergy are also on the rise. The most notable example is peanut allergy, which was previously rare in many countries. The increase may be due to altered dietary habits in previously unexposed populations coupled with changes in processing procedures resulting in increased allergenicity of peanut antigens, but the issue remains unresolved.

The review below focuses primarily on the underlying immunological mechanisms governing host responses to ingested antigens. We focus initially on what has been learned from basic studies in animal models, what has been deduced from extrapolation of these systems to immunocompetent human adults, and what is known of the immunology underlying sensitization to dietary versus inhalant allergens during childhood.

Immunological Tolerance to Dietary Antigens in Experimental Animals: the Phenomenon of Oral Tolerance (OT)

The first formal description of experimental OT dates to the work of HG Wells in 1911 (1). His studies in the guinea pig involving repeated feeding of egg white protein (ovalbumin [OVA]), and the results were prototypical of several generations of subsequent laboratory research, namely, that the repeated antigen feeding of immunologically naive animals elicited initial "hypersensitivity" responses. In some animals, these responses resulted in fatal anaphylaxis, but in the majority the symptomatology was transient and was followed by permanent unresponsiveness to the antigen. We now recognize the initial hypersensitivity as a hallmark of the "Th2 default" response of the mucosal immune system to a soluble protein antigen (2), and the ensuing state of antigen-specific unresponsiveness as indicative of the subsequent onset of immunological tolerance, i.e., OT.

The species currently most studied in the laboratory is the mouse, and a comprehensive picture is now emerging of the characteristics of murine OT. Most aspects of the adaptive immune response may be experimentally down-regulated by antigen feeding, in many cases via a single dose. The range of susceptible immunologic phenomena include cellular immunity measured as delayed-type hypersensitivity (DTH [3–5]), contact sensitivity (6), cytotoxic $CD8^+$ T cell responses (7, 8), production of cytokines (9), and antibody secretion (10–12). There is some evidence that local secretory IgA responses to the eliciting antigen may be preferentially "spared" in this tolerance induction process (12) via generation of IgA T helper cells in gut-associated lymphoid tissues (GALT) during tolerogenesis (13). This may be part of a generalized mucosal protection process,

because IgA antibodies would block antigen penetration through the gut wall and thus limit the scope for allergic sensitization.

Many host and environmental factors have been identified as partial determinants of susceptibility to OT induction. Prominent among host factors are hormonal balance (14, 15), genetic background (16), and in particular, postnatal age. A transient temporal window defining increasing susceptibility to sensitization to dietary antigens is operative in the mouse during the early postnatal period (17–19). This seems to be due to some as yet undefined mechanistic failure in the OT process. It appears likely that the failure involves one or more components of the host adaptive immune response, as competence for OT generation in neonatal mice can be conferred by adoptive transfer of adult spleen cells (20). Host immune competence is a key feature underlying efficient OT, as demonstrated by the fact that administration of cytotoxic immunosuppressants seriously compromises the process (3, 14).

Additional exogenous factors that interfere with OT induction include inflammatory adjuvants (21), low dose irradiation (4), and changes in host microbial flora (22, 23). This will be further discussed in more detail below.

The type and dose of antigen are also important factors in the induction of OT. Whereas tolerance can be readily induced to all thymus-dependent soluble protein antigens, replicating and particulate antigens tend to induce active immunity. The inflammatory response elicited by replicating antigens bypasses OT mechanisms, and this can be mimicked with inert soluble protein antigen by coupling to adjuvants and/or microbial toxins (24, 25). The tolerogenic dose range for some antigens can also be within the immunogenic range for others, as shown in recent studies contrasting cow's milk whey proteins and OVA (20). It is also noteworthy that, while OT can be induced over a wide range of dosages and using varying feeding frequency regimes, continuous exposure leads to the most profound tolerance (26, 27). Furthermore, very low doses below the tolerogenic range can in some circumstances prime animals for subsequent immune responses (28).

Antigen Presentation and Processing in OT Induction

Several pathways are operative in sampling and processing of ingested antigen, and it is possible that the particular pathway that is dominant within an individual immune response may ultimately determine the nature of ensuing immunity. There are three principal pathways for sampling of antigens from the luminal surface of the gut: between lining epithelial cells, through the epithelial cells themselves, or via microfold cells (M cells) with subsequent delivery into Peyer's patches (PP). In each situation, distinct populations of potential antigen-presenting cells (APCs) are encountered, and it is not clear what the contribution of each is in the OT process.

The full range of known professional APCs have been identified throughout the gut wall and associated lymphoid tissues, comprising dendritic cells (DCs), macrophages, and B cells (29–31). Many sites contain multiple APC populations. For example, at least three populations of DCs with APC activity have been defined in PP (32–34), one of which has been proposed to selectively prime T cells for interleukin-10 (IL-10) production ([34]; see below).

Gut-derived DC are currently the focus of intense interest, because of their role as potential candidates for generation of the rate-limiting tolerogenic signal(s) in the OT process. In other organ systems, DCs are recognized as the ultimate "gatekeepers" of the immune response (35). These cells are the most potent APCs for activation of T cells in primary immune responses, and they are increasingly being implicated in regulation of tolerance to both self and exogenous antigens. Recent studies have demonstrated that administration of the growth factor F1t3L to mice markedly expands the numbers of DCs in the intestine and associated lymphoid tissues, and at the same time increases susceptibility to OT induction (36).

An additional antigen presentation pathway that may also contribute to OT development involves the direct absorption into the circulation of breakdown products of ingested antigens (i.e., low molecular weight peptides) that are potentially tolerogenic (37–39). Indirect evidence suggests that these peptides may exert some of their effects within the liver (40–42), which is traversed by large numbers of recirculating T cells. There is also evidence for a role for antigen-presenting enterocytes in OT induction (43–46). These cells can internalize and process antigens and can express surface major histocompatibility complex (MHC) class II molecules, but they appear to lack the costimulator molecules necessary for full-blown T cell activation (43).

Cellular Mechanisms Governing the Induction and Maintenance of OT

A variety of mechanisms exist for OT development and persistence in experimental murine systems.

The initial evidence that OT is an active process came from studies involving adoptive transfer of OT to naive recipients via $CD8^+$ splenocytes from tolerant donors (47). However, this maneuver failed in many laboratories until careful studies revealed that several apparently distinct mechanisms operate at the two extremes of the antigen exposure dose response curve (48). At one end of the spectrum, high dose oral antigen exposure leads to functional elimination of antigen-specific T cells via either deletion or anergy induction. Clonal deletion of OVA-specific $CD4^+$ T cells via in vivo apoptosis has been demonstrated in OVA-fed OVA-transgenic mice (49); however, subsequent adoptive transfer studies in transgenic T cells have yielded conflicting results (50, 51). Tumor necrosis factor-tumor necrosis factor receptor (TNF-TNFR) interaction has also been suggested to play a central role in OT-induced apoptosis in one of these models (52).

An alternative to deletion is induction of T cell anergy, in which antigen-responsive cells are functionally paralyzed. This also occurs in the high dose range, most likely via aberrant antigen presentation by MHC class II-bearing APCs that lack key co-stimulator molecules such as CD80/CD86 (53). These anergized cells do not apoptose, but instead persist in the periphery. They presumably maintain their surface antigen receptors but lose the capacity to clonally expand and secrete the full repertoire of cytokines following an encounter with antigen. In particular, in some model systems cytokine secretion by anergized T cells appears limited to IL-10. Consistent with the potential importance of this mechanism, evidence exists in some models of OT for the presence of tolerized T cells whose ability to respond to antigen in vivo can be restored by exogenous IL-2 (54). Further supporting evidence has been provided in transgenic mice (55).

In contrast, exposure of animals to low dose oral antigen may induce a form of "low-zone tolerance" involving active antigen-specific suppression of immunity that can be adaptively transferred by $CD8^+$ T cells in some systems, and by $CD4^+$ T cells in others (56). However, OT induction proceeds normally in CD8 knockout mice (8), suggesting that the role of these cells may be restricted to maintenance, rather than induction, of OT. Additionally, there is evidence to suggest that a subset of the T cell receptor g/d [TCRγ/δ] T cells may also participate in the OT process (57, 58).

Currently, much of the OT literature is focused on $CD4^+$ T regulatory cells and the cytokines they secrete. Two principal subtypes have been identified, namely, Th3 cells, which secrete transforming growth factor beta (TGF-β) with or without IL-10 (59, 60), and Tr1 cells, which secrete IL-10 (61). IL-4 has also been implicated in the OT inducing activity of Th3 cells; however, its role may be restricted to that of a non-essential growth factor (62) given that IL-4 knockout mice can still generate OT (63).

TGF-β has many roles in the control of epithelial growth and differentiation and in local control of secretory IgA production in the gut (30, 31, 64). The latter explain the selective preservation of secretory IgA antibody production during systemic OT induction (12, 13). Additionally, TGF-β has a number of potent immunosuppressive effects (65), including those targeted at APC functions. IL-10 is increasingly recognized as a powerful anti-inflammatory and immunomodulatory agent that plays a critical role in local homeostasis in the gastrointestinal mucosa, particularly via its damping effects on Th1 activity (66, 67).

One interpretation of these findings is that many of the mechanisms defined experimentally may represent redundancies. However, it is more likely that each constitutes one component of a multi-layered, integrated regulatory process, each of which are individually "selected" as dictated by prevailing conditions of antigen dosage and exposure frequency (20). An intriguing additional possibility is that some of the recently described "T regulatory" populations may in fact be partially anergized T cells, which, despite functional downregulation, have conserved their ability to secrete certain cytokines, such as IL-10 and TGF-β.

OT in Humans: How Well Do Mouse Models Mimic the Human Situation?

As noted above, the baseline "default" response of laboratory mice appears biased toward a Th2-like cytokine profile, admixed with TGF-β production (2). This Th2 default is also consistent with the pioneering studies of Wells in guinea pigs (1), wherein the first manifestations of immunological reactivity in a subset of his test animals was what we now recognize as IgE-mediated

anaphylaxis. However, this Th2 bias is clearly a transient state in most situations, given the fact that the hallmark of successfully induced OT is tolerance in the IgE antibody class.

Selective priming of T cells in PP for production of IL-4 and TGF-β is a hallmark of this bias toward baseline Th2 in murine immune responses to oral antigen (68, 69). These cells are hypothesized to migrate to peripheral sites and function as T regulators to dampen Th1 immunity (70). Such studies have not been performed in humans. However, recent studies indicate that, unlike the murine situation, the immunological milieu in human gut associated lymphoid structures such as PP is strongly Th1-biased (71), at least in adults. Thus, freshly isolated T cells from the human gut lamina propria produce high levels of interferon-gamma (IFN-γ) relative to IL-4, IL-5, and IL-10 (72–75), and high levels of IL-12 production are observed in human PP (71, 76). This intrinsic difference between mouse and humans may be a direct reflection of the markedly differing levels of microbial stimulation in humans versus specified pathogen-free mice housed under controlled conditions and exclusively fed sterilized food (71).

Although these observations suggest substantial differences between human and murine immune responses to oral antigens, it is clear from several lines of evidence that the fundamental process of OT nevertheless occurs in humans. Notably, feeding of keyhole limpet hemocyanin to immunologically naive volunteers selectively down-regulated subsequent cellular immune responses to the antigen (77, 78). Furthermore, prospective studies have shown transient IgE antibody production to foods to be common during the first 2 years of life and then down-regulated thereafter (79). Also, deliberate parenteral immunization of volunteers with the common dietary antigen bovine serum albumin elicited little or no antibody production (80). Additionally, clinical trials aimed at amelioration of autoimmune disease by autoantigen feeding have provided varying levels of clinical effects (64), and have also shown induction of Th3 responses in blood lymphocytes that are comparable to those reported in the murine model (60).

In addition, recent comparative studies on T cell responses of PP-derived T cells (PPTs) versus peripheral blood T cells (PBTs) from normal subjects indicated consistent lymphoproliferation in PPTs in response to the dietary antigen β-lactoglobulin (BLG), in contrast to low- or non-responsiveness in PBTs (76). Moreover, the peripheral blood mononuclear cell (PBMC) responses were dominated by Th1 cytokines (76), mirroring the overall Th1-biased milieu of gut-associated lymphoid tissues (71). This apparent OT at the periphery with the concomitant presence of antigen-specific IFN-γ-secreting T cells in lymphoid compartments draining the intestinal mucosa, mimics precisely the situation reported for mice fed repeated doses of OVA (9), and may be indicative of the contribution of locally activated regulatory T cells in the maintenance of systemic tolerance. It is clear that additional fundamental studies are needed on human immune responses to dietary antigens, and in particular on the potential interactions between these and parallel responses to microbial stimuli provided by the local commensal flora.

Food Allergy in Humans: the Clinical Reality

Dietary antigen-induced enteropathies are believed to be central to the pathogenesis of a broad range of chronic inflammatory diseases in humans, including IgE mediated food allergy, celiac disease, inflammatory bowel disease, and other enteropathies. The discussion below will focus on allergy-like manifestations of aberrant immunity to dietary antigens, and emphasizing issues relating to the initiation of dietary allergies in early life. From a clinical point of view it is important to appreciate that similar clinical symptoms may be induced by immune reactions, various biochemical intolerances, and toxic reactions. Thus, food intolerances caused by non-immunologically mediated mechanisms are often erroneously called "food allergy." For example, the majority of adults in the world are lactose intolerant; in these persons, drinking milk can induce symptoms that may be mistakenly interpreted as evidence of food allergy.

Allergy to food antigens in an infant is often the first manifestation of atopy and may be the forerunner of IgE mediated allergy to inhalant allergens. IgE mediated food allergy and atopic dermatitis (AD) in infancy may thus be the first steps in the "atopic march" (81–83). In most instances, clinical tolerance to the food develops within the first 3 years of life; however, the atopic march continues with manifestations of allergic asthma and subsequently allergic rhinoconjunctivitis. The reasons for this switch from the gastrointestinal to the respiratory tract are poorly understood.

Clinical tolerance does not develop equally to all food allergens. For unknown reasons, IgE-mediated allergy to cow's milk and egg are uncommon after the age of 4 years, whereas allergy to soy protein tends to last for a longer period, and for most patients peanut allergy and celiac disease are regarded as lifetime conditions. Active celiac disease can relapse at any age if the individual is exposed to gluten for some time.

The resolution of clinical food allergy is accompanied by the development of tolerance to food antigens. This process has been studied mostly for allergens causing IgE-mediated reactions, and less is known about the kinetics of immune responses to antigens involved in the pathogenesis of celiac disease and other enteropathies. Prospective studies in which immune responses to food antigens were studied through the first several years of life showed that transient IgE antibody responses to such antigens as egg and cow's milk are common in healthy non-atopic infants (79). In contrast, the IgE antibody responses are of higher magnitude and more prolonged in infants who develop food allergy and/or who will manifest respiratory allergy during later childhood. Indeed, high levels of IgE antibodies to egg or milk in an apparently healthy infant predict the appearance of respiratory allergy some years later (79, 81–83). Only prospective studies could reveal any qualitative differences in the development of immune responses to usually transient allergens like milk and egg proteins versus allergens associated with chronic sensitivity, such as peanut proteins.

Cellular Mechanisms Underlying Control of T Cell Immunity to Dietary Antigens in Humans: Lessons from Studies on Responses to Aeroallergens

As noted above, studies on antibody production indicate that immune responses against environmental allergens are initiated very early in life in most individuals, and these observations have prompted detailed investigations in many laboratories on the nature of underlying T cell immunity during this life phase. The salient findings are reviewed below.

First, it is now clear that T cells responsive to both dietary and inhalant allergens, as measured by lymphoproliferation, are present in cord blood from virtually all subjects (84–88). Additionally, T cell cloning and subsequent genotyping studies indicate that the responsive cells are of fetal origin and exhibit a Th2-polarized and/or Th0 cytokine profile (89, 90). These T cells may have been primed by processed antigen crossing the placenta, perhaps bound to maternal IgG (88–91). Evidence showing the presence of detectable levels of allergen in complex with IgG antibodies in cord blood supports this suggestion (92). However, it is also feasible that these T cell responses may be directed against cross-reacting antigens or anti-idiotypic antibodies, and further research is required to resolve this issue.

Second, it is evident that these early T cell responses are subject to a variety of regulatory mechanisms postnatally that are driven by direct exposure of the infant immune system to incoming environmental allergens. Given the experience from animal models, it is likely that these regulatory mechanisms are dictated by the concentration, frequency, and routes of allergen exposure; the age—and hence developmental status—of the individual at the time of exposure; and potentially by allergen structure (e.g., susceptibility to proteolytic degradation). The relevant immunoregulatory mechanisms involved probably span the full range from classical low-zone tolerance (essentially Th1/Th2 cross-regulation) to high-zone tolerance phenomena (anergy and/or deletion via apoptosis), and will inevitably include important, but as yet uncharacterized, contributions from recently described subsets of T regulatory cells that appear to participate in numerous immunological control mechanisms.

The overall dynamics of these immunoregulatory processes are now at least partly understood. In particular, cross-sectional and prospective studies indicate that, in atopic children, consolidation of Th2-polarized immunity against inhalant allergens is initiated in early infancy (93, 94) and may be completed by the end of the preschool years in children who develop clinical allergy (95). However, a recent prospective study from Estonia, with a low prevalence of allergy, indicates that other regulatory mechanisms may also be operative (96). During the first 2 years of life, the incidence of positive skin prick tests was similar to that in recent studies from Western Europe, whereas at 5 years the prevalence was only 3%. At the same time the prevalence of circulating IgE antibodies to milk or egg increased to 36% and 47% to inhalant allergens. The discrepancy between positive skin prick tests and circulating IgE antibodies is interesting in a country with a low prevalence of atopic allergy and Th1-dependent

type 1 diabetes, and with a lifestyle similar to that prevailing in Scandinavia some 30–40 years ago. The findings may also suggest that clinical tolerance to a food does not exclude the presence of IgE antibodies and other indicators of Th2 immunity. It is possible that a traditional lifestyle is associated with an early induction of a general regulation of T cell immunity. This notion is supported by the recently reported close correlation globally between the prevalence of wheezing and type 1 diabetes (97).

In contrast to what appears to be positive selection for different forms of active T cell immunity against inhalant allergens during infancy, the majority of subjects manifest active down-regulation of T cell responses to dietary allergens such as egg, as demonstrated by diminishing lymphoproliferative responses and by a progressive reduction in the number of egg-specific T cell epitopes recognized in vitro (93, 94, 98). This finding suggests that control mechanisms akin to high-zone tolerance (anergy/deletion) operate in the mouse, although additional regulatory pathways may operate in parallel.

Microbial Stimulation in the Gastrointestinal Tract as a Potential Modulator of Human OT

Studies from our laboratories were the first to present direct evidence suggesting that genetic risk for allergy was associated with delayed postnatal maturation of T helper cell function (99). Both the cloning frequency of $CD4^+$ T cells and the capacity of cloned $CD4^+$ T cells to secrete IFN-γ and IL-4 were reduced in infants with positive atopic family history (AFH^+), and the reduction was most marked in IFN-γ, indicating an overall Th2 bias in T cell function in this group (99). Several studies have since reported similar findings in cord blood (94, 100–103). T cell function in fetal life is constitutively Th2 biased as part of a set of control mechanisms to limit potential damage to the placenta via toxic Th1 cytokines (104), and the more pronounced Th2 bias in AFH^+ children may reflect inappropriate persistence of one or more of these control mechanisms after birth (88, 105).

In addition, interaction with microbes, especially the normal microbial flora of the gastrointestinal tract, is the principal environmental signal for postnatal maturation of T cell function (in particular, the Th1 component) (106, 107). Recognition of these signals is mediated by a series of Toll-like receptors expressed on cells of the innate immune system, and other receptors such as CD14, and it is noteworthy that a polymorphism in the CD14 gene has recently been associated with high IgE levels (108). Recent international studies have drawn attention to the wide variations in allergy prevalence between different countries (109, 110), and that these changes may have occurred over the last 20–30 years (111). On the basis of the findings reviewed above, we have suggested that variations in patterns of microbial colonization of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in allergy prevalence throughout the world (106). Ongoing cohort studies in our laboratories and elsewhere are focusing in detail on this complex question. These suggestions are supported by the observation that germ-free mice do not develop tolerance in the absence of a gut flora (23), and by the demonstration of differences in the composition of the gut flora between infants living in countries with a high and a low prevalence of allergy (112, 113) and between healthy and allergic infants (114–117). Although all the studies confirm such differences, no particular protective or potentially harmful bacterial species have been identified so far. In the two prospective studies (116, 117) and one cross-sectional study (114), the presence of bifidobacteria were associated with less allergy, while the presence of *Clostridium difficile* has been linked to allergy (115, 116). The administration of lactobacilli to mothers or their infants was recently reported to be associated with less AD during the first 2 years of life (118). Although the study was prospective and placebo-controlled, the findings need to be confirmed because the study design and interpretation was somewhat unclear.

Maternal Influences

There is a close immunologic interaction between a mother and her offspring, not only during pregnancy, but also as long as the baby is breastfed. Human milk contains numerous immunologic components, including IgA and other antibodies, and various chemokines (119) and cytokines mainly with anti-inflammatory and IgA-stimulatory properties, such as TGF-β, IL-8, and IL-10 (120). It is well established that human milk often contains food antigens that may induce IgE antibody formation. Less is known about the immunologic consequences of introducing foreign

antigens while the infant is still breast-feeding. As indicated by studies of immunity to infectious agents, it is possible that this represents a mechanism by which immune responses are modulated (121). In the early 1990s, there was a pronounced increase in the incidence of celiac disease among Swedish infants (122). Prior to the increase in celiac disease, gluten typically had been gradually introduced while the baby was still breast-fed. Then, practices changed to an avoidance of gluten during the first 6 months. When the national recommendations were changed back to gradual introduction of gluten, the incidence of celiac disease dropped rapidly.

Concluding Remarks

The development of immunological tolerance to food antigens is a complex process and depends on an intense interaction between the host and the environment, including through microbial stimulation. It is intriguing that microbial stimulation, in particular via the gastrointestinal tract (106), has also been implicated as an etiologic factor in respiratory allergic diseases. This suggests that microbial stimuli exert effects beyond the mucosal tissue microenvironments adjacent to sites of exposure, and presumably can influence systemic precursor compartments such as bone marrow and thymus (107). The underlying mechanism(s) are likely to include stimulation of functional maturation of cells within the innate and adaptive immune systems during the early postnatal period (105–107), a process that may ultimately determine the overall efficiency of immune/tolerance induction during early life, with major effects reaching into adulthood. A full understanding of the underlying mechanisms may open new venues for the prevention not only of food allergy, but also conceivably of respiratory allergies.

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In Vitro Diagnostic Methods in the Evaluation of Food Hypersensitivity

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Introduction

The technical feasibility and clinical utility of in vitro determinations of antibodies and other markers will be discussed, with specific emphasis on food allergy. Allergic diseases, including reactions to foods, represent increasing problems in the western world, with symptoms that may not be easily distinguished from other disorders. The term hypersensitivity is defined as something that induces objectively reproducible symptoms or signs, initiated by exposure to a defined stimulus at a dose tolerated by normal subjects (1). Hypersensitivity can be differentiated into allergic hypersensitivity, which involves an immune mechanism, and non-allergic hypersensitivity, where immune mechanisms are excluded. Different tests must be applied to distinguish between such conditions. An allergic hypersensitivity can be either IgE-mediated or non-IgE-mediated; an example of non-IgE-mediated hypersensitivity is celiac disease (CD), which involves immune cells and antibodies of IgG and IgA isotypes. In distinguishing between the two types of allergic hypersensitivity, a given test should identify the IgE- or IgG/IgA-related allergic mechanisms in allergic patients from those of other patients suffering from similar symptoms.

Most patients are sensitized to more than one allergen that may trigger their clinical symptoms. It is often difficult to distinguish which is the clinically offensive allergen. Furthermore, allergic symptoms related to IgE antibodies depend not only on those IgE antibodies but also on a number of related and unrelated confounding factors. These factors include inflammation, organ function, presence of infection, physical and psychological stress, and hormonal influences. For patients with food allergy and intolerance, double-blind placebo-controlled food challenge (DBPCFC) is considered the gold standard (2, 3). However , this technique does not distinguish among allergic hypersensitivity involving IgE antibodies, antibodies of IgG/IgA isotypes and cellular immune mechanisms, and those of intolerance, including enzyme deficiencies or other unknown mechanisms exhibiting similar degree of hypersensitivity.

A clinical diagnosis of IgE-mediated allergy should be based on the patient's history, symptoms, findings on physical examination, and laboratory test results. Diagnosing patients with IgE-mediated allergy would differentiate them from those having several other disease etiologies presenting with similar symptom profiles. Because there is no working gold standard for true allergy diagnosis, only one for food hypersensitivity and CD, such diagnosis is complicated. With the recognition that the prevalence of allergic problems is increasing and differential diagnosis by history and physical examination is difficult, tools should

be developed for revealing the mechanisms behind the symptoms. The present communication discusses well-defined blood tests for specific IgE or IgG/IgA antibodies and other markers, representing objective means to identify food-specific allergies in individual patients. The presence or absence of such antibodies or markers can be determined with high sensitivity and precision. Such information represents only one piece of information among others that must be used to compile a definitive clinical diagnosis.

Antibodies to various allergens may be present without obvious clinical disease. Nevertheless, the presence in a very young child of minute levels of specific IgE antibodies, especially to hen's egg white and, to a lesser extent, cow's milk, can be used as a predictor of evolving sensitization and allergic disease (4). In contrast, the presence of IgG/IgA antibodies to a specific food may just be the result of an increased exposure to the substance or allergen, sometimes associated with a leaky gastrointestinal mucosa, but without an obvious link to a clinical disorder (5–7). One exception is the IgG/IgA antibodies to gliadin and tissue transglutaminase (tTG) in CD (8).

For markers of inflammation, the situation is less consistent, and sampling is often problematic. However, there are methods for determining markers from 1) mast cells, e.g., histamine, tryptase, and leukotrienes (9–11); 2) eosinophils, e.g., the eosinophil granular constituents such as eosinophil cationic protein (ECP), eosinophil protein X (EPX), eosinophilic peroxidase (EPO), major basic protein (MBP), and leukotrienes (9–11); 3) basophils, e.g., histamine and leukotrienes (9, 10); and 4) neutrophils, e.g., myeloperoxidase (MPO), human neutrophil lipocalin (HNL), lactoferrin, and lysozyme (11), although these are often not well established as clinical diagnostic methods but are to be considered more as research tools (9–11).

For conditions originating with enzyme deficiency, the situation is even worse. Except for lactose intolerance, most tests to determine enzyme deficiency are less well proven. For example, in histamine intolerance mediated by a deficient diamine oxidase system (12), standardized methods have not been established, despite the fact that the clinical condition is recognized and often presents as headache after ingestion of certain histamine-, phenylethylamine-, or serotonin-containing foods such as red wine. Because the enzyme is located primarily in the jejunal mucosa, the problems are induced by gastrointestinal exposure. The diagnostic tests for this condition are of a research character and are difficult to use in clinical practice (12).

Markers and Methods with Confirmed Value

IgE Antibodies

It is well known that the presence of IgE antibodies to a specific food indicates a certain probability of a clinical reaction to that food, although the risk levels are unique in each patient. Virtually any food may lead to a reaction, although only a small number of foods, such as hen's egg, cow's milk, peanuts, soy, wheat, tree nuts, and fish account for about 90% of the reactions in children (13–15). In adults, peanuts, tree nuts, fish, and shellfish may be the most common, although sensitivity to other allergens may also be present and must be revealed by case history together with testing (16–18). Negative results obtained with in vivo tests and skin prick tests (SPTs) are informative with a very high negative predictive value (NPV) (>95%) (19, 20). Positive test results show great variation in sensitivity for different allergens and patient groups when interpreted as positive. The quantification of IgE antibodies, as shown below, has been demonstrated to provide more information, as demonstrated by Sampson and coworkers (21, 22) as well as by other investigators (23–25). However, it is important to emphasize that the age of the food-allergic patient affects the interpretation of the IgE antibody level (23, 24). The prevalence of allergic conditions does not seem to influence interpretation (26).

Monitoring IgE levels may be clinically important in the follow-up of patients with allergy to cow's milk. Thus, in patients destined to outgrow their allergies to cow's milk protein, the levels of IgE antibodies to whole cow's milk, and even more so to casein, were lower than in those patients with lasting allergy (27).

IgG/IgA Antibodies in CD

CD is a food hypersensitivity disorder the etiology of which is currently being resolved. It involves an autoimmune enteropathy triggered by the ingestion of gluten in susceptible individuals. The typical intestinal damage, such as loss of villi and hyperplasia of crypts, resolves completely upon elimination of gluten from the diet. Historically the diagnosis of CD has included the need for

several intestinal biopsies as outlined in the first European Society of Pediatric Gastroenterology, Hepatology, and Nutrition guidelines (28). Because of the development of more sensitive and specific in vitro tests for specific IgA and IgG antibodies to gliadin, endomysium, and tTG, the guidelines have been revised and the number of biopsies needed has been reduced (29). The combined use of IgA and IgG anti-gliadin antibodies (AGA) measurements available in different in vitro assay formats (e.g., enzyme-linked immunosorbent assay [ELISA]; ImmunoCAP) has been shown to be sensitive and specific in relation to the presence of clinical disease (8).

Histochemical staining methods used to detect IgA antibodies to endomysium have been considered even more specific and sensitive, but also more difficult to perform in a standardized manner. The recent demonstration that tTG is the main target of the autoimmune response (30) has led to the development of several specific IgA ELISAs based on guinea pig or, preferably, human tTG, giving very high sensitivities and specificities. Tests with recombinant human tTG seems to perform the best and are a useful tool in both small children and adults (8, 31). In the cases of IgA deficiency, specific IgG antibodies to gliadin and tTG are of special value (32).

Allergen and Antigen Properties in the In Vitro Diagnosis

Allergen Sources

The ability of a test to detect specific IgE antibodies depends on the presence of all relevant allergen components in the test system. Food is prepared from both animal and plant origin and the antibody patterns of patients sensitized to food are often even more complex than those seen for inhalant allergens. The use of native allergen source material of the highest quality and containing all relevant allergen components is of primary importance. The mode of preparation of extracts for preservation of allergenic potency during processing is vital (33–35).

Intact macromolecules from the partly digested food may pass through the intestinal mucosa into the circulation and act as allergens (5–7). Attention has also been drawn to the possible creation of neo-allergens during processing and digestion of foods, for example, the allergenic determinants are enhanced and/or formed by roasting peanuts (36). In some cases, food allergens are destroyed during processing, as exemplified by the fact that some patients may tolerate the cooked food, but not its raw counterpart (37, 38). To be able to detect all patients with differing antibody specificities, a native food source material that is representative of the natural exposure should be used.

The IgE antibodies are produced as a consequence of exposure to allergens in the environment. Regional differences in food habits may result in different patterns of IgE antibody specificity (34, 39, 40). However, increasing international trade, and use of tropical food, ornamental plants, and herbs widens possible exposure far from what is common in the local environment.

Currently, considerable research efforts are directed at characterization of individual food allergen components; today more than 70 food allergen components have been defined and listed by the International Union of Immunological Societies (IUIS) (http://www.allergen.org). The use of separate components or combinations of components may lead to new and better tools in the diagnosis of food allergy in the future. More than 40 of these components have been cloned and may in the future be available as recombinant proteins (41).

Antigen Sources

Gliadin, Transglutaminase: The common antigen source for determination of gliadin-specific IgA and IgG antibodies is crude or purified fractions of wheat gliadin. Gliadin is obtained as the alcohol-soluble fraction from wheat gluten prolamins. Prolamins from other closely related cereals such as rye, barley, and oats show some degree of cross-reactivity but are not commonly used for CD diagnostics (42, 43). For measurements of IgA and IgG antibodies to tTG, guinea pig-derived antigen was used initially, but human tTG has been shown to give higher sensitivity and specificity (8, 44).

Histamine, Tryptase, Leukotrienes: In determination of histamine, the metabolites also need to be considered. Thus, histamine released in the tissue or in the blood is gradually degraded to methylhistamine. When secreted into the urine, other metabolites are also formed that do not necessarily possess a similar immunochemical or pharmacological reactivity in current assays, leaving results sometimes difficult to interpret

(45). Leukotrienes and prostaglandins can also be preferentially determined in urine using immunoassays and high-performance liquid chromatography (HPLC). The most frequently encountered markers are the leukotrienes LTD4, LTC4, LTE4, and the prostaglandin D 2α (PGD2α) metabolite 2α,11β-PGF2α (46). A special test, the so-called CAST-ELISA, has been developed for measurement of the release of the leukotrienes from blood cells (9, 10).

Granular constituents from eosinophils (ECP, EPX, EPO, MBP), neutrophils (MPO, HNL, lactoferrin), monocytes (lysozyme), and mast cells (tryptase) need to be isolated or cloned from human cells, because there is limited cross-reactivity between species (47).

Cross-Reactivity

Cross-reactivity between allergen-specific IgE and related allergens in vitro is also seen with SPTs. Consequently, the clinical relevance between different allergens must be determined individually for each patient, taking the clinical history and provocation/elimination diet results into account. Proteins with similar functions in different plant species may have a similar structure (48). The IgE antibodies may detect such similarities between allergens from different sources as a function of biology and chemistry resulting in allergic reactions despite the fact that no apparent exposure to the allergen can be identified. Among foods there are several groups of cross-reactive allergens. The pollen-related food allergies to fruit and vegetables are well known, but cross-reactions have also been demonstrated between shellfish and other animals, between fruit and latex, and between different fruits (17, 41, 49–52).

As discussed in Chapter 3, many food allergens from plant sources are proteins belonging to the "pathogenesis-related" (PR) protein family, e.g., Bet v1 homologs that have been identified in

Table 7–1.
Examples of Common Food Allergens

Protein Classification	*Property*	*Allergen Source (Allergen)*
PR 2	β1–3-Glucanases	Fruits, banana, latex (Hev b 2)
PR 3	Type 1 (basic) and type II (acidic) chitinases	Avocado (Pers a 1), banana, chestnut
PR 4	Chitinases	Turnip, elderberry
PR 5	Thaumatin and osmotin-like proteins (antifungal)	Cherry (Pru av 2), apple (Mal d 2), bell pepper
PR 6	Protease and amylase inhibitors	Soy, wheat, barley, rye, rice
PR 9	Peroxidase	Wheat, barley
PR 10	Bet v 1 homologs similar to ribonucleases	Apple (Mal d 1), cherry (Pru av 1),carrot (Dau c 1), celery (Api g 1), pear (Pyr c 1), hazelnut (Cor a 1.04), apricot (Pru ar1)
PR 14	Lipid transfer proteins Lipid metabolism	Peach (Pru p 3), plum, cherry, apple (Mal d3), apricot, maize, broccoli, carrot, rapeseed
Profilin	Actin binding, signal transduction	Celery (Api g 4), potato, hazelnut, apple, pear (Pyr c 4), tomato, cherry (Pru av 4), soybean (Gly m 3), peanut (Ara h 5)
Parvalbumin	Ca^{2+}-binding proteins	Cod (Gad c 1), salmon (Sal s 1)
Tropomyosin	Ca^{2+}-binding proteins	Shrimp (Met e 1, Pen a 1, Pen i 1), lobster (Hom a 1), squid (Tod p 1), abalone (Hal m 1), scallop, crab (Cha f 1)
Seed storage proteins	2 S albumins, vicilins, conglutins	Mustard (Sin a 1, Bra j 1), castor bean (Ric c 1), rapeseed (Bu III), brazil nut (Ber e 1), walnut (Jug r 1, Jug r 2), peanut (Ara h 1, Ara h 2, Ara h 3, Ara h 4, Ara h 6, Ara h 7), soy
Protease	Proteolysis	Papaya (papain), pineapple (bromelain), fig (ficin), kiwi (Act c 1), soy (Gly m 1)

a great number of pollens and fruits (53). Another group of PR proteins include the chitinases that are present in latex and fruits (53). Other allergens known to induce cross-reactivity between pollens and fruits are profilins with highly preserved protein structures (54). Lipid-transfer proteins (LTPs) compose another group of very stable proteins present in fruits and vegetables and cause cross-reactions both in vitro and in vivo (39, 55). In foods of animal origin, tropomyosins and serum proteins are known to be cross-reactive (49). Future research on the basis of recombinant (or purified native) components is needed to provide further information about correlations between structure and allergenic reactivity, and ideally will lead to the development of more specific tools for diagnosis. Table 7–1 shows some common food allergens.

Carbohydrate structures on glycoproteins also may be involved in cross-reactivity between foods and pollens (CCD) (50, 56, 57). Some of these carbohydrates have been identified and at least two important epitopes have been described that contain xylose and fucose (57). An important and widely discussed issue is that IgE antibodies in a blood test may be bound to a univalent structure such as a carbohydrate, whereas biological activity, such as that shown in a skin test, may be negative because of the univalency of the test material (58). However, this does not prove that clinical reactions will not occur when the individual is exposed to allergenic material containing the carbohydrates in a different multivalent conformation that can induce the biological activation of cells and mediators, triggering clinical reactions. Proteins carrying multivalent carbohydrate epitopes can induce histamine release (59), and these kinds of structures in some foods may be important in the clinical response (60). Thus, it cannot be concluded that the IgE antibodies directed at carbohydrate structures are without biological and clinical significance.

Epitopes

Clinical sensitivity to a certain food allergen often changes over time. It is estimated that about 80% of children outgrow their cow's milk allergy (16), although only 20% outgrow their peanut allergy (61). Some results suggest that IgE antibodies from individuals with persistent allergy may be directed against different epitopes than those in patients with transient allergy (62). Epitopes may be continuous (linear or sequential) or conformational (involving different parts of peptide chains due to folding on the peptide chain), and the specificity of an antibody depends on the uniqueness of the epitope. The measurement of specific IgE to single epitopes may provide a new way of not only diagnosing, but also predicting allergic reactions in food-allergic children. In the future we may see tests identifying antibodies to different epitopes and then predicting whether the allergy is transient or persistent. To obtain such information by monitoring epitope-specific IgE antibodies over time, the test system needs to be quantitative and give correct results over the whole measuring range.

Performance Characteristics of Laboratory Tests

Standardization of Allergen and Antigen Extract in Antibody Tests

It is important to know exactly why the individual is reacting. Therefore, the extracts of allergen, antigen, or other markers of the inflammatory process used in the assays need to be standardized. These markers can be assessed with biochemical methods and/or by demonstrating antibodies in sera from known allergic individuals. Common methods include immunoblotting and inhibition of binding to the solid phase. It is of utmost importance to verify the reproducibility of different allergen batches produced. In particular, because the antibody specificities in different patients are unique to various allergen components (63), the reproducible presence of all components on the solid phase of the assay system must be assured to obtain results relevant for a clinical interpretation.

Interactions Between Antibody and Antigen

The immunological methods used to determine the presence and levels of antibodies and antigens in solution and on a solid phase matrix follow simple chemical rules. Many assays today utilize a solid phase for easy separation of reacted and non-reacted reagents. Similar chemical rules regulate the interactions between receptors on cells and their ligands. From the law of mass action applied to a heterogeneous solid phase immunoassay, it can be concluded that when the value of the allergen concentration multiplied by

the equilibrium constant exceeds 10, more than 90% of the antibodies are bound and the reaction becomes antibody affinity-independent (63). Therefore, all allergen components in an allergen extract used in the method need to be in large excess to provide such high binding capacity for all antibodies regardless of antibody affinity and antibody class. A few commercial assay systems fulfill these criteria (64), which enables them to quantitatively measure all IgE antibodies present in serum samples without being distorted by background noise or inhibited by simultaneously occurring IgG antibodies (65). For instance, in two of the most extensively studied systems for IgE antibody determination, it was shown that 85%–100% of the allergen-specific IgE antibodies present in allergic serum samples were bound to the solid phase surface (66). Furthermore, using the same two systems, immunoblotting experiments revealed that all IgE antibody specificities present in a serum sample are similarly bound to the allergens on the solid phase, giving a representative quantitative result (66). It is important to emphasize that such efficient binding of all relevant antibodies indeed is not true for all assay systems in use today (64). In particular it has been demonstrated that the relatively binding efficiency of the surface of a microwell used in many ELISA systems is too low to be able to pick up all antibodies. The reaction becomes affinity-dependent, making the dilution curves not parallel; a true quantification is therefore impossible and gives results difficult to interpret (67). Therefore, serum samples must always be diluted to reach optimal concentration conditions in such systems. For instance, IgA and IgG antibodies to gliadin and transglutaminase can be accurately quantified in such systems after 100-fold dilutions. Table 7–2 shows examples of some tests' principles.

Calibration

Much effort has been focused on assays that can identify allergen-specific IgE antibodies because of their clinical importance in mediating immediate hypersensitivity reactions, including

Table 7–2.
Tests for Discrimination of the Presence of Allergy and Tests for the Identification of the Offending Allergen

Aim of the Test Is to Identify	*Principle of the Test*	*Basic Technology*	*Major Test System*	*Allergen Coupling*	*Detection System*
Presence of atopic condition	Multi-IgE antibody tests; e.g., PhadiatopIncluding allergens from several different sources	Heterogeneous assay using a solid phase for separation of allergen-bound specific IgE antibodies, labeled anti-IgE reagents	Phadiatop	Cellulose foam	Radioactivity Enzyme/fluorescence
			AlaTOP	Soluble polymer	Enzyme/absorbance Enzyme/luminescence
Presence of sensitization to spe cific allergens	IgE antibody tests to allergens from one source material	As above	Pharmacia CAP System	Cellulose foam	Radioactivity Enzyme/fluorescence
			UniCAP	Cellulose foam	Enzyme/fluorescence
			Advia Centaur	Biotin-labeled allergen in solution	Chemiluminescence
			AlaSTAT	Soluble polymer	Enzyme/absorbance
			Immulite 2000	Soluble polymer	Enzyme/luminescence
			Hycor	Paper disc	Enzyme/absorbance
			CLA-MAST	Cellulose threads	Enzyme/luminescence
Presence of sensitization to specific allergen component	IgE antibody tests to one single allergen component	As above			
Presence of antibodies to specific antigens	IgA/IgG/IgG4 antibody tests to single antigens	Heterogeneous assay using a solid phase for separation of antigen-bound specific Ig antibodies, labeled anti-Ig reagents	ELISA tests	Polystyrene	Enzyme/absorbance/fluorescence
			Pharmacia CAP System	Cellulose foam	Enzyme/fluorescence
			UniCAP	Cellulose foam	Enzyme/fluorescence

(*continued*)

Table 7–2. (Continued)
Tests for Discrimination of the Presence of Allergy and Tests for the Identification of the Offending Allergen

Aim of the Test Is to Identify	*Principle of the Test*	*Basic Technology*	*Major Test System*	*Allergen Coupling*	*Detection System*
Presence of inflammation mediators from different cells	Histamine from basophils and mast cells	Solid phase with catching antibody, labeled antimediator reagents	RIA	Microparticles	Radioactivity
			UniCAP	Cellulose foam	Enzyme/fluorescence
			ELISA tests	Polystyrene	Enzyme/absorbance/fluorescence
	Tryptase from mast cells		RIA	Microparticles	Radioactivity
	Lipid mediators such as leukotrienes and prostaglandins		CAST-ELISA	Polystyrene	Enzyme/absorbance
			ELISA tests	Polystyrene	Enzyme/absorbance/fluorescence
	Eosinophil mediators such as ECP, EPX, EPO		UniCAP	Cellulose foam	Fluorescence
			RIA	Microparticles	Radioactivity
	Neutrophil mediators such as MPO, HNL		RIA	Microplates	Radioactivity
	Lymphocyte mediators such as cytokines		ELISA tests	Polystyrene	Enzyme/absorbance/fluorescence
Cellular immune response	T cell proliferation	Cell cultivation with specific allergen/antigen stimulation and analysis of cell proliferation			

anaphylactic reactions, and their low levels in patients' sera. Since the first test for IgE antibody determination became available, there has been considerable development in the field. The original radioallergosorbent test (RAST), which became available in 1974, included a calibrator consisting of serial dilutions of a serum sample containing IgE antibodies to birch pollen. This was used to construct a calibration curve providing results in arbitrary units (Phadebas RAST unit/mL) and internally calibrated against the World Health Organization (WHO) International Reference Preparation for Human IgE 69/204 (68). Newer generations of test systems usually replace the allergen-specific IgE antibody reference with a calibrator directly traceable to the WHO International Reference Preparation for Human IgE 75/502, which is one prerequisite for quantitative measurements of IgE antibodies (63). In addition, specific absorption of antibodies should result in a parallel decrease of the content of total IgE (63, 66, 69). Some procedures utilizing the so-called modified RAST have tried to increase the sensitivity, but the results reported by such tests are both unexpectedly positive and unexpectedly negative, indicating a high degree of imprecision (64, 70).

For tests measuring IgG and IgA antibodies and other markers, development of calibration has been studied less extensively. However, several systems have applied calibration curves that provide determinations in relative units in a semiquantitative manner and allow the comparison of results from time to time. Because there are no international reference preparations for allergen-specific IgG or IgA antibodies, the same concept used for specific IgE has also been used in some systems, i.e., the use of a calibration curve consisting of total IgG or IgA for which there are WHO reference preparations available. The prerequisite for using this kind of calibration is that dilutions of samples are parallel to the calibrator curve in the system used. This approach can ensure stability and reproducibility over time.

Validation

For IgE antibody determinations, specific recommendations for performing tests were recently detailed in a publication from the National Committee for Clinical Laboratory Standards (NCCLS) (71). The recommendations include procedures for quality control for daily performance in clinical laboratory setting, and minimal performance targets of 15% coefficient of variation of IgE antibody assays. The College of American Pathologists has similar recommendations for IgG and IgA antibody determinations, as well as for determinations of other markers (69). According to the guidelines by NCCLS, a quantitative assay should

meet criteria that include recovery of antibodies, precision, linearity and parallelism of dilution curves, and calibrators over the measuring range. It states that all assay designs at the present time include a solid phase for separation of bound and unbound IgE antibodies, and all allergen components used must be in excess.

The question of whether different specific IgE antibody blood tests really give interchangeable results has been addressed. Results from proficiency testing programs in Europe have been published that assess the performance of several commercial systems for the measurement of IgE antibodies specific to different allergens (72). The testing indicated that the results from different assay systems are often not equivalent and interchangeable, although it has been demonstrated that some systems possess good performance characteristics (64, 70). In contrast, several other systems and assays do not live up to acceptable standards. However, such proficiency testing is more common for inhalant than for food allergens.

Other tests of IgG and IgA antibodies and inflammation markers have been much less standardized. This makes comparisons between results obtained with different tests and methods more difficult.

Clinical Application of Laboratory Tests

The Application of Sensitivity and Specificity of a Test

The performance of a particular test is usually given as its sensitivity and specificity compared with clinical disease. There is a considerable documentation for the presence of IgE antibodies in allergic disease, more than for many other test systems, and IgE antibody tests may therefore be taken as example for the discussion. Thus, for IgE antibody tests, very good results of sensitivity and specificity have been documented for a variety of allergens (73). Even for the early tests that were developed and marketed, data showed a good correlation between the levels of specific IgE antibodies and skin test reactivity or symptom scores (74).

Sensitivity is defined as the proportion of test-positive patients in relation to the total number of patients who have the disease, whereas specificity is the proportion of test-negative persons in relation to the total number of patients who do not have the disease. The positive predictive value is defined as the proportion of truly positive patients in relation to the total number of test positive patients (Table 7–3). (75)

Table 7–3.
Concepts in Clinical Validation of a Test

		Test Status +	Test Status −	
Gold Standard	+	A	B	A+B
	−	C	D	C+D
		A+C	B+D	A+B+C+D

True positive: A
True negative: D
Clinical sensitivity: A/(A+B)
Positive predictive value: A/(A+C)
Prevalence = Prior probability: (A+B)/(A+B+C+D)
Efficiency = Concordance: (A+D)/(A+B+C+D)

False positive: C
False negative: B
Clinical specificity: D/(C+D)
Negative predictive value: D/(B+D)

IgE antibody test values of more than 90% for sensitivity, specificity, and predictive values have been obtained for certain test systems documented with several hundred patients (76). In other situations, low levels of antibodies cannot easily be associated with clinical disease (21, 22). Furthermore, in adults there may be clear-cut clinical evidence of food allergy without any detectable IgE antibodies (77).

Information on predictive values are highly dependent on the prevalence of disease in the population (75). Thus, for patients from a population with similar prevalence of atopic allergy to a given allergen, good predictive values can be obtained. Studies have confirmed the association between the levels of specific IgE antibodies and the degree of allergen exposure and development of symptoms (23–25, 69, 74, 76, 78, 79) However, it is difficult to relate the presence or absence of IgE antibodies exactly to the presence of clinical disease in an individual patient, especially because there is no absolute gold standard for IgE-mediated clinical food-allergic disease. In CD, the gold standard is biopsy and clinical improvement with gluten-free diet (29).

Quantification of the Marker

An alternative derivation of the positive predictive value is obtained by the use of logistic regression on the binary dichotomized results from the test and from the gold standard (80). To utilize the quantitative information from a test, the logistic model can be used, with an alteration from the binary dichotomized result of the test to the actual

quantitative result, or to the logarithm of the actual quantitative result. A test quantifying a marker will provide a continuous description of the relation between the probability of having the disease associated with a certain marker, and the concentration of that marker (Fig. 7–1). The logistic model can be formulated as logit $Pr(Y=1 \mid X) = \alpha + \beta X$.

The estimated relationship can be interpreted as the probability that the patient is diagnosed as being allergic to an allergen as a function of the level of the specific marker. A low level will give a low probability for a positive diagnosis and a high concentration will give a high probability for a positive diagnosis. According to mathematical theories, the slope in the model will represent the logarithm of the odds ratio.

With the aim to decrease the number of time-consuming DBPCFCs for diagnosing patients with food allergy, Sampson (21, 22) described the use of clinical "decision points" in a given food. Applying the probability model for the same data, such models give information regarding the decision points, and important information indicating that even a low concentration of food-specific IgE antibodies may be associated with a certain risk of clinical reactivity.

Different shapes of the relationship will indicate different identification patterns of symptoms; a steep curve indicates identification of symptoms even with low levels of the marker, whereas a flatter curve usually indicates that higher levels of the marker are required to make a diagnosis, or that no clear identification of the disorder can be attained with the test. Similar arguments can be made for IgA and, to a lesser extent, for IgG antibodies to gliadin and transglutaminase and for other markers in which different concentrations result in different likelihoods of disease. The actual shape of these relationships must be studied for each individual marker.

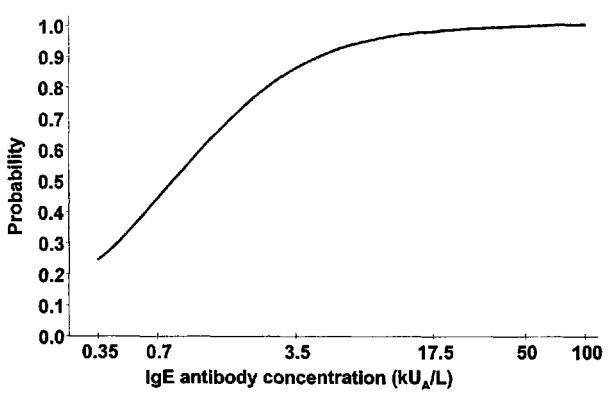

Figure 7–1. Logistic relationship between antibody concentration and the probability of clinical reaction.

When interpreting results from a dichotomized evaluation, it is crucial to carefully examine all conditions that the results are based on: the population used in the study, the number of subjects, etc. For all studies involving specific markers such as antigen-specific IgE, IgG, or IgA antibodies, it is also mandatory to specify the basis for defining a truly positive individual, i.e., the "gold standard."

Compared with a dichotomous use of the decision points, the probability curves give more information about how the level of antibodies are related to the likelihood of reactivity to food hypersensitivity. Because the logistic model describes the relationship between the quantitative measure of sensitization and the dichotomous measure of diagnosis, i.e., "yes" or "no," it does not depend on the prevalence or prior probability in the same manner as the dichotomized calculation of the positive predictive value.

Markers and Methods with no Confirmed Value

Total Serum IgE

Measurements of total serum IgE are used to give a very rough indication of whether there are any prerequisites of IgE-mediated disease in a patient. Because of the considerable overlap between IgE levels in allergic patients and normal controls, and those with other disorders that may increase serum IgE (e.g., parasite infections), total serum IgE does not add considerable insight into the diagnosis of food allergy.

IgG/IgA Antibodies in Atopic Allergy

Tests of IgG and IgG4 antibodies do not give any indication of the causes of clinical symptoms in cases of immediate IgE-mediated reactions. Thus, IgG and IgA antibodies to foods are commonly found in both food-allergic patients and healthy persons. Such antibodies appear to be secondary to exposure to the food antigens/allergens and have not been shown to have any clinical value in the diagnosis of food allergy (81–85). As an example, patients with CD often have high levels of IgG antibodies to cow's milk proteins. Dur-

ing the acute phase of the disease, they may have high levels of milk-specific IgG, and when the patient is in remission (after implementation of a gluten free diet), levels decline (5, 7).

Histamine and Basophil Histamine Test

Histamine released into the tissue and blood is gradually inactivated to methylhistamine. The relative amount of histamine and its metabolites over time following a clinical reaction is difficult to establish. Methylhistamine, which cross-reacts with histamine to some extent, can be determined with radioimmunoassay, although the half-life of both species is in minutes (45). In the urine, histamine is not present, and methylhistamine or some other metabolite must be determined.

The basophil histamine test determines the release of histamine from peripheral blood basophils induced by cross-linking IgE antibodies bound to their specific receptors on the cells (86). Also, complement activation and direct activation in some cases of idiosyncratic reactions to aspirin can release histamine. Because of the difficulty of establishing optimum doses of allergens for release of histamine, and the difficulty of obtaining fresh blood cells, the test has been limited to academic and research settings. Development of whole blood semi-automated systems may, to some degree, circumvent the problem with high "spontaneous" histamine release from basophils in food allergic individuals who ingest small amounts of the offending allergenic food (87). A good performance of this test method has been demonstrated compared to IgE antibody determinations in serum and food challenges, although the results were never more predictive than IgE antibody determinations in blood or than SPTs (88). When patients were compliant with diets excluding their offending allergenic food for several months, "spontaneous histamine release" decreased considerably (89). In cases of in vitro passive challenges of peripheral blood cells with allergen, the results have been less conclusive. Furthermore, about 5%–10% of the population have nonresponsive basophils that fail to release their histamine following allergen challenge in vitro (90).

Tryptase, ECP, and EPX

Tryptase is found almost exclusively in mast cells. It has a much longer half-life in peripheral blood than histamine or histamine metabolites (91). Unfortunately, in food-allergic reactions, the only situations in which tryptase determinations have been useful has been in anaphylactic reactions, where elevated levels have been documented in a minority of patients in both research and clinical practice settings (91, 92). However, the majority of fatal anaphylactic reactions where the tryptase level was measured in the peripheral blood demonstrated no elevation of plasma tryptase (93).

Eosinophil markers such as ECP and EPX in peripheral blood have also been used as research tools following food challenges in allergic individuals. Increased levels of these markers have been reported after positive challenges, sometimes in connection with decreased numbers of total eosinophils (94). Recently, some investigators have been evaluating levels of EPX in the feces of patients undergoing challenges with suspected foods. It is still too early to evaluate the clinical usefulness of this procedure (95).

Leukotrienes

Leukotrienes and, to some extent, prostaglandins have been used to monitor inflammation in allergic situations, mostly in patients with airway allergy, asthma, and drug allergy. Both LTE4 and the PGD2 metabolite 9α,11β-PGF2 can be determined in the urine, although there is rather limited information on this in relation to food-allergic reactions (46). A specific test, CAST-ELISA, has been developed to measure the leukotrienes LTC4, LTD4, and LTE4 released from peripheral cells (9–11). Even if results have been reported from applications in food allergy, all these methods need further documentation in clinical situations before they are widely applied in clinical routine.

Cytokines

Serum cytokines have not yet proven useful for clinical information. This may be due to complexities related to the time they are obtained in relationship to the reactions and the different cells being activated (77). Some studies have reported an imbalance of interleukin-4 (IL-4) and interferon-γ (IFN-γ) in children (96) and adults (97). Furthermore, IFN-γ and IL-2 have been reported to be elevated in food-allergic reactions (98). However, much more information is needed before such assays can be used in clinical routine. Table 7–2 shows various antibody and inflammation marker tests.

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8

In Vivo Diagnosis:
Skin Testing and Challenge Procedures

Scott H. Sicherer

Introduction

The oral food challenge (OFC) is the tool at the physician's disposal that can most definitively diagnose an adverse reaction to food. Quite simply, the patient either does or does not tolerate the food when ingested, and the diagnosis is secured. Although potentially definitive, OFCs can be dangerous if severe reactions are induced. Therefore, physicians also rely on patient histories and a number of additional tests to help determine the likelihood of a true allergy or adverse reaction to food prior to, and sometimes in place of, undertaking an OFC. For allergic reactions that are mediated by IgE antibody, the tests most familiar to the allergist are the skin prick test (SPT), a focus of this chapter, and the radioallergosorbent test (RAST) considered elsewhere (Chapter 7). Numerous additional tests may be needed in various clinical scenarios (e.g., stool culture, endoscopy with biopsy, pH probe, breath hydrogen) to assist in determining if an adverse reaction to food is the cause of a clinical problem. In addition, refinements on currently available tests, clinical evaluations of proposed tests (e.g., patch tests with food), and additional novel tests are under investigation to improve and expand the diagnostic armamentarium. Despite the potential for inaccurate histories and various limitations of in vitro and in vivo tests, the OFC can provide a final diagnostic answer. OFCs designed to be double-blind and placebo-controlled so as to reduce subject and observer bias are considered the "gold standard" for the diagnosis of food hypersensitivity.

Historical Background

The typical diet includes several meals and snacks distributed throughout the day. Because the frequency of food intake is high, any sudden adverse physiological event or chronic illness could be incorrectly ascribed to food. Once a patient makes an erroneous association between a food and a symptom, it may be difficult to dissuade the patient from their notion of cause and effect. In a paper published in 1950, Graham and colleagues (1) performed experiments that would be difficult to undertake today for ethical reasons. Subjects with strong beliefs about their reactions to foods were given water by nasogastric tube and told they were receiving the test food, and were given the test food and advised that the water was being instilled. Reactions to the tests correlated with suggestion. To address this potential for subject bias, masked ingestions were introduced by Loveless in several studies in the 1950s (2, 3). In an accompanying editorial, Lowell (4) emphasized the need for blinded challenges to demonstrate cause-effect relationships in food allergy. Charles May is credited with bringing double-blind, placebo-controlled oral food challenges (DBPCFCs) into routine clinical and research use (5).

By the late 1980s, a number of seminal points concerning the epidemiology of food hypersensitivity were confirmed and refined through the use of blinded OFCs. Challenges confirmed the role of food allergy in chronic disease such as childhood atopic dermatitis (AD) (6) and in immediate reactions (7), and determined that 6%–8% of young children experience genuine adverse reactions to

foods, but that most of the sensitivities resolved in early childhood (8). The types of symptoms elicited by foods were confirmed to be most commonly associated with the skin (hives, AD), gut, and respiratory tree and not commonly with behavioral problems (6–11).

The foods that affected children were generally confined to a rather small group that included cow's milk, egg, soy, wheat, peanut, and tree nuts; older individuals were affected by peanut, nuts, and seafood. The inaccuracy of the patient's history in regard to the relationship of food allergy to chronic disease was also underscored by several studies that documented an accuracy generally under 40% (7, 12–14). In addition, food additives/preservatives were not a frequent cause of problems (11).

Now, blinded oral challenges are a fundamental tool for scientifically establishing a number of important features of food hypersensitivity reactions. Studies have broadened our understanding of the spectrum of food hypersensitivity disorders. A growing number of studies point out the role of food hypersensitivity in isolated gastrointestinal disorders (15); however, food allergy is not a frequent cause of isolated chronic respiratory disease (16). The number of foods proven to cause reactions is ever expanding and includes: celery (17), carrot (18), apple (19), and melon (20), among a large number of others. Despite advances of in vitro and in vivo diagnostic tests, the OFC has remained the final endpoint to determine clinical tolerance or reactivity to food. Table 8–1 summarizes the early and recent advances in our understanding of food hypersensitivity obtained through OFCs.

Table 8–1.
Features of Adverse Reactions to Foods Determined Through Studies using OFCs

Epidemiology	6%–8% of children 1%–2% of adults Most common foods: egg, milk, peanut, tree nut, seafood, soy, wheat Increasingly wide variety of foods
Associated disorders	Anaphylaxis (acute skin, gut, respiratory and cardiovascular reactions) Atopic dermatitis (~35% with moderate skin disease) Numerous gastrointestinal disorders
Infrequently associated disorders (2%–5%)	Isolated chronic respiratory disease Chronic urticaria
Clinical symptoms only rarely, or possibly not associated	Behavioral disorders Neurological disorders

Skin Prick Tests

Tests to detect food-specific IgE antibody are central to identifying or excluding foods responsible for immediate-type, and some chronic disease-inducing, food-allergic reactions. The most familiar, convenient, and commonly used method is prick-puncture skin testing. The intradermal form of allergen skin testing was introduced by Blackley (21) over 100 years ago, and the prick test was described by Lewis and Grant in 1924 (22). The technique is simple, but specific variations exist. While the patient is off antihistamines for an appropriate length of time, a device such as a needle, bifurcated needle, probe, or lancet is used to puncture the epidermis through an extract of a food. Appropriate positive (histamine) and negative (saline-glycerin) controls are also placed. The test site is examined 10–20 minutes later. A local wheal and flare response indicates the presence of food-specific IgE antibody. A mean wheal diameter 3 mm or greater compared to a saline control is generally considered positive (23), but interpretation will be discussed in more detail below. Of course, the test would not be expected to be positive for food reactions that are not mediated by IgE antibodies. Clearly, the SPT is an invaluable screening tool for the allergist. However, the clinician using SPTs for the diagnosis of food hypersensitivity must be aware of the utility and limitations of the test in order to use it to the best advantage for clinical and research purposes.

Technical Considerations

Virtually every step along the way of performing and interpreting skin tests for food and other allergens may affect the final interpretation. The selection of skin test reagent is of primary importance. Unfortunately, standardized food extracts are not currently available despite a clear, long-standing recognition of the need (24, 25). Commercial extracts are usually prepared as glycerinated extracts of 1:10 or 1:20 dilution. With the lack of standardized extracts, it is well recognized that variations exist in allergen distribution and concentration between lots and companies (26, 27). The problem of protein stability must also be considered. An example demonstrating the lability of certain food extracts is the evaluation of food

allergy in pollen-food syndrome (oral allergy syndrome to fresh fruits and vegetables). Patients may react to the uncooked, but not the cooked form of the food; this may similarly be reflected in skin test results, because commercial extracts may lack the ability to display the labile proteins involved (28). For the evaluation of allergy to fresh fruits and vegetables, and possibly other foods, many authorities have suggested the use of fresh foods (e.g., fresh milk, egg white, fruits, and vegetables) (29). The SPT can be performed using liquid foods, by creating an in-house extract, or using a prick-prick technique (pricking the fruit and then the patient, thereby transferring the juice) (30). Presumably, such in-house reagents are more concentrated and this may increase sensitivity, a possible drawback in some circumstances, and may increase the risk of side effects from the test itself. The effect of allergen concentration on wheal size is somewhat tempered by the fact that wheal size increases by a factor of 1.5 for each log increase in concentration (31).

The materials used for pricking the skin, and the technique used with any given device, may also influence the results. A variety of devices are on the market for introducing the allergen into the epidermis. As may be imagined, the more penetration, the more likely there will be a response, so the area and depth to which the allergen is introduced are important. Therefore, the configuration of the device, the pressure applied by the operator, and the time over which pressure is applied must be considered (32). Test results also vary according to the location on the body on which they are placed. For example, the back is about 20% more reactive than the arm (33). Studies that evaluate histamine reactivity indicate that wheals become detectable in early infancy and increase in size with age until adulthood (34, 35). These physical and patient variables become relevant when comparing study results and making clinical decisions. In practice, consistency of materials and procedures, and review of precision (coefficient of variation should be <20% for wheal diameter) should be undertaken by comparing repeated tests by personnel administering them. Intradermal allergy skin tests with food extracts give an unacceptably high false-positive rate, and have been associated with systemic reactions including fatal anaphylactic reactions, so they should not be used (36).

Another variable factor in SPTs is the timing at which they are read and the manner in which they are measured and reported. The histamine test peaks at 10 minutes while allergen wheal size generally peaks at 15–20 minutes (37). One suggested method of measurement is to determine the greatest wheal (or flare) diameter and its perpendicular maximum diameter, and to calculate the mean of these two measurements (37). In practice, reporting of results often varies by investigator and may be reported as mean diameter, mean diameter compared to histamine control categorically (e.g., 1+, 2+, etc), or as a calculated area. Studies must be evaluated carefully because individual investigators may report data on the basis of a variety of methods that may not be directly comparable (e.g., mean wheal diameter versus largest diameter). The diluent control must also be considered because of irritation and dermographism, so a positive test (reflecting IgE) is generally regarded as one with a mean wheal diameter at least 3 mm greater than the histamine control. Despite the numerous potential confounding variables involved in the SPT procedure, the clinical utility is excellent. Technical issues that can affect SPT sensitivity are shown in Table 8–2.

Diagnostic Value

The ability of a test to indicate the presence or absence of disease depends on intrinsic characteristics of the test itself and features of the population to which it is being applied. The SPT is excellent for detecting food-specific IgE antibody; when it is negative, it is highly likely that there is none and that no IgE antibody-mediated allergic reaction to the tested food would occur (i.e., it has excellent negative predictive accuracy). Obviously, a negative result does not exclude the possibility of cell-mediated allergic reactions or intolerance. To complicate matters for the allergist and patient, the presence of IgE to a food often does not equate with clinical reactions; that is,

Table 8–2.
Features That Affect Sensitivity of SPTs

Feature	*Correlation with Sensitivity*
Extract concentration	Direct
Device used	Variable
Pressure applied during application	Direct
Location	Back > volar aspect of arm
Reporting progressively larger reaction sizes (e.g., wheal 4 mm instead of 3 mm) as categorically positive	Inverse

there is often (~50%) clinically inconsequential sensitization.

The sensitivity and specificity of a test provides information about its ability to identify a known condition. Sensitivity refers to the proportion of patients with an illness who test positive, and for IgE-mediated food allergy, the sensitivity of the SPT is usually high (>80%). Specificity refers to the proportion of individuals without the disorder who test negative, and for IgE antibody-mediated food allergy, specificity of the SPT is usually lower than the sensitivity but usually better than 50% (23, 29, 38). Sensitivity and specificity are affected by intrinsic properties of the test (Table 8–2), but the clinically important issue for the physician concerns the probability that a patient has food allergy if the test is positive (positive predictive value, PPV) or does not have food allergy if the test is negative (negative predictive value, NPV). The predictive accuracy is affected by the prevalence of the disorder in the population being tested (or as applied to the individual, the prior probability that the person being tested has the disorder). In studies using referred patients with an increased probability of disease, and a definition of positive SPT as one with a mean wheal diameter of 3 mm or greater, SPTs have an excellent NPV (~95%), but the positive predictive accuracy is on the order of only 50% (39).

The definition used to indicate a positive test (or degree of positive) will also affect the PPV and NPV. For example, increasing skin test wheal size correlates directly with increasing IgE antibody and the risk of clinical reactions. Therefore, if one were to analyze skin test sizes (rather than just labeling them categorically as positive or negative at a mean wheal size of 3 mm), sensitivity and specificity would vary with each incremental change in size. In general, as the definition of a positive test requires a larger wheal, specificity increases and sensitivity decreases. Receiver operator curves are used to display the association of test size defined as positive with sensitivity and specificity that must be determined experimentally (Fig. 8–1). The uppermost left quadrant on the curve is the point where combined maximum sensitivity and specificity could be achieved. Similarly, as "cut-off" for positive result increases, so does PPV, while the NPV simultaneously decreases. Because these indices of predictive value are population-dependent, the predictive value drops (illness is overestimated) when results obtained in a referral center (high prevalence) are applied to individuals who are not as likely to be clinically allergic.

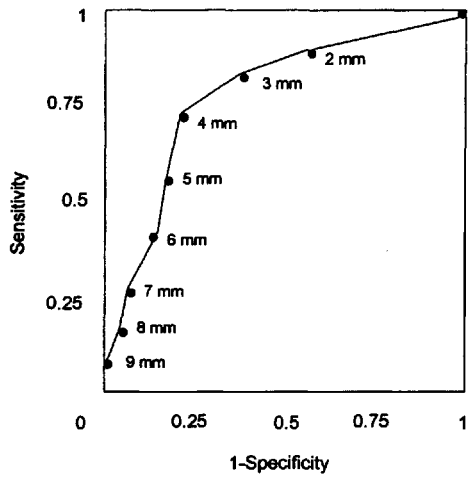

Figure 8–1. A receiver operator curve showing a hypothetical experiment in which SPT sensitivity and specificity were determined for various wheal sizes. When different skin test sizes are considered as a positive "cut-off," there is a trade-off between sensitivity and specificity. The single point at which sensitivity and specificity is maximized is the one closest to the upper left corner (4 mm in this example). When the skin test size meets and exceeds 9 mm in this example, specificity is 100% and all patients would be expected to react to this food.

An additional way of interpreting a test is to consider the chance that a person with food allergy would have a positive test compared to the chance that one without food allergy would have a negative test. This "likelihood ratio" is independent of population prevalence, but to use it for predicting food allergy, one must have a sense of pretest probability in the individual tested (i.e., the effect is similar to population prevalence of disease on PPV and NPV). If one knows the likelihood ratio of a skin test and the pretest probability of food allergy, it is possible to calculate a posttest probability by multiplying the likelihood ratio by the pretest probability (40). Although the specific data are not worked out for most foods, the concept is clinically vital, and it underscores the importance of a careful history. Consider, for example, three individuals: one had three severe allergic reactions to egg requiring epinephrine, another has AD and no history of a reaction to egg, and a third sometimes has headaches when he eats egg. Each patient is tested by SPT to egg white and has a 4-mm wheal. When there has been recurrent anaphylaxis to egg, the meaning of a 4-mm wheal in response to egg is that it confirms reactivity, because the pretest probability is high. In a chronic

condition such as AD, a modest-size skin test result may reflect clinical reactivity in only about half of patients (depending also on age), and may be a relevant positive in this scenario needing confirmation by other means. The test result in the situation of isolated headaches is most likely of no clinical concern, because the pretest probability is essentially zero. Considering again the patient with multiple episodes of egg-related anaphylaxis, if there were no wheal to egg the clinician would not be likely to trust the result because the pretest probability is so high and the correct course of action would be to repeat the test and consider a supervised OFC if the test were negative. These features underscore the importance of medical history when evaluating test results. Likelihood ratios can be calculated for increasing skin test wheal sizes, which in turn can assist in broadening the ability to predict reactions in various clinical scenarios. However, more studies are needed to provide reliable data for a large number of foods (41). Such data would be particularly helpful for the interpretation of skin tests performed to foods with homologous proteins (see Chapters 3 and 34) in persons who have a bona fide allergy to one of a group of related foods.

An argument has been made that in certain circumstances, very large SPTs may have 100% positive predictive value. This concept was demonstrated in a study (41) showing that for young infants, reactions to egg, milk, and peanut were certain to occur if the skin test wheal was ≥8 mm for cow's milk and peanut and ≥7 mm for egg. The scenario reflects increasing likelihood ratio with increasing sizes of skin tests (likelihood ratios over 12.5 for all three allergens with wheals 6 mm or greater in the referral population). This result requires replication in further studies. When considering the clinical use of such study results, it is also important to consider the variables mentioned previously concerning method of interpretation, skin test device, reagents, study population, etc. The clinical utility of SPTs are maximized when two decision point wheal sizes are considered in the interpretation: one with high NPV and another with high PPV. When considered together, this may reduce the need for further evaluations (e.g., OFCs).

Patch Tests

Patch tests are classically used to evaluate cell-mediated responses to various chemical sensitizers. This methodology has been investigated for food allergy by applying whole food proteins in a manner similar to patch tests for contact allergens in a format termed the atopy patch test (APT). Although workers use different regimens, the tests are generally performed by applying the suspected agent to the surface of the skin in a metal cup (Finn chamber) under an occlusive dressing and leaving it in place for 24 hours. The test site is evaluated at the time of removal and 1–2 days later for evidence of inflammation that can be scored by severity. Controls are applied to determine possible irritant reactions. The APT can hypothetically induce T cell responses reflecting those that occur in subacute and chronic AD (42) or perhaps in gastrointestinal food hypersensitivity (43). Early studies of the APT in cow's milk allergy in infants showed improved utility for determining delayed responses to OFCs compared to SPTs that were better correlated with immediate symptoms (44). Numerous subsequent studies, such as those performed to evaluate the diagnostic value of skin tests, have shown the test to be of modest utility with a wide range of sensitivities and specificities in various settings. In several studies, positive APTs were associated with delayed reactions, a phenomenon for which the sensitivity of SPT is generally low (0.04–0.26) (38, 44, 45). In one study of children with AD (46), the APT had the highest PPV for allergy to egg and milk compared to SPT or RAST for both immediate and delayed reactions. However, the results vary widely among workers, because much lower PPV (40%–63%) and specificities (0.71–0.87) have been reported (38, 44, 47). Like studies of SPTs, the variety of results using APTs may reflect variations in patient populations (age, type of atopic disorder), definition of positive tests, reagents, and study techniques.

There are several practical issues in using the APT. Their use requires two to three physician visits and a fairly large area of intact, rash-free skin, and they are cumbersome and more costly than SPTs. Clearly, the APT shows some promise as a diagnostic tool, but the method needs to be further standardized and its utility compared to results using other diagnostic methods.

Oral Food Challenges

The first point to consider before undertaking an OFC is whether the procedure is indicated (aside from those performed purely for research purposes). The OFC ultimately either confirms or

refutes a specific food as causing clinical disease. The issues to consider when deciding whether to undertake an oral challenge, and what challenge setting to use (e.g., open, single or double-masked), are summarized in Table 8–3. Diagnostic tests considered in this chapter and elsewhere (Chapter 7), results of elimination diets, and historical points are central to decision making. In some settings oral challenges may be optional or contraindicated. Severe anaphylaxis to an isolated ingestion, with a positive test for specific IgE antibody to the causal food is one example of a relative contraindication for oral challenge. However, in some circumstances even a patient with this convincing history may require a challenge, for example, if enough time has passed and laboratory indices are favorable for the possibility that tolerance has developed. If the food being eliminated is not nutritionally or socially important (e.g., kiwi), then challenge may be unwarranted. The same rules may apply if several members of a food family are being eliminated, but the food family is not a major part of the diet (e.g., elimination of all tree nuts when an allergy to one is certain). Overall, a variety of safety and social issues should be considered. This section will consider several of the important issues derived from the history and result of elimination diets that must be considered before undertaking an OFC. Specific details for undertaking and interpreting oral challenges are reviewed.

The History and Physical Examination

The history and physical examination are undertaken before the selection of any diagnostic tests. The clinician must determine from the history whether the complaints are likely to be associated with food allergy, intolerance, or toxic effects, or are not related to foods whatsoever. Furthermore, the physician must construct a priori assessments of the chance that foods do play a role, which foods may be involved, and whether the pathophysiology—if it is related to a hypersensitivity reaction—is IgE antibody-mediated, cell-mediated, or both. A careful history should focus on 1) the symptoms attributed to food ingestion (type, acute vs. chronic); 2) the food(s) involved; 3) consistency of reactions; 4) the quantity of food required to elicit symptoms; 5) the timing between ingestion and onset of symptoms; 6) the most recent reaction or patterns of reactivity; 7) the manner in which the food was prepared (raw or cooked); 8) potential contamination with known allergens; and 9) any ancillary associated activity that may play a role (e.g., exercise, alcohol ingestion). It is convenient and possibly quite illuminating to have patients keep a symptom diary and chart the foods they consume with and without symptoms, and to collect ingredient labels from the foods they eat. The information gathered from the general history and diet records are used to determine the best mode of diagnosis or may lead to dismissal of the problem based on the history alone. Ancillary history and physical examination indicating atopic disorders (AD, asthma, allergic rhinitis, other food hypersensitivities) would increase the chance that symptoms are related to a food-allergic reaction.

In the case of acute reactions following the isolated ingestion of a particular food with classic food-allergic symptoms, such as acute urticaria or

Table 8–3.
Issues to Consider Before Undertaking an OFC

Category	*Variables*	*Factors*
Indication to challenge	Probability to pass (risks)	History, physical examination, test results, nature of allergen, natural history of disease
	Needs (benefits)	Social Nutritional
Challenge type	Open	Numerous foods to screen, disorder with objective symptoms, allowance for bias
	Single-blind	Less prone to bias than open
	DBPCFC	Least prone to bias, most definitive approach for subjective symptoms
Challenge location	Home	Adding foods in chronic or behavioral disorders with no risk of acute/severe reactions
	Office	Challenges at low risk for severe reaction
	Hospital/ICU	Challenges that are more likely to elicit reactions requiring medical intervention

anaphylaxis, the history may clearly implicate a particular food and a positive test for specific IgE antibody (SPT/RAST) would be confirmatory and exclude the need for OFC. If the ingestion was of mixed foods and the causal food was uncertain (e.g., a meal with five ingredients), the history may help to eliminate some of the foods. For example, foods frequently ingested without symptoms are generally excluded as potential triggers when evaluating symptoms associated with acute reactions. Tests for food-specific IgE antibodies may help to further narrow the possibilities. In chronic disorders such as AD or asthma, it is more difficult to pinpoint causal foods (48). The history is helpful, but because these disorders have a waxing and waning course, and considering limitations in diagnostic laboratory tests, false associations to food ingestions are common (49). A similar problem arises with gastrointestinal reactions that are often delayed in onset following ingestion of causal foods or are chronic with a relapsing course. Although true association is uncommon, the evaluation of reactions to food dyes and preservatives usually requires OFCs. Finally, patients may attribute a host of medical complaints to food ingestion in disorders that are not proved to be pathophysiologically linked to food allergy (e.g., arthritis, fatigue, behavioral problems, etc.). In all of the circumstances involving chronic complaints, the OFC is capable of revealing or excluding relationships to foods. Such determinations are crucial because patients may undertake unnecessary dietary alterations that can have nutritional and social consequences (50, 51). Overall, the approach to diagnosis in chronic disorders, where most readily available diagnostic tests are of limited value, requires elimination diets and OFCs to confirm suspected associations.

Food Elimination Diets

When food hypersensitivity is under consideration, an elimination diet is often warranted before undertaking OFCs (52). There are three types of elimination diets (Table 8–4); the type used will depend on the clinical scenario being evaluated and the results of tests for IgE antibody. The first type involves the elimination of one or several foods from the diet. This may be the obvious course of action when an isolated food ingestion (i.e., peanut) causes a sudden acute reaction and the test for IgE to the food is positive. This would also represent a therapeutic intervention. However, eliminating one or a few suspected foods from the diet when the diagnosis is not so clear (e.g., in asthma, AD, chronic urticaria) can be a crucial step in determining if food is causal in the disease process. If symptoms persist, the eliminated food(s) is (are) excluded as a cause of symptoms. The length of trial depends on the type of symptoms, but 1–6 weeks is usually the time interval required.

The second type of diet involves eliminating a large number of foods suspected of causing a chronic problem (usually including those that are common epidemiologically as causes of food-allergic reactions as described above) and giving a list of "allowed foods." This "oligoantigenic" diet is useful for evaluation of chronic disorders when a larger number of foods are suspected. In most cases, this is the situation with AD or chronic urticaria. An example of such a diet is given in Table 8–4, but individualization is almost always needed. The advantage of this diet is that a nutritionally balanced, palatable diet is maintained while most possible causal foods are removed. The primary disadvantage is that, if symptoms persist, the cause could still be attributed to foods left in the diet. For finicky eaters, it may be helpful to assess

Table 8–4.
Types of Elimination Diets Used to Evaluate the Role of Adverse Food Reactions in Chronic Disease

Diet	*Description/target*	*Example*
Specific food(s)	1) Diet targeted to one or several suspected foods 2) May be therapeutically necessary as final treatment	Elimination of egg in toddler with atopic dermatitis; elimination of food dyes and preservatives in child with chronic urticaria
Oligoantigenic	Palatable, balanced diet devised according to patient preferences, but eliminating a large group of common or suspected allergens (e.g., egg, milk, peanut, seafood, etc.)	Allow lamb, broccoli, squash, sweet potato, rice, corn, beets, cooked apple and pear, sugar, salt, and vegetable oil for 6 weeks in patient with reflux and atopic dermatitis
Elemental	Amino acid-based formula (or, less ideally, an extensive hydrolysate) as sole nutrition	Used for 8 weeks to evaluate severe eosinophilic gastroenteropathy in a 3-year-old with failure to thrive

exactly what foods are favorites and try to allow foods of low risk that are enjoyed by the patient and can be used for meals and snacks.

The most limited type of diet is an elemental diet in which calories are obtained from a hydrolyzed formula or, preferably, from an amino-acid based formula. A variation is to include a few foods likely to be tolerated (however, this adds the possibility that persistent symptoms are caused by these foods). Unfortunately, except for the most serious disorders that warrant its use, this is a severe diet to impose and is extremely difficult to maintain in patients beyond infancy. In extreme cases, nasogastric feeding of the amino-acid based formula can be done, although some patients can tolerate the taste of these formulas with the use of flavoring agents provided by the manufacturers. This diet may be required when the diets mentioned above fail to resolve symptoms but suspicion for food-related illness remains high. It is also required in disorders associated with multiple food allergies such as the allergic gastroenteropathies (53).

Information about strict adherence to the diet must be carefully reviewed with the patient. It is common for families to make errors. Patients and families must be educated about label reading, cross-contamination, and the fact that the food protein, as opposed to sugar or fat, is the ingredient being eliminated (for example, lactose-free milk contains cow's milk protein). If there is no improvement with elimination, then the foods eliminated are not likely to be a cause of the complaints. However, if resolution of symptoms is achieved, OFCs may be warranted as a next step in identifying which foods from among those eliminated are or are not tolerated.

Food Challenges

Preparing for Challenges

The risks and benefits of the challenge procedure must be discussed with the patient and his or her family. As mentioned previously, numerous factors must be considered including the odds for passing, the nutritional and/or the social need for the food, and ability of the patient to cooperate with the challenge. The next step to consider is the location or setting in which to undertake the challenge. In some circumstances, the food may actually be administered without physician supervision at home. For example, when vague complaints, or ones not usually associated with food allergy (headache, behavioral issues), are being evaluated and there is no risk of an acute anaphylactic reaction, and especially when symptom onset is perceived to be delayed, foods (even in a double-blind, placebo-controlled structure) could be added at home. However, whenever there is an even remote possibility of an acute and/or severe reaction, physician supervision is mandatory. In considering this issue, a distinction should also be made between "challenging" a food as opposed to merely reintroducing a food back into the diet. For example, if many foods were eliminated for a chronic, non-IgE-mediated disease and acute reactions are not an issue, adding the previously tolerated food back to the diet at home is not a "challenge" and would not be suspected to cause a problem. In a similar manner, a patient with a chronic disease that responds to elimination, but was not severe and not IgE-mediated (abdominal pain, reflux, allergic eosinophilic esophagitis and gastroenteritis), can be challenged at home by adding one food back into the diet at a given time, and monitoring for recurrence of symptoms for 5 days.

Except in the uncommon circumstances described previously, OFCs are undertaken under direct medical supervision. In this scenario, a physician or trained health care worker evaluates symptoms during a challenge. The decision to undertake a supervised challenge includes, but is not limited to, the evaluation of disorders that include a potential for severe reactions. The next issue at hand is whether the challenge is considered of "low risk" and can be done in an office setting or should be conducted in a location with the capacity to manage severe anaphylaxis (e.g., hospital, intensive care unit). Whether an intravenous line should be in place before commencing the challenge must also be considered. These decisions are based on the same types of data evaluated for the consideration of food allergy in the early diagnostic process: the history, SPT results, etc. In any setting, it must be appreciated that oral challenges can elicit severe, anaphylactic reactions, so the physician must be comfortable with this possibility and be prepared with emergency medications and equipment to promptly treat such a reaction. In the office setting, such preparations are similar to those recommended in the context of offices that administer allergens by injection for immunotherapy (12, 54).

If the challenge is considered "high risk" (e.g., positive test for IgE, previous severe reaction, asthmatic patient), then it is best to perform it in a very controlled setting (e.g., a hospital). In high-

risk challenges, it may also be prudent to have intravenous access before commencing challenges. One research group reviewed their records of 349 food challenges in children with AD and recommended intravenous access for challenges when the history indicated a prior need for medical intervention or when particular tests for IgE antibody indicated a fairly high risk for reactions (55). In addition to potentially severe reactions elicited by OFCs undertaken for IgE antibody-mediated food-allergic reactions, one type of non-IgE antibody-mediated reaction can also be severe: food protein-induced enterocolitis syndrome (56, 57). This cell-mediated disorder results in a symptom complex of poor growth and profuse vomiting and diarrhea with or without microscopic blood in the stool while the causal food is part of the diet (57, 58). The disorder is usually diagnosed in infancy and the most common causal proteins are cow's milk and soy; rarely, other foods, such as rice, egg, fish, and poultry, are responsible. When severe, particularly with re-feeding after a period of elimination, as is the case during the OFC, reactions may include lethargy, dehydration, and hypotension, and may be complicated by acidosis and methemoglobinemia (58–60). Food challenges for this non-IgE-mediated syndrome are typically performed with 0.15–0.6 g of the causal protein (usually cow's milk or soy) per kilogram of body weight, and reactions of profuse vomiting and diarrhea typically begins 2–4 hours after the ingestion and are accompanied by a rise in the absolute neutrophil count of over 3500 cells/mm^3. Because of these characteristics and the potential for shock, intravenous access should be obtained in advance to allow for fluid resuscitation. With this disorder, the challenge is best performed in a hospital setting.

Challenge Methods

Patients must be given specific preparatory instruction prior to undertaking the challenge. Patients avoid the suspected food(s) for at least 2 weeks, antihistamines are discontinued according to their elimination half-life, and chronic asthma medications are reduced as much as possible prior to undertaking the challenge. Beta-agonists are eliminated for a relevant time period before challenges are undertaken. The patient should be examined carefully before challenge to confirm that they are not already having chronic symptoms, and to determine their "baseline." It would not be prudent to undertake a challenge in an individual with, for example, mild wheezing, for both the ability to judge a reaction and for safety concerns. For some diseases (e.g., severe AD) hospitalization may be necessary to treat acute disease and establish a stable baseline before challenges.

Challenges can be done openly, with the patient ingesting the food in its natural form, single-blind, with the food masked and the patient unaware whether the test substance contains the target food, or by DBPCFC, where neither patient nor physician knows which challenges contain the food being tested. In the latter two formats, the food must be hidden in some way, such as in another food or in opaque capsules. When challenges are undertaken for research purposes, the DBPCFC is the preferred format because of the low chance for bias from either the patient or physician who must monitor symptoms. However, there are several factors that weigh in deciding which type of challenge to use. Although the open challenge is most prone to bias, it is easy to perform because no special preparation is needed to mask the food. Indeed, if the patient tolerates the ingestion of the food, there is little concern about bias. It is only when symptoms, especially subjective ones, arise that the issue of bias come into play. Therefore, open challenges are a good option for screening when several foods are under consideration; if a food is tolerated, nothing further is needed. However, if there is a reaction to an open challenge used in the clinical setting, and there is concern that the reaction may not have been physiological, the format could be altered to include blinding and controls. Single-blind challenges help to alleviate patient bias and may be an option to increase efficiency (since a second placebo arm is not always needed).

Despite attempts and discussions to make a uniform international protocol for performing OFCs, no consensus has been reached, and many published studies use variations on a general theme (12, 61, 62). In all challenges, the food is given in gradually increasing amounts. For most IgE-mediated reactions, the author and colleagues (63, 64) give a total of 8–10 g of the dry food or 100 mL of wet food (double amount for meat/fish) in gradually increasing doses at 10–15 minute intervals over about 90 minutes followed by a larger, meal size portion of food a few hours later. The doses may be distributed, for example, in portions, such as [0.1%, 0.5%], 1%, 4%, 10%, 20%, 20%, 20%, 25%. However, researchers and clinicians have used a variety of other challenge regi-

mens (lower starting doses, variations in the degree of dosing increases, different time intervals, etc.) with good success (65, 66).

The starting dose varies among studies, but clinical correlation may be helpful. To place this in perspective, it is reported that highly sensitive cow's milk-allergic patients may react to trace milk contamination (e.g., 8.8–14 parts per million) in commercial products, but these are generally not patients with a profile conducive to oral challenges (67, 68). In a study of adult peanut-allergic patients undergoing DBPCFCs, 50 mg of peanut was generally the lowest dose that elicited objective reactions (one patient experienced subjective symptoms at only 100 μg of peanut) (69). We reviewed challenge data for 513 positive challenges to six common allergenic foods in children with AD (70). Starting doses were usually 500 mg, but at the physician's discretion, starting doses were sometimes 100 or 250 mg. The percentage of children reacting at the first dose (500 mg or less) was as follows: egg, 49%; milk, 55%; soy, 28%; wheat, 25%; peanut, 26%; and fish, 17%. Twenty-six milk challenges and 22 egg challenges were positive at a first dose of 250 mg; three milk challenges and seven egg challenges were positive at a first dose of 100 mg. Eleven percent of the reactions that occurred on first dose were severe. The dose that elicited a reaction was not predictable with SPT size or IgE antibody concentration. Based on these results, starting doses of 100 mg or less were recommended. To be particularly cautious, one could argue for starting doses that begin under the thresholds reported to induce reactions. Unfortunately, the published thresholds vary by logarithmic differences among studies and data are not available for most foods. However, reactions are usually not reported under 0.25 mg of protein for peanut, 0.13 mg for egg, and 0.6 mg for milk (mg of protein varies according to the form of the food) (71). Some workers begin challenges by placing the food extract on the lower lip for 2 minutes (labial food challenge) and observing for local or systemic reactions in the ensuing 30 minutes (65, 72, 73). The development of a contiguous rash of the cheek and chin, edema of the lip with conjunctivitis or rhinitis, or a systemic reaction is considered a positive test (65). Negative labial challenges are generally followed by an OFC.

The physician or health care worker records the dose given, the time of administration and any symptoms that arise during the challenge (12). Frequent assessments are made for symptoms affecting the skin, gastrointestinal tract, and/or respiratory tract. With children, early indications of a reaction can include subtle signs such as moving the tongue in the mouth to rub an itchy palate, or ear pulling due to referred pruritus. While some families believe increased physical activities (hyperactivity) are a sign of food allergy, a common early response for children as they begin to experience a reaction is that they become suddenly quiet or assume a fetal position as a prodrome to more objective symptoms. Children with AD may develop a maculopapular rash in predilection areas of eczema. Objective monitoring can be done with peak flow or spirometry. Challenges are terminated when a reaction becomes apparent and medications are given, as needed. Generally, antihistamines are given at the earliest sign of a reaction, and epinephrine and other treatments are given if there is progression of symptoms or any potentially life-threatening symptoms, but this is open to the judgment of the supervising staff, who must take the patient's history into account. In some cases, families or individuals may question whether it is necessary to treat the symptoms at all, or may even ask to proceed with more doses to see "how bad" the reaction could be. This is not advisable for obvious safety reasons and also because the reactions are not likely to reflect what a subsequent exposure may cause in an uncontrolled setting. If the history indicates delayed reactions or reactions after prolonged ingestion, adjustments can be made to make the challenge more closely resemble the suspected course for reactions. In some cases this could include supervised ingestion over several days.

The successful administration of OFCs to young children requires a great deal of preparation, ingenuity, and patience. Young children may become stubborn and refuse to ingest the challenge food. Prior planning with the family to select palatable or familiar forms of challenge foods, or vehicles to hide foods in if the challenge is masked, can be helpful in improving the experience (74). For example, milk protein may be mixed and hidden in soy frozen dessert products. Having additional challenge vehicles, for example liquid and solid forms of the challenge substance, readily at hand may prevent delays. Allowing the use of well-cleansed utensils and dinnerware that are familiar to the child (e.g., a favorite cup or plate) makes the challenge more natural appearing. Diversions such as toys, games, or videotapes are helpful. Since splattered or drooled food can elicit a local skin reaction from direct skin contact (but not necessarily from ingestion), it is helpful to

have wet napkins on hand and straws for liquid challenges. Similarly, when performing OFCs with children, it is better to feed them rather than to let them feed themselves and risk splattering.

The setup for a DBPCFC is more complicated than that needed for open or single-blind challenges. Although the procedure is more labor intensive, it can be carried out in an office setting if the challenge is not high risk (12). The procedure still introduces graded doses, but in this case either a challenge food or a placebo food are administered. The aid of a third party is needed to prepare the challenges so that the observer and patient are kept unaware whether a true or placebo challenge is being undertaken. A coin flip can be used by the third party to randomize the order of administration. The food is hidden either in another food or in opaque capsules. Suggestions for materials to have on hand for creating masked challenges are shown in Table 8–5. It is beneficial to stretch the imagination in trying to best mask foods, especially foods with strong odors. It is easiest to use opaque capsules, but oral symptoms are then bypassed and some patients are unable to ingest enough capsules. It is often easier to mask liquid into liquid and to use powder or dehydrated forms of foods that can be folded into solid vehicles. Certain flavoring agents such as mint can also help to mask odors. It is important to select vehicles that are clearly tolerated by the patient. If a gritty food is being hidden in a vehicle, then a similarly gritty food should be added as placebo to the carrier vehicle. For example, oat as an allergen mixed in apple sauce may be matched to corn meal in apple sauce. It is also important to appreciate that certain preparation methods (canning, dehydration) may alter the allergens, hence an open challenge with a meal-size portion of the food prepared in its natural state for consumption following a negative DBPCFC is essential. It is preferable not to use fatty foods as vehicles because they can delay gastric absorption.

Depending on the particular food hypersensitivity disorder under consideration, timing of dose administration can be adjusted. For example, when evaluating potentially IgE antibody-mediated reactions, two challenges may be performed on a single day with 2–4 hours between challenges (one is placebo and one is active, so one food is tested each day). The practice of interspersing placebo and active food proteins during a single challenge (i.e., random ordering of sequential doses that may or may not contain the causal protein) should be discouraged because it can be difficult to determine if a reaction shortly after a particular dose—possibly a placebo dose—was actually a delayed response from an active dose administered previously.

The DBPCFC is considered the "gold standard" for diagnosing food allergy (12, 39, 75) Any test, however, can have limitations. The false positive and false negative rate for the DBPCFC based primarily on studies in children with AD is 0.7% and 3.2%, respectively (76, 77). To help exclude false negatives, it has long been suggested to include an open feeding under supervision of a meal-size portion of the tested food prepared in its usual manner, as a follow-up to any negative DBPCFC (5, 62, 78). When one is evaluating subjective symptoms, there is a greater likelihood of false positive or negative determinations. Increasing the number of challenges (additional placebo and true foods) helps to diminish the possibility of a random association, but this can be a very labor-intensive approach (79). Although the DBPCFC can elucidate the relationship of symptoms to foods, it is not specific for food hypersensitivity. Any adverse reaction to food (intolerance, pharmacologic effect) can be evaluated, so demonstration of an immunological explanation is still needed to label a reaction as a food allergy. Oral challenges are almost the only methodology to adequately evaluate reactions to food additives (coloring and flavoring agents, preservatives) (80–82). The same can be said for symptoms not likely to be associated with food allergy (behavior, etc.).

Table 8–5.
Equipment and Common Foods to Stock for Use in Creating Masked Food Challenges

Equipment	*Common Allergens*	*Useful Carrier Agents*
Paper plates, cups, utensils	Peanut flour, peanut butter	Proprietary formulas (hydrolyzed casein, amino acid)
Mixing bowls	Powdered egg white	Baby foods (squash, carrot, potato)
Scale	Powdered or fresh milk	Apple sauce
Mortar and pestle	Soy milk, soy flour	Juices
Blender	Wheat breads, flour	
Microwave	Baby foods	

Post-Challenge Issues

There are several issues that need to be addressed when an OFC results in a reaction. The disappointment engendered should be openly discussed. Sometimes patients can be partly consoled to know that their hard work at avoidance was necessary and successful. Patients often wish to know if future reactions could be severe, a question whose answer may not be related to the result of the challenge, because dosing is gradual in the test rather than sudden and possibly high during accidental exposures. In some cases, it may be apparent that patients were not symptomatic with small exposures during the challenge and may have a margin of error in terms of potential accidental exposures. Patients and families may also inquire as to the possibility that the challenge could "boost" or prime their allergy. Although there are no published data to clearly support or refute this concern, the OFC is ultimately the only way to know whether the food is tolerated, and it is performed clinically when risk assessments are favorable for passing, thus making this concern essentially moot. A plan for re-evaluation with laboratory tests and OFCs should be discussed depending on the usual natural course for the food in question, and on patient-specific determinants such as age and other food allergies. Review of food avoidance measures is also helpful, and a re-evaluation of any nutritional impact that avoidance may have engendered should be undertaken.

Patients who have passed a challenge often need additional counseling about how to introduce or reintroduce the food. In some cases, a remaining fear could result in continued avoidance. There may also be concerns about redeveloping the food allergy, a situation that is quite rare. Patients with remaining food allergies must be cautioned specifically about any increased risk of exposure to an allergen that is commonly associated with the food that they are now able to ingest. For example, a patient with milk and egg allergy who passes an OFC to wheat must be warned to carefully check wheat products, now new to the patient, that may also contain milk and egg. When there are no remaining food allergies, patients may be loath to stop carrying epinephrine, and this should be discussed as well with consideration for a period of continued availability to reduce stress.

Summary

In vivo tests are primary tools in the armamentarium available to the clinician for the diagnosis of adverse reactions to foods. Skin testing is safe, cost-effective, and when properly performed and interpreted, highly informative for the diagnosis of IgE antibody-mediated disorders. OFCs are the most definitive test available to date for the final confirmation of these disorders. Although oral challenges are time consuming and may elicit severe reactions, they can be safely and efficiently performed with the proper preparation and remain the mainstay of diagnosis for clinical and research settings.

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Part 2

Adverse Reactions to Food Antigens: Clinical Science

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9

Immediate Reactions to Foods in Infants and Children

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Introduction

Immediate hypersensitivity reactions in children are the most easily demonstrable and understandable adverse food reactions. They comprise a subgroup of the pathophysiologic reactions that have been described and attributed to foods. A number of classification schemes are used to characterize adverse food reactions. One reasonable approach that uses a mechanistic scheme has been proposed (1). This classification begins with two broad groups of adverse food reactions: toxic, which may affect any individual, and non-toxic, which include the immunologic reactions discussed in this chapter. The two broad groups of immune-mediated non-toxic reactions are IgE-mediated and non-IgE-mediated. The IgE-mediated reactions are usually divided into immediate-onset reactions (immediate in time) and immediate plus late phase (in which the immediate onset symptoms are followed by symptoms that are prolonged in time or ongoing). In this latter pattern the immediate reactions seem to become blunted or less obvious as the chronic lesions persist. Although this phenomenon has been described in both animal models and in vitro systems, the full explanation for this observation is not clear.

The non-IgE-mediated adverse reactions to foods are less well defined at the molecular and cellular level. These conditions appear to have multiple and possibly concurrent or sequential immunopathologic mechanisms that explain the complexities of these conditions. A classification scheme is presented in Table 9–1. When considering the physiology of immediate hypersensitivity reactions, perhaps the best model is the skin prick/puncture test (SPT). When allergen is placed on the skin, and the skin is gently indented (some reactions will occur without puncturing the skin), the allergen is able to permeate the skin and cross-link allergen-specific IgE antibodies that are present on cutaneous mast cells. This results in the release of pre-formed chemical mediators that cause an "immediate" wheal and flare reaction, which occurs within a few minutes. The same mechanism may occur in several organ systems and results in symptom within those organs. The cutaneous wheal and flare reaction that produces localized or generalized urticaria is a systemic manifestation of immediate hypersensitivity. A few patients will develop generalized erythema without wheals. In addition, local contact urticaria may occur from mere contact between the food and the skin. The gastrointestinal (GI), respiratory, and cardiovascular systems are the other major target organs that may be involved in immediate hypersensitivity-induced symptoms. The symptoms for each organ system are presented in Table 9–2 and considered in more detail below.

Atopic dermatitis (AD) seems likely to involve both immediate and late-phase IgE-mediated reactions with an extensive pathology in some individuals. Eczematous lesions may vary from very mild to extremely severe, and it is the latter that are most likely to involve food hypersensitivity. One difficulty in detecting the involvement of food in severe AD is the duration of the lesion and the apparent diminution of the immediate re-

Table 9–1.
Classification of Adverse Reaction to Food

Toxic reactions (see text)
Non-toxic reactions
Non-immune
Immune
IgE-Mediated
Immediate
Gastrointestinal (nausea, vomiting, diarrhea, cramps)
Cutaneous (urticaria, angioedema, atopic dermatitis)
Respiratory (rhinitis, sneezing, cough, wheezing, asthma)
Anaphylaxis
Immediate and late-phase
Atopic dermatitis
Allergic eosinophilic gastrointestinal conditions
Non-IgE-mediated
Gluten-sensitive enteropathy (celiac disease)
Food protein-induced enteropathies/enterocolitis

sponse that would be anticipated to follow ingestion of an allergenic food.

Gluten-sensitive enteropathy, or celiac disease (CD) and the more transient protein enteropathies (e.g., food protein-induced enterocolitis syndrome, food-induced proctocolitis syndrome, etc.) are probably the best examples of non-IgE-mediated adverse reactions to food. These conditions are immunologically mediated and extensive research is exploring these mechanisms. They may involve a sequence or combination of immunologic events, thus making them difficult to classify as a single immunologic mechanism.

This chapter will consider the important aspects of "immediate," IgE-mediated food hypersensitivity reactions in children, and will place them in the context of clinical practice with respect to their mechanisms, diagnosis, and current treatment.

Table 9–2.
Presumed IgE-mediated Food Hypersensitivity Reactions Elicited During Blinded Food Challenges

Generalized Reactions
Anaphylaxis
Food-dependent exercise-induced anaphylaxis
Cutaneous Reactions
Urticaria
Angioedema
Atopic dermatitis
Food-dependent, exercise-induced urticaria/angioedema
Respiratory Reactions
Sneezing
Rhinorrhea
Pruritis of the upper respiratory tract
Laryngeal edema/laryngospasm
Cough, wheezing, breathlessness, asthma
Ocular Reactions
Watering and pruritis of the eyes
Scleral edema
Chemosis
Gastrointestinal Reactions
Abdominal pain, nausea, vomiting, diarrhea
Colic
Allergic eosinophilic gastroenteritis (with gastroesophageal reflux)

Prevalence

General Observations

The incidence and prevalence of food hypersensitivity reactions in children and adults have become increasingly popular topics to the media and general public. However, large population studies using acceptable epidemiological methods have been reported infrequently. The public's perception of the frequency of food hypersensitivity seems significantly discrepant from the data in published studies. In one report from a popular magazine, about 25% of the population believed that at least one family member had "food allergy" (2). A more recent estimate based on a household questionnaire suggested that 15% of households contained at least one individual reporting food allergy (3). A French study used a more scientific questionnaire based on a representative population sample and found a prevalence of about 3% (4).

Prevalence also has been examined by looking at populations of varying ages. A population-based study evaluating only children estimated that 35% had "food-related allergic symptoms" (5). In a study of children born consecutively and enrolled in a longitudinal evaluation of adverse reactions to foods, 28% reported one or more symptoms that were related to food ingestion. However, only 8% of these reactions were shown to be food-related by oral challenge and IgE antibodies could be implicated only a minority (6). The most common foods producing symptoms, none of which could be shown to be IgE-mediated, were fruits and vegetables. These reactions were all transient and had disappeared by 3 years of age.

Prevalence of Reactions to Specific Foods

Prevalence has also been sought for reactions to specific foods. Milk allergy studies (sometimes including the term "milk intolerance," which generally refers to non-IgE-mediated reactions) con-

tain the best data. A Danish study of cow's milk allergy found that 2.2% of infants had challenge-documented reactions to milk during the first year of life (7). In a group of 1386 Dutch infants, 2.8% exhibited signs and symptoms of cow's milk allergy (8). Studies from the Isle of Wight, in a well-defined population of infants, found a similar prevalence (9). These studies had the advantage of being prospective, population-based studies, thus the percentages reported are more accurate and less diverse. Sicherer and co-workers (10) used random digit dial methodology and individual interviews to estimate the prevalence of peanut and tree nut allergy in the US. This study found that about 1.1%, or over three million individuals, have peanut or tree nut allergy. In the UK, about 0.5% of children reportedly have peanut hypersensitivity (11). In a large German multi-center study, 1.3% of children were found to be allergic to egg (12).

A frequently posed question is whether the incidence/prevalence of food hypersensitivity is increasing. If the population-based studies could be repeated in the near future and then at intervals of every 5–10 years, we might have an effective answer to this question. One population-based study of children with peanut allergy from the Isle of Wight suggests that the prevalence of peanut allergy among 4-year-olds may have doubled in the past 6 years (13). The reason that these conditions may be increasing remains as elusive and controversial as the general hypotheses to explain the increase in all atopic/allergic disease.

Prevalence of Reactions for Atopic Conditions

Another useful way to consider prevalence is to ask what is known about prevalence of food hypersensitivity in various conditions. For example, what is the prevalence of food hypersensitivity in AD or in asthma? At present the best studies have examined the role of food hypersensitivity in AD. As might be expected, the findings are associated with the severity of disease. The more severe the AD and the younger the child, the more likely that food hypersensitivity is involved in the pathogenesis. In a study by Burks et al (14) of children attending university-based dermatology and allergy practices, one third of the children were found to be food allergic. Eigenmann et al (15) studied children with moderate to severe AD referred to a university-based dermatology clinic and found that 37% of the children were food allergic. The value of these two studies lies in the critical nature of the testing. Subjects underwent double-blind placebo-controlled food challenge (DBPCFC) or had highly suggestive histories of isolated ingestion of food with typical onset of symptoms. They concluded that children with more severe AD that was poorly responsive to treatment warranted evaluation for food hypersensitivity.

Data on prevalence of food hypersensitivity in asthma is more elusive. There is some information from a highly selected population of asthma patients evaluated in a residential treatment center using DBPCFC. However, this group is not representative of the general population of asthmatic subjects. Nevertheless, it is instructive to see that 40% of these children experienced wheezing as one symptom during DBPCFC, but only 3% had isolated wheezing as the sole manifestation of a food hypersensitivity reaction (16). In a study by Onorato et al (17), 300 individuals ranging in age from infancy to 80 years were examined. This study also included DBPCFC. Only 2% of this population had a positive food challenge, with reactions limited to subjects between 4 and 17 years. The foods involved included egg, wheat, and corn. Novembre et al (18) studied 140 children with histories suggestive of food-induced asthma. Wheezing was elicited in 6% of the children aged 2 to 9 years during DBPCFC. Taken together, these studies suggest that the prevalence of food-induced asthma is low, but that it is important not to overlook the possibility that asthma may be elicited as the only symptom of an immediate hypersensitivity reaction to food. Patients who have (or had) AD, have gastroesophageal reflux, and require daily anti-inflammatory therapy and/or are poorly controlled are most likely to be food allergic.

Prevalence of Anaphylaxis to Food

Sorensen and colleagues (19) reviewed all cases of anaphylactic shock, occurring outside the hospital, in the Thisted Hospital catchment area in Denmark. They identified 3.2 cases per 100,000 inhabitants per year, of which 5% were fatal. These investigators found that 40% of the cases had been given an incorrect International Classification of Diseases (ICD) code at the time of discharge. In a retrospective survey, Yocum and Khan (20) reviewed all cases of anaphylaxis treated in the Mayo Clinic Emergency Department over a 3½ year period. One hundred seventy-nine patients

were identified. They concluded that anaphylactic reactions to food were the leading single cause of anaphylactic reactions outside of the hospital, occurring more frequently than reactions to bee sting and drugs combined. In another study, Yocum and colleagues (21) reviewed the medical records of subjects in the Rochester Epidemiology Project, a population-based study, for the five years from 1983 to 1987. On the basis of their review, they reported an incidence of anaphylaxis of 21 per 100,000 person-years and an anaphylaxis occurrence rate of 30 per 100,000 person-years. Because the Olmsted County population is reportedly representative of the US white population, and because there is no evidence of significant differences in the occurrence of anaphylaxis among races in the US, this survey would suggest that 83,000 cases of anaphylaxis occur in the US each year (population 280 million) (22). In this study, 36% of the reactions were reportedly due to food allergic reactions. Extrapolating from these data, one would predict that there are about 29,000 anaphylactic episodes due to food allergy in the US each year, resulting in about 2000 hospitalizations and 150 deaths.

In 2001, Brown et al (23) reported a retrospective review of 142 patients age 13 years and older who presented to an emergency department in Brisbane, Australia, during the year 1998–1999. The incidence of anaphylaxis was one per 439 emergency department visits and one adult presentation per 3400 individuals in the catchment area. One individual died, giving a case fatality rate of 0.7%. Foods were responsible for 17% of the cases, and the causative foods identified included fish, seafood, nuts, mango, and lemon.

Reports of food-associated exercise-induced anaphylaxis are increasing, raising the concern that this condition is becoming more common as well. It is therefore very important to establish or eliminate the potential role of exercise when evaluating individuals with food-induced anaphylaxis. This problem is being reported in children, especially teenage females, as well as in adults.

As may be discerned from the preceding discussion, food-induced anaphylaxis is a significant problem and is probably significantly underreported. Following the report of fatalities and near fatalities by Sampson et al (24), an attempt was made to establish a national registry to record and research deaths due to food anaphylaxis. Over a period of 6 years, only 32 cases were discovered and evaluated, and most of these were found through the media, not from reports by medical personnel. The age range was 2 to 32 years with the large majority being adolescents and young adults (25). The need to improve the reporting of these fatalities cannot be overemphasized.

Pathogenesis

As noted in the introduction, immediate hypersensitivity reactions to foods are nearly all mediated by IgE antibodies. Again, it is instructive to think of the classical allergy SPT as the model of immediate hypersensitivity. IgE is bound to the FcεRI receptor in tissue mast cells and circulating basophils. Other tissue cells also bind IgE. When the allergen is introduced into the skin, the IgE molecules are cross-linked, which then activates the tissue mast cells, causing them to release mediators including histamine, prostaglandins, and leukotrienes. The interaction of histamine with receptors on blood vessels leads to vasodilatation with fluid leakage that results in the formation of a wheal. An axon reflex causes the flare that is seen surrounding the wheal. An important reason to understand this process is that young children often exhibit urticaria when foods come in contact with their skin. However, when they consume these foods, even in customary quantities, no reaction occurs. This is a most important clinical observation that may result in confusion when attempting to determine whether children with contact urticaria to a food, who have not eaten the food, should be allowed to consume it or if it should be excluded from their diet. In the clinical setting it is surprising that a child could have a large positive skin test to a food and yet not develop urticaria when he or she ingests the food. In considering how systemic immediate hypersensitivity reactions to foods occur, it is obvious that the GI tract plays a crucial role in either allowing absorption of food allergens or in excluding them from the systemic circulation. One of the puzzles of food allergy is that an individual may be sensitized to numerous food allergens as detected by SPT or specific circulating IgE antibodies measured by the radioallergosorbent test (RAST), and yet not develop symptoms when the foods are consumed. It seems highly probable that the first line of host defense is the intestinal barrier. This barrier includes not only the physical structure of the intestinal mucosa, but also the mucus layer, secretory IgA, and digestive enzymes, as discussed in Chapter 1. When food proteins and peptides cross the intestinal barrier, they may be cleared by

specific IgA or specific IgG that form complexes that allow the antigens to be removed before they interact with IgE molecules. However, some of these antigens do reach target cells in the skin, intestine, lung, and cardiovascular system where their interaction with mast cell-bound IgE results in mediator release and ensuing symptoms.

Walzer and his colleagues (26) conducted some intriguing studies in the early part of this century. In an elegant series of investigations, they used the Prausnitz-Küstner test to demonstrate that individuals that were sensitized with serum from a fish-allergic subject and then fed fish developed wheal and flare responses at the site of sensitization. In a most interesting experiment, Walzer (27) injected "reagin-bearing serum" from an allergic subject into the ileocolostomy or ileostomy stump of patients and then fed them the incriminated food. Within minutes of the oral administration of the antigen, pallor and hypersecretion of the sensitized area began. After a few more minutes, edema was noted and increased for about an hour, followed by marked hyperemia and then profuse secretion of mucus. The reaction lasted about an hour. Itching or burning of the surrounding skin accompanied some of these reactions. This is only one of the experiments performed but it is a revealing demonstration of the pathophysiology of an IgE-mediated reaction done decades before anyone had a remote idea of the nature of reagin.

The exact sequence of immunologic events that occur after an allergenic food protein encounters a sensitized cell is still to be determined. However, it is clear that multiple cytokines and cell products are released that may lead to inflammation in the organ systems affected. For example the effect of major basic protein (MBP) has been demonstrated in the skin of children with food allergen-induced AD (28).

Animal models are now being created to mimic these clinical and immunological findings observed many years ago. These models will allow the mechanisms of these diseases to be unraveled and, hopefully, treatments will soon be found (29–32).

Undoubtedly there is a significant genetic component to the development of immediate hypersensitivity to food allergens. In a recent twin study Sicherer et al (33) showed that concordance for peanut allergy is quite different in monozygotic versus dizygotic twins. The monozygotic group had a pairwise concordance of 64%, whereas the dizygotic group was much lower, being measured at a pairwise concordance of only 7%. Thus, despite the importance of genetics, this study clearly demonstrates the crucial role of the individual's interaction with the environment, in this case exposure to and processing of food proteins.

Despite the hundreds of different foods in the diet, relatively few foods are involved in most immediate hypersensitivity reactions: egg, cow's milk, wheat, soy, peanut, tree nuts, fish, and shellfish. However, this list is most characteristic of North American populations. In other parts of the world where other food proteins are more commonly ingested, other foods are more prevalent as important food allergens, e.g., rice in Asia and fish in Scandinavia. Although there are reports of allergic reactions to most foods, certain properties tend to make some foods more allergenic than others. These characteristic properties are water solubility, heat stability, acid stability, and glycoprotein structure with molecular weights in the range of 15,000 Da to 60,000 Da.

Clinical Manifestations

Immediate hypersensitivity reactions to foods occur primarily in the GI, cutaneous, cardiovascular, and respiratory systems. Generalized severe reactions such as anaphylaxis involve multiple systems and are often a life-threatening catastrophe. A list of the symptoms by system appears in Table 9–2.

Cutaneous Manifestations

Urticaria is one of the most recognizable symptoms of immediate hypersensitivity. Acute urticaria triggered by food proteins typically appears within minutes of the ingestion of a food. Their brief duration, typically less than 24 hours, is an important characteristic of food-induced hives. By contrast, chronic urticaria with or without angioedema is rarely due to food allergy. This most troublesome condition is often attributed to food allergy, but it generally falls to the allergist to reassure patients that chronic hives are rarely food related.

AD is more often food related, especially in young children (34–38; see also Chapter 11). As noted above, several investigators have shown that AD, especially when it is severe, is often food related and should at least be considered (14, 15, 39) However, many youngsters are sent for allergy testing for the mildest of eczematous conditions that are limited to small areas of their body. These children are much less likely than those with severe symptoms to have true food allergy.

Respiratory Manifestations

Respiratory manifestations of immediate hypersensitivity reactions include rhinitis, sneezing, cough, laryngospasm, wheezing, tightness of the chest, and breathlessness (40–42; see also Chapter 14). Ocular symptoms are often included with respiratory symptoms. In severe reactions, swelling of oral structures may occur that threaten the integrity of the airway. Although isolated respiratory symptoms do occur, they are uncommon and most often accompany symptoms in another target organ, e.g., skin or GI tract.

During food challenges, lower airway symptoms are often produced in conjunction with upper respiratory, GI, and cutaneous symptoms. The timing of these reactions clearly suggests an IgE-associated "immediate" hypersensitivity. However it also seems likely that typical "late-phase" reactions can be initiated by immediate reactions. James et al. (40) studied a population of 320 children with food allergy, asthma, and AD. Of this group, 205 children exhibited reactions during 567 positive DBPCFCs. In this highly atopic population, 27% of positive food challenges precipitated pulmonary symptoms. These youngsters all had strong evidence of IgE-associated responses. Atkins et al (41) demonstrated a probable biphasic reaction in an adult who reacted strongly to cottonseed protein during a DBPCFC (Fig. 9–1) James et al (42) found significant changes in airway reactivity following methacholine challenges in some of their food-allergic, asthmatic subjects after blinded food challenges. This study reported two intriguing findings. One finding was that chest symptoms (i.e., cough and/or wheezing) appeared in individuals whose forced expiratory volume in 1 second (FEV_1) did not change significantly; the second finding was that some individuals exhibited an increase in methacholine sensitivity despite absence of chest symptoms during the DBPCFC. The meaning and significance of this observation remains to be clarified.

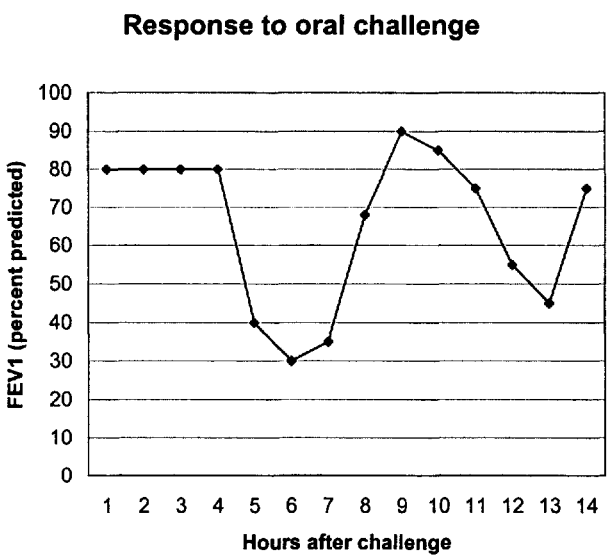

Figure 9–1. Change in FEV_1 after cottonseed protein challenge, showing a probable biphasic reaction with a fall at 4 hours and again at 10 hours.

GI Manifestations

GI symptoms of immediate hypersensitivity to foods include abdominal pain, nausea, vomiting, and diarrhea. Although colic in young children due to immediate food-related hypersensitivity is probably unusual, there are enough blinded challenge studies to present a convincing argument that it does occur (43–47; see also Chapter 16). A brief elimination diet may bring about a dramatic improvement in an infant's colic symptoms within a short time (hours to a few days), offering dramatic help to distressed families.

Recently milk allergy was implicated as a cause of constipation (48). The finding of IgE to milk protein in these children is intriguing. Although this report has some convincing data, it remains for other investigators to confirm this observation before it gains widespread acceptance.

The allergic gastroenteropathies with or without eosinophilia are discussed elsewhere (see Chapters 17 and 18); however, at least some of these conditions may involve triggering by IgE. The subsequent symptoms may involve a sequence of immunologic processes that make them look less like immediate hypersensitivity and more like a delayed (in time) process (49). In contrast, some of the presentations of GI syndromes are extremely difficult to associate with food ingestion and have no features of immediate hypersensitivity (50).

Cardiovascular Manifestations

The cardiovascular manifestations of immediate hypersensitivity are primarily hypotension and the attendant changes in the circulation, leading rapidly to circulatory failure and death if not promptly and aggressively treated. It is important to note that severe food allergic reactions may present with the patient in shock, with no other

symptoms or signs. The difficulty in recognizing the presentation may be one reason that fatal reactions to foods are underreported.

Anaphylaxis

Anaphylaxis is the most severe form of immediate hypersensitivity and can be fatal. There is not a uniformly accepted definition of anaphylaxis. Some authors include reactions that are exclusively cutaneous. Others require there to be respiratory or cardiovascular symptoms (see Chapter 15). When reading the literature it is important to know the definition being used in any given study. Recognition of the reaction is crucial in life-threatening circumstances. The list of symptoms that may occur as part of a severe allergic reaction include all those listed in Table 9–2. A recent study from multiple emergency departments illustrated some of the missing information that results in inadequate identification (51). (The study also illustrated the inadequacy of treatment in the emergency department, and the inadequacy of recommendations for treatment and evaluation after discharge.) Taken together, this information strongly supports the notion that identification and reporting of anaphylaxis to food is vastly underreported.

The characteristics of fatal and near-fatal anaphylaxis are becoming clearer (24, 25). The age range of the fatal and near-fatal cases is primarily between 15 and 35, and the sex distribution appears to be about equal. The overwhelming majority of subjects from whom there are data have asthma. Unfortunately, there are no adequate data on the status of the individual's asthma at the time of the food ingestion that resulted in death. Almost all of the patients were known to have immediate hypersensitivity to food, and the fatal ingestions were accidental. The symptoms began promptly in all subjects, although the initial symptoms were sometimes mild and tended to be ignored initially. When the symptoms progressed they became severe quickly. None of the fatal cases in the report of Sampson et al (24) received epinephrine promptly. In contrast, four of the 32 subjects reported by Bock et al (25) did receive epinephrine promptly and it was not life saving. Exactly why the epinephrine failed is not clear because it would appear that, in most situations, prompt administration of epinephrine quickly resolves the symptoms. The foods incriminated in each of the studies were nuts or peanut in the vast majority of cases. In Sampson's series, 10 of 13 subjects were allergic to peanut or tree nut, whereas in the Bock report, 30 of 32 were allergic to peanut or a tree nut. In nine very young children with food-induced anaphylaxis the predominant foods were milk and egg (peanut was not yet being consumed) (52). A study of pediatric anaphylaxis in Italy found some different features. In 95 episodes that occurred in 76 children, milk and seafood were the most important triggering foods (53). A 5-year retrospective chart review at an urban university children's hospital in the US identified 55 episodes of anaphylaxis in 50 inpatients and outpatients. Food was the second most common inciting agent after latex, but nuts, peanuts, and seafood were the foods most often implicated (54).

Some patients diagnosed with idiopathic anaphylaxis may subsequently be found to have a food trigger. In the case of a spice causing anaphylaxis, a positive skin test with a ground preparation of the food under suspicion may narrow the list of possible offenders and allow testing that might ultimately identify the causal food.

An intriguing feature of food-induced anaphylaxis is the lack of detection of serum tryptase (24). This is in sharp contrast to subjects that develop anaphylaxis due to insect stings and drug allergy, and it raises the possibility of differences in the molecular mechanisms of anaphylaxis due to different triggers. Clearly an immediate release of histamine has been measured in several studies; however, the elevation is very brief, making this determination difficult to use clinically or even in research settings (55).

Oral "Allergy" Symptoms: the Pollen-Fruit Cross-Reactivity Syndrome

The "oral allergy syndrome" (OAS) has been described with increasing frequency. It is particularly prevalent in individuals with allergen reactivity to pollens of birch, mugwort, and ragweed; however, there are a number of reports of oral symptoms with other fruits and vegetables that may "cross-react" with other inhaled allergens (56). There are also reports of foods causing oral symptoms in the absence of identified inhalant allergen cross-reactivity (57, 58). The term becomes confusing when the reported symptoms extend beyond the oral structures, tongue, lips, and mouth, and caution should be used when patients describe throat tightness, throat closing, or difficulty breathing. It has been suggested that the term "pollen-fruit cross-reactivity syndrome" would be

a more accurate term for this problem. (This subject is fully discussed in Chapter 13).

Food-Associated Exercise-Induced Anaphylaxis

Anaphylaxis following exercise and food ingestion was observed more than 20 years ago (59), although it seems to be reported with increasing frequency. There are two main patterns: 1) anaphylaxis after exercise following ingestion of a specific food, and 2) exercise-induced anaphylaxis following the act of eating, but without any particular "culprit" food being identified. In each pattern, the individual usually exercises within 2 hours of eating the incriminated food. This condition has been well described in adolescents and young adults, whereas reports in younger children remain quite rare (60). Considering the frequency of severe food allergy in school-age children, this infrequency of association between exercise and food ingestion may be due to the lack of vigorous, sustained physical activity in younger children.

Nevertheless, it is worth considering the possible association of exercise in younger children when the history is inconsistent with food alone as the trigger.

In adolescents in whom the condition has been studied and reproduced, skin testing to the foods under suspicion is quite helpful. The offending food should elicit a positive skin test response, thus helping to shorten the list of suspected culprits during the evaluation. A case report illustrates the potentially complicated nature of making the diagnosis accurately. Aihara et al (61) describe a 14 year old in whom the simultaneous ingestion of two foods followed by exercise was necessary to initiate the anaphylaxis. When each food (wheat and umeboshi, pickled Japanese plums) was consumed separately or in combination with exercise, no symptoms occurred. But when both foods were ingested and then followed by exercise, the youngster experienced difficulty breathing with a 40% drop in FEV_1, urticaria, and pruritis of the upper respiratory system. Another difficult case (62) was observed in a teenager in whom the specific food could not be determined but in which a meal containing a list of ingredients followed by exercise elicited a severe allergic response. This challenge-proven confirmation of the history without being able to find the specific food or foods is another illustration of the complicated nature of this problem and potential need for intervention in a sophisticated setting.

The mechanism remains obscure but some theories have been proposed. 1) Patients have a lower mast cell threshold for mediator release. This is suggested by the fact that increased serum histamine and increased eosinophilic cationic protein have been isolated in patients after oral food challenges and exercise. 2) There is an increased absorption and circulation of food allergens with exercise after ingestion due to increased splanchnic blood flow, leading to mast cell degranulation. This action may be potentiated by nonsteroidal anti-inflammatory agents that cause an increase in GI tract permeability, thus allowing more intact food antigen absorption. 3) Autonomic dysfunction and the ability of gastrin to cause increased cutaneous mast cell mediator release may also play a role.

Currently, management of these individuals involves the correct identification of the offending food(s), and complete avoidance of their ingestion for several hours before vigorous physical activity. Some patients are advised to avoid exercise for at least 4 hours after eating.

Diagnosis

The diagnostic evaluation of cases that may represent immediate hypersensitivity begins with the history. The history has two purposes. The first is to elicit a detailed account of the previous observations for each food. The second purpose is to ascertain enough information about the reaction to attempt to reproduce it during a food challenge. Details such as the quantity of each food, the timing to onset of the symptoms, the duration and severity of the symptoms, frequency of occurrence, most recent occurrence, and response to treatment are very important. As the history is elicited, it is essential to consider the other diagnostic categories that may represent immediate onset adverse reactions to foods that may not involve the immune system. In young children, GI symptoms that are associated with food ingestion include the intolerance syndromes due to secondary and, less frequently, primary enzyme deficiencies. It is common in all ages for lactose intolerance to be mistaken for milk allergy. In view of the familial frequency of lactose intolerance, it is worth the time to explain the difference to families and to help them understand how the proper diagnosis is determined. Other GI illnesses, espe-

cially in young children, may lead to diarrhea and be confused with food hypersensitivity. A commonly confused condition is gastroesophageal reflux disease. Frequent vomiting, spitting up, or complaints of nausea may accompany this condition. In addition, it may trigger a chronic cough and be an exacerbating feature of asthma. When a cough and vomiting are prominent, food allergy is often a concern of the families. These same symptoms rarely may be due to pyloric stenosis and gastroesophageal fistula (H-type). Distally in the GI tract, chronic diarrheal illnesses with or without abdominal pain is often attributed to food allergy. One group of these diseases is strongly associated with food proteins, and the other group is not. The former includes the protein gastroenteropathies, with or without eosinophils infiltrating the intestinal tissue (63). This spectrum includes gluten-sensitive enteropathy that is a delayed-onset illness and has not been shown to involve IgE (64, 65). The intermediate syndromes more clearly involve immune mechanisms and may involve IgE. These conditions are covered in detail in Chapter 17. The other group of disorders includes neoplasia, inflammatory bowel disease, dumping syndromes, and the pancreatic insufficiency of cystic fibrosis. Prolonged diarrhea following viral gastroenteritis is occasionally referred to allergists because of suspected immediate hypersensitivity to food proteins, but it is rarely due to immediate food hypersensitivity.

A number of toxins may mimic immediate hypersensitivity reactions to foods. Probably the most notorious is scombroid fish poisoning (66). High levels of histamine are produced in fish that have been improperly refrigerated. The ingestion of histamine may mimic anaphylaxis, with generalized flushing, prolonged and severe vomiting, and cardiovascular collapse. The fish most often responsible include tuna, skipjack, mackerel, mahi-mahi, sardines, anchovies, and herring.

Ciguatera toxin poisoning may present with tingling of the lips, tongue and throat, followed by nausea, vomiting, abdominal cramps, diarrhea, headache, and occasionally chills and fever (67). The latter symptoms should suggest diagnoses other than food hypersensitivity. This toxin is a lipid-soluble, heat-stable chemical produced by algae that are consumed by reef fish such as grouper, snapper, sea bass, and numerous other species, and is then ingested by humans when they consume these fish. Proper diagnosis is usually based on a meticulous history from the patient who can recall details of what was consumed, often months before.

Aged cheese may contain high levels of amines that mimic histamine poisoning (68). Tyramine, phenylethylamine, nitrates, and nitrites are reported to cause headache in individuals with migraine. (It is interesting to note that no systematic blinded challenges using these compounds have been reported in migraine headache subjects to confirm this commonly held belief.) The methylxanthines, theophylline, theobromine, and caffeine may be found in a number of beverages and when consumed in large amounts, may cause tremor, nervousness, and tachycardia. Chocolate contains phenylethylamine, which is identified as the cause of the headache from chocolate. The vasoactive amines epinephrine, norepinephrine, tyramine, dopamine, histamine, and 5-hydroxytryptamine are found in bananas, tomatoes, avocados, cheese, pineapples, and wine. The quantity of the food that must be consumed to cause symptoms is not clear, but symptoms from these foods are unlikely to be confused with typical immediate hypersensitivity reactions.

This chapter covers only immediate food hypersensitivity, so discussion is limited to foods that promptly trigger the onset of symptoms. Therefore, if the allergic symptoms are reported to occur daily, the allergenic food must be consumed daily. One helpful feature is the consistency with which an incriminated food triggers a symptom complex. However, food hypersensitivity reactions are subject to a dose/response relationship. Usually a low dose of allergen is sufficient to trigger allergic symptoms, although exceptions to this principle occur when the threshold of reactivity is relatively high. Although this is generally not the case for tree nuts and peanuts, where minute quantities will trigger the symptoms, it may be true for dairy products, especially as younger children lose their reactivity to cow's milk protein. For example, children who have symptoms from drinking a cup of milk may tolerate milk-containing foods such as pancakes or cookies. Sampson and colleagues have begun to identify two groups of cow's milk-allergic subjects that may have genetic differences in their reactivity and ability to "outgrow" the problem. The first group has "transient" milk allergy, and the second has a "persistent" allergy and typically is identified as being older and having more severe symptoms (69–71).

Other factors may occasionally be associated with inconsistent histories of reaction. These factors may include reactions to the more labile fruit and vegetable proteins that are susceptible to the heat of cooking. Reactions to these foods have

been noted more often in some studies in older children (72). Recently it was found that the nature of cooking can affect peanut allergens, and that boiling peanuts may result in less allergenicity than roasting them, as is more common in the US (73). This finding may account of the lower incidence of peanut allergy in Asia, where peanuts are boiled. There may occasionally be delayed absorption of some food proteins or effects on absorption from alcohol and medication. It may be possible for antihistamine consumption to affect the presence of oral and cutaneous symptoms. However, the authors have seen many children exhibit significant reactions to accidental food allergen ingestion even though they were taking antihistamines. As noted above, exercise can be a significant confounding factor.

Foods may be cross-contaminated with other foods and erroneously implicated when another trigger is responsible (74). For example, if fish is cooked in soy oil, the fish protein is concentrated in the oil and then coat French fries cooked in the same oil with fish protein. There are several reported cases of non-dairy dessert products becoming contaminated with cow milk when the common machinery used in preparation of the two products was not adequately cleaned between the manufacture of the dairy and the non-dairy foods (75). Contamination with allergenic proteins from utensils has been implicated in severe allergic reactions, e.g., when a knife used to spread peanut butter is dipped into the jelly jar, making the jelly unsafe for a peanut-allergic family member. Similar examples abound, and these potential problems must be communicated to families as part of the avoidance education process.

If IgE-mediated hypersensitivity is suspected after obtaining the history and performing the physical examination, skin testing to suspected foods or the most common food allergens may help determine the probability that a food is provoking immediate hypersensitivity. Skin tests are most helpful when they are negative, because they virtually exclude IgE-mediated allergy. Studies from major centers have demonstrated that SPT using allergen extracts of concentrations of 1:10 to 1:20 weight/volume are a very accurate means of detecting the presence of IgE antibodies to food (76–79). It is important to explain to patients that positive skin tests do not mean that the patient will experience an allergic reaction to the responsible allergen (e.g., food, pollen, dander), they simply detect the presence of allergen-specific IgE antibodies. More than 60% of individuals with positive skin tests will be able to ingest the food without experiencing symptoms.

Skin tests to be performed should be based upon the patients' histories. Rarely is a large panel of food skin tests required or desirable, and may more often lead to confusion in highly atopic children. For most fruits and vegetables, skin testing with both commercial extracts and fresh foods is necessary and will increase the utility of food skin testing (80). In cases where an isolated food ingestion leads to the prompt onset of allergic symptoms, and no other explanations are possible, a positive food skin test may be considered diagnostic. It is rare that this scenario will lead to the unnecessary removal of a food from the diet.

It has been suggested that foods applied to the oral mucosa and/or to the lip may help to accurately diagnose food hypersensitivity and eliminate the need for food challenges (81). Individuals experiencing oral symptoms from a food will almost always stop eating that food immediately. They often remove the food from their mouths. Further research in this area may confirm the utility of labial challenges, but some individuals have been found to have oral symptoms from contact of a food with oral tissues, but they do not develop systemic symptoms when the food is ingested or mixed with another food (82). Some individuals experiencing mild oral symptoms may not have systemic symptoms if the food continues to be consumed.

Over the last three decades, available in vitro testing methods (RAST, enzyme-linked immunosorbet assay [ELISA], and basophil histamine release assays) have been found to have comparable sensitivity to skin testing, but more expensive and time consuming. A modification of the RAST, the clinical assay Pharmacia (CAP) fluorescent enzyme immunoassay, is an assay quantitating food-specific IgE that can be used to diagnose immediate hypersensitivity to a few foods (milk, egg, peanut, and fish) without performing challenges (83–86; see also Chapter 7). Despite a thorough history, it is not uncommon that the food causing allergic symptoms is not apparent. In fact, it may not be clear that food hypersensitivity is the problem. In this situation, elimination diets may be more useful than in vivo or in vitro testing. Strict elimination diets, "oligoantigenic" (few food) diets, or even elemental diets will bring about resolution of the symptoms if the patient's usual diet contains the responsible food. It is important to counsel the patient and explain the purpose of these diets. If the patient has some notion of the

timing between meals and the onset of symptoms, this is useful information. However if the patient is uncertain, then the physician and patient must at least agree on how long to perform the diet to rule out potential allergy. Ordinarily elimination diets should last no longer than one or two weeks, but a few GI hypersensitivities require more prolonged diets to bring about resolution of the symptoms (50). If an elimination diet is to last longer than 2 weeks, a dietitian should be consulted to assure that the diet is nutritionally adequate. Elemental diets may be quite definitive but are much more difficult for patients to accept. It requires a very motivated patient (or family) to truly adhere to an elemental diet, which is usually a liquid diet with one or two foods included to make it tolerable.

If the elimination diet makes a significant difference in the symptoms of concern, and the child has not experienced anaphylactic symptoms and there is no danger of a severe reaction, then it is reasonable to return to the normal diet. Symptoms should return when the normal diet is resumed if a food is responsible. If symptoms do not return, the problem has resolved and no explanation may ever be found. However, if the symptoms do return, then it is possible to conclude that some food or foods (rarely more than a few) are triggering the symptoms. See Chapter 8 for further discussion of dietary intervention and challenges. Food challenge remains the only definitive way to be certain that an incriminated food is truly causing symptoms.

The DBPCFC remains the gold standard for research and allows comparison with other tests that have been developed and will be developed in the future (82, 87–94). In practice, open challenges are useful to rule out foods that have been suspected of causing problems. Single-blind challenges are useful in the office and clinic setting especially for the evaluation of immediately hypersensitivity complaints. If there is a severe reaction with a prompt onset of symptoms in a typical pattern (hives, vomiting, severe respiratory symptoms), then challenge to that food will not be needed. For objective symptoms such as urticaria, upper respiratory symptoms, asthma, or prompt-onset vomiting and diarrhea, a single unequivocal positive result during blinded food challenge is strong evidence of confirmation of the history. For subjective symptoms, it is necessary to demonstrate a number of positive active challenges and negative placebo challenges to substantiate the diagnosis (95). A positive challenge only indicates the presence of an adverse reaction to a food; it does not necessarily identify the mechanism. Challenges are an important component of the allergist's arsenal of diagnostic tools but must be undertaken with caution in case a severe reaction should occur. Allergists equipped to treat severe reactions to allergy injections should already have the appropriate equipment for treating anaphylactic reactions (96, 97)

Natural History and Treatment

Treatment of immediate food hypersensitivity has two components. The first component involves prevention of further reactions, and the second involves treatment of acute reactions. At this time the only proven approach to treat food allergy is avoidance of the offending food(s). This may be accomplished with continuous education of children, families, and those who care for the allergic child. Special diets other than elimination of the documented food have not been shown to be safe. Occasionally a so-called rotation diet will appear to be successful because the patient may have a high threshold of reactivity and the amount of the food on any given day is below this threshold. There is no evidence that this is a safe approach, and it may delay the loss of reactivity in some children with milk allergy and perhaps allergy to other foods.

At this time there are no medications that will prevent reactions in food-allergic individuals. Antihistamines may mask some oral and cutaneous symptoms, but in general are insufficient to dramatically alter allergic reactions. Well controlled studies evaluating cromolyn sodium (Gastrochrom) for the treatment of food allergy do not support its use, despite some anecdotes that have been published (98).

Natural History

As discussed more thoroughly in Chapter 32, studies of the natural history of food allergy have shown that for many foods, clinical reactivity is lost over time. It appears that younger children are more likely to lose their reactivity, and that it is more likely to be lost to milk, egg, soy, and wheat than to peanuts, tree nuts, or seafood. In a group of children with atopic respiratory disease, 44% lost their food hypersensitivity over 1 to 7 years by historical report (99). Similarly, 37 youngsters with GI symptoms due to milk hypersensitivity were studied by food challenges one and four years after the initial diagnosis, and a loss of reactivity was found in 12 of 37 (32%) (100). In a study of

children with AD and food hypersensitivity, 19 (25%) of 75 children lost clinical reactivity when rechallenged after 1 year on a diet eliminating the challenge positive foods. After 2 years on elimination diet, 23 (31%) of the children had lost their symptomatic reactivity to all allergen that previously had elicited positive clinical responses (101). It is of note that many children lose their clinical reactivity but maintain food-specific IgE as detected by SPT, which makes skin testing of little use for monitoring ongoing clinical reactivity. Recently, it was found that a small proportion of children with peanut hypersensitivity lose their reactivity (102, 103). Up to 20% of children in one study seemed to lose their reactions to peanut (104). Studies are under way to determine whether youngsters who outgrow peanut allergy have certain characteristics that predict this probability. The only clear factor appears to be young age. It appears rare that children who react to peanut after age 5 "outgrow" their reactivity. In contrast to other food allergies, a recent study indicated that 3 of 30 children who appeared to have "outgrown" peanut allergy redeveloped allergic symptoms (105).

Immune Modification

Immunomodulatory therapy for IgE-mediated food allergy is under development (see also Chapter 42). Prototype studies with extracts of peanut have been performed in animal models. Previous attempts to use whole peanut allergen preparations were successful in reducing the clinical and immunological reactivity of peanut-allergic individuals, but the side effects were too severe for this to be a practical approach (106, 107).

Investigators are studying new concepts for desensitization in food allergy. One approach provides a global strategy for treating IgE-associated food allergy. This approach involves the use of humanized anti-IgE antibody therapy. This form of therapy has the advantage of treating all inhalant and food sensitivities, regardless of allergen specificity. Early studies of anti-IgE therapy in asthma and allergic rhinitis (108–111) and peanut allergy (112) show great promise. Whether anti-IgE therapy will eventually allow food-allergic subjects to ingest foods to which they have reacted, or whether it will only reduce their reactivity enough to make accidental ingestion less hazardous, will be the subject of ongoing investigations.

Symptomatic Treatment

When a reaction does occur, the treatment chosen depends on the severity of the reaction. Severe bronchospasm, laryngospasm, or cardiovascular collapse (anaphylaxis) require prompt intramuscular administration of epinephrine, and there is no good substitute at this time. Recent studies have shown the importance of injecting the epinephrine intramuscularly preferably in the lateral thigh. Simons et al (113–116) have made several important contributions to the understanding of dosage and route of epinephrine that should be used in children (and adults), and have demonstrated the need for additional dosage levels for infants and younger children. They have also demonstrated that inhaled epinephrine is not an adequate substitute for injected epinephrine (117). A growing list of references shows the inadequacy of both physician/medical personnel training and patient instruction in this area. The importance of adequate training and adequate response plans is crucial (118–120). Adding bronchodilators, anti-cholinergic medication, and antihistamines will be helpful, but it appears that only epinephrine is life-saving.

Conclusion

Immediate food hypersensitivity is a significant health problem for a large number of individuals throughout the world. The mortality from food-induced anaphylaxis is tragic and should be preventable. Improved education in this field of both the medical professional and the public in general must become a higher priority. Fortunately, research that will lead to treatments that improve quality of life and protect some lives is promising, and the future in this area appears bright.

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10

Food Allergy in Adults

Dean D. Metcalfe

Introduction

Adverse reactions to food and drink have appeared in writings for centuries. Not until 1921, however, did Prausnitz and Küstner demonstrate that the factor responsible for an "allergic" reaction to a food was present in serum and could be transferred to a nonsensitive individual (1). This observation was the basis of the PK test (no longer performed), which is now understood to involve the passive transfer of IgE. In 1950, blinded and placebo-controlled food challenges on individuals suspected of having mild food allergy were reported (2). The physicians conducting these tests were among the first to correlate clinical reactions to foods to abnormal in vivo responses, which are now, for the most part, accepted as having an immunologic basis.

Food allergy (hypersensitivity) is an abnormal reaction resulting from heightened immunologic responses to glycoprotein components within foods, referred to as "allergens". The term "food intolerance" is often applied to any abnormal response to an ingested food, whether immunologic or not. Food intolerance may also result from a toxic or pharmacologic property of a food; alternatively, it may represent an abnormal metabolic response of the host to a food component.

True food hypersensitivity in adults can be divided into two general subgroups on the basis of the presumed immunologic mechanisms involved: food allergen-specific IgE responses, and non-IgE-dependent immunologic responses corresponding, respectively, to immediate and delayed-in-time reactions (Table 10–1). IgE-dependent reactions are further classified as to symptom complexes developed in the primary target organs. Delayed reactions in adults do not include food protein-induced colitis and enterocolitis, because such diseases have been described in infants and children but are not generally recognized in the older population. Whether such pathologic entities exist in adults but are hidden within the spectrum of inflammatory bowel disease remains to be demonstrated.

Prevalence

Data on the prevalence of food hypersensitivity reactions in specific adult populations are available. The public's perception of the number of food-allergic individuals is clearly far greater than controlled studies have demonstrated. As an example, a Dutch study examining the prevalence of food allergy and food intolerance in adults using questionnaires, clinical follow-ups and double-blind, placebo-controlled food challenges (DBPCFC) estimated the prevalence of food allergy and intolerance together to be 2.4% (3). The prevalence of tree nut and peanut allergy as determined in the U.S. by random digit dial telephone survey was found to be 1.1% of the general population (4). The prevalence of food additive intolerance appears to be even lower, an estimated 0.01% to 0.23% (5).

IgE-Mediated Immediate Reactions

IgE-mediated immediate hypersensitivity reactions to food occur rapidly following ingestion or inhalation of a food antigen to which an individual is sensitive. A number of target organs may be affected. Reactions include urticaria, angioedema, rhinoconjunctivitis, laryngeal edema, asthma, oral

Table 10–1.
Categorization of Immunologically Mediated Reactions to Foods in Adults

Disease	*Primary Target Organs*	*Effector Systems*
I. Immediate Reactions		
Rhinoconjunctivitis	Eyes, upper respiratory tract	IgE: Basophils/mast cells
Oral allergy syndrome	Mouth	IgE: Mast cells
Urticaria/angioedema	Skin	IgE: Basophils/mast cells
Atopic dermatitis	Skin	IgE: Mast cells, eosinophils
Asthma	Lower respiratory tract	IgE: Mast cells, eosinophils, lymphocytes
Gastrointestinal reactions	Gastrointestinal mucosa	IgE: Mast cells
Systemic anaphylaxis	Skin, respiratory tract, gastrointestinal tract, cardiovascular system	IgE: Basophils/mast cells
II. Delayed Reactions		
Allergic eosinophilic gastroenteritis	Gastrointestinal mucosa, submucosa	IgE: Mast cells, eosinophils, lymphocytes
Gluten-sensitivity enteropathy	Gastrointestinal mucosa	IgA, IgG: Lymphocytes
Dermatitis herpetiformis	Skin	IgA: Lymphocytes, neutrophils

allergy syndrome (6), vomiting, diarrhea, and systemic anaphylaxis (Table 10–1). In some instances, urticaria or anaphylaxis depends on a co-stimulus to exacerbate symptoms, such as in food-dependent exercise-induced anaphylaxis (7–10). The presumption is that the threshold for mast cell activation is lowered by the accessory stimulus and allergen absorption is increased, although no data have been gathered as yet to support these hypotheses.

Pathogenesis

Mast cells, basophils, and IgE all function in immediate hypersensitivity reactions to food. A rise in plasma histamine has been associated with the development of urticaria, laryngeal edema, wheezing, vomiting, diarrhea, and hypotension after blinded food challenges (11). Elevated levels of serum mast cell tryptase have been reported in association with fatal anaphylaxis induced by food (12). Interaction between IgE on mast cell surfaces with food extracts has been demonstrated by immediate wheal and erythema reactions in the dermis after local injection of these extracts. Gastrointestinal (GI) mucosal reactions similar to those that follow mast cell degranulation in human skin have been reported in vivo (13, 14). Intraluminal administration of food antigens in sensitive individuals leads to a rapid increase in histamine and tryptase (14).

Upon high-affinity IgE receptor (FcεRI) aggregation, mast cells exert their effects on tissues by releasing preformed mediators such as histamine, tryptase, and tumor necrosis factor (TNF), as well as newly synthesized arrays of mediators such as prostaglandins, leukotrienes, and cytokines that may contribute to the IgE-mediated late-phase response (15, 16). Basophils also release histamine and tryptase in response to FcεRI cross-linking (17). Mast cells additionally appear to modulate, even down-regulate, subsequent inflammatory events through the synthesis and release of such molecules as interleukin-1 receptor antagonist (IL-1ra) (18).

Increased intestinal mucosal permeability following mast cell activation facilitates the entry and distribution of a food antigen to other target organs, initiating degranulation of mast cells at those sites. Nevertheless, an alteration in mucosal permeability is not necessary and does not serve as the underlying defect in food allergy. This observation is consistent with clinical data that have failed to demonstrate significant defects in IgA synthesis, to find associated GI diseases including achlorhydria, or to generate convincing evidence of immune complex diseases in patients with immediate reactions to foods.

The most reliable clinical correlates of immediate reactions to foods are a family and personal history of atopy and the presence of positive skin tests to foods and inhalants. This evidence indicates that the basis of the production of IgE in response to foods relates to inherited patterns of IgE synthesis and regulation. Patients with IgE-mediated food allergy, however, do not consistently give a family history of hypersensitivity to specific foods, suggesting that the development of IgE directed to food antigens is multifactorial. Environmental exposure to antigens appears to contribute to the amplitude of the IgE response (19). Other influences include viral infections and exposure to environmental adjuvants.

Allergens in Foods

Almost every major food allergen identified is a protein or glycoprotein with a molecular weight between 10 and 40 kilodaltons (kDa). These allergens tend to resist denaturation by heat or acid and degradation by proteases. In adults, the most common food allergens causing systemic reactions are peanuts, tree nuts, crustaceans, fish, and egg (20).

Peanuts are one of the most allergenic foods. They are responsible in many reports of fatal and near-fatal anaphylaxis (21, 22). Peanuts exhibit a complex makeup of over 32 different proteins. Three major allergens identified are Ara h1, Ara h2, and Ara h3 (23, 24). These proteins are relatively resistant to heat. Soybeans are also a major crop of the legume family and contain allergenic components. In one instance, an allergic reaction to soybeans was traced to a reaction to Kunitz soybean trypsin inhibitor (25). Other members of the legume family include a variety of green beans, kidney beans, garbanzo beans, garden peas, black-eyed peas, and lentils; significant antigenic cross-reactivity among these members of the family has been demonstrated in vitro and by skin test reactivity. Results of DBPCFC, however, demonstrate that clinically important cross-reactivity to legumes is rare (26). Clinical hypersensitivity to one legume does not warrant dietary elimination of all legumes in the absence of clinical sensitivity.

Shrimp antigens have also been isolated that are allergenic in some individuals (27–30). Pen a 1 is a 36 kDa muscle glycoprotein and is the major allergen in shrimp, making up about 20% of shrimp soluble protein. A similar protein from Indian shrimp is termed Pen i I. Amino acid sequence analysis indicates it has significant homology with the muscle protein tropomyosin from *Drosophila melanogaster* (28, 29). T cell epitopes have been described. Tropomyosin is identified as the allergen responsible for cross-reactivity among some crustaceans (shrimp, lobster, crab, and crawfish) (30), and cross-reacts with cockroach tropomyosin (31).

Fish are among the most common causes of food-allergic reactions in adults (20). Allergen M (Gad c I) from codfish, the first extensively studied allergen, is a parvalbumin found in the muscle of fish and amphibians. Gad c I is a heat-stable, partially protease-resistant, single-polypeptide-chain protein with a molecular weight of approximately 12 kDa (32–34). Synthetic peptides corresponding to amino acids 13–32, 49–64, and 88–103 have been shown to bind IgE from cod-allergic subjects (35). One study utilizing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analyses showed immunologic cross-reactivity between Gad c I and 10 other common fish species (36). In this study, two of 11 patients who had multiple positive skin tests to various fish reacted to three fish upon DBPCFC, and one patient reacted to two fish. Unlike many other food allergens, fish allergens appear to be relatively susceptible to degradation during food processing (37). Patients who are allergic to fresh-cooked tuna and salmon sometimes tolerate canned tuna and canned salmon.

Chicken egg may also cause food allergic reactions in adults. The egg albumin is more allergenic than egg yolk. The major egg allergens appear to be ovalbumin, ovomucoid, and conalbumin (38–42). Ovomucoid is heat-stable. In some individuals, IgE can be found that is directed to egg yolk proteins (43). Cross-allergenicity has been demonstrated among eggs from different birds (43).

Clinical Patterns of Target Organ Responses

IgE-mediated immediate reactions to ingested food may involve one or more target organs, including the skin, respiratory tract, GI tract, and cardiovascular system. Oropharyngeal reactions, which are often observed first, are typically characterized by pruritus and urticaria in and around the mouth. Uvular edema may also be present. Only rarely does the initial exposure to antigen result in obstructed airflow. This development of oropharyngeal symptoms with food ingestion, termed the oral allergy syndrome (OAS), is most commonly the result of ingestion of fresh fruits and vegetables (6, 20). Patients sensitive to birch pollen have been reported to develop the syndrome after the ingestion of raw potatoes, celery, carrots, apples, hazelnuts, and kiwi fruit (6, 44), and ragweed-sensitive patients may develop symptoms after contact with melons and bananas (6).

GI manifestations of immediate food reactions are commonly seen and may sometimes be the only problem noted. Entry of the food allergen into the stomach and the intestine may result in nausea, vomiting, cramping pain, abdominal distention, flatulence, and diarrhea.

Systemic anaphylaxis is the constellation of signs and symptoms that result from systemic IgE-mediated mast cell and basophil activation. It oc-

curs within minutes, but occasionally has been reported to occur hours after ingestion of an offending food (45). Patients develop pruritus, urticaria, angioedema, laryngeal edema, bronchospasm, cramping abdominal pain, diarrhea, hypotension, vascular collapse, and cardiac dysrhythmias. Early recognition of the problem and prompt intervention are the keys to treatment. In one study, a common occurrence was failure to administer epinephrine immediately (21). Systemic anaphylactic reactions in adults are most often associated with the ingestion of peanuts, tree nuts, fish, and crustaceans. Asthmatics are at high risk.

In addition to foods, other factors such as exercise may be necessary to uncover a reaction. Anaphylaxis has been reported following ingestion of certain foods such as shrimp, wheat, and celery before exercising (46–49). In some patients, attacks are associated with meals, but no specific foods are implicated.

Cutaneous manifestations of IgE-mediated food allergy, which include acute urticaria and angioedema, are common. Chronic urticaria is much less frequent. In one report of 554 adults with urticaria, food allergy was demonstrated in only 1.4% (50).

Respiratory symptoms including asthma have been observed during oral challenge with suspected foods. Although rhinitis and asthma are frequently suspected to have a food-related origin, the frequency of such reactions appears to be low (51) and rarely occurs without other organ involvement (52, 53).

Relatively few studies have analyzed immediate reactions to foods in adults and/or followed protocols that examine the entire clinical picture. In one comprehensive study, however, 45 patients with a history of immediate reactions to foods were evaluated by history, physical examination, laboratory studies, skin testing, and DBPCFC (52, 53). Of the patients studied, 56% reported that as many as three foods could elicit a reaction. Allergic reactions to foods had an average age of onset of 19.8 years. All occurred in patients with an allergy history, although few had histories of reactions in childhood. Most reactions had persisted over an average of 14.8 years. Reactions generally involved the GI tract alone or in combination with the skin or respiratory tract. Twenty-five patients participated in DBPCFCs. Positive challenges were observed most frequently to crustaceans and peanuts. Doses of challenge foods provoking symptoms ranged from 5 g to 100 g. The clinical signs and symptoms noted on food challenge reproduced those reported by history. Reactions were usually mild and self-limited.

Atopic Dermatitis

Atopic dermatitis (AD) is an inflammatory condition of the skin that is characterized by a chronic relapsing course, extreme pruritus, age-related dermal distribution patterns, and association with allergic rhinitis and asthma (54, 55). The role of food hypersensitivity in AD has been investigated primarily in children. Approximately one third of children with AD seen in university dermatology and allergy clinics have been observed to have food hypersensitivity contributing to their skin symptoms (54). Far fewer adults with AD appear to benefit from food elimination diets, although comparable studies to those done in children need to be performed in adults.

Diagnostic Tests

The diagnosis of an immediate IgE-mediated reaction to a food depends on the history, appropriate exclusion diet, skin testing or antigen-specific IgE in vitro tests, and blinded provocation. Food-specific IgG or IgG4 antibody levels, and food antigen-antibody immune complex measurements appear to have no convincing diagnostic value in predicting or verifying immediate food hypersensitivity.

In Vitro Tests

Examples of in vitro tests to measure antigen-specific IgE include the radioallergosorbent test (RAST), the enzyme-linked immunosorbent assay (ELISA), and the basophil histamine release assay. In vitro tests are the procedure of choice when medications would interfere with skin testing, when skin disease is so extensive as to preclude skin testing, or when skin testing could place the extremely sensitive individual at risk (56). Although modifications of the RAST have been developed, all involve antigens coupled to a solid phase such as a paper disk. Patient sera are allowed to react with this solid phase. The amount of bound antigen-specific IgE antibodies is calculated by adding labeled anti-human IgE antibodies. The ELISA is a variation of this methodology. Antigen may be quantified using these assays by measuring the ability of an extract to inhibit binding of specific antibody to the solid phase.

In Vivo Tests

Skin testing with food extracts provides a simple and reliable method of demonstrating food allergen-specific IgE antibodies. The negative predictive value of food allergen skin testing is greater than 95%, whereas the positive predictive value is low (56). Skin testing to food antigen is usually performed by the skin prick test, in which a drop of 1:10 or 1:20 glycerinated food extract, a positive histamine control, and a negative saline control are placed on the skin. The skin is then punctured through the drop with a sterile needle. Skin testing using appropriately diluted extracts may be performed by the intradermal technique. Such testing is more likely to produce clinically irrelevant positive tests (57), however, and is associated with a higher frequency of systemic reactions than is observed with the skin prick test. IgE-mediated food allergy is unusual when skin tests prove negative. The use of extracts of fresh fruits and vegetables is often necessary to exclude the oral allergy syndrome in cases of labile allergens.

Oral Challenge

DBPCFC is another diagnostic procedure used to evaluate a variety of food-related complaints and clinical correlations of food allergy (58). Suspect foods should be eliminated for 10–14 days before DBPCFCs, and antihistamines should be discontinued and other medications minimized. The food challenge is administered in a fasting state, starting with a dose unlikely to provoke symptoms. The dose is then doubled every 30–60 minutes (or more), depending on the type of reaction suspected and the length of time required to produce symptoms. After the patient has tolerated 10 g of lyophilized food blinded in capsules or liquid, the food should be given openly in usual quantities under observation to verify lack of sensitivity (58). A randomized challenge with an equal number of placebo and food antigens is necessary to control for a variety of confounding factors. DBPCFCs should be conducted in a clinic or hospital setting only if trained personnel and equipment for treating systemic anaphylaxis are present, and only with the patient's informed consent. Patients with a convincing history of systemic anaphylaxis to a specific food should not be challenged with that food. In adults, blinded challenge may be used to convince a patient that the food of concern to them does not provoke symptoms.

Management

Strict elimination of offending foods is the mainstay of therapy, as it lowers the risk of immediate food hypersensitivity reactions. Severe elimination diets may lead to malnutrition and should be instituted with nutritional guidance. Patients must be instructed on how to read food labels to detect hidden food allergens. In addition, patients with specific food allergies must exercise extreme caution when eating outside the home, and especially at restaurants. Even after a patient asks restaurant employees about the presence of a specific food, experience demonstrates that a reaction can still occur because the ingredients are not known or recognized by the chef. No appropriately designed trial has demonstrated clear efficacy and indications for the use of prophylactic medications or oral desensitization in the management of allergic reactions to foods.

The treatment of food-induced anaphylaxis is essentially the same as that for anaphylaxis due to a medication or insect sting (59). A patient with potential anaphylactic reactivity must be taught to self-administer epinephrine, and must keep an epinephrine-containing syringe and an antihistamine available at all times. A patient may sometimes inadvertently ingest a food to which he or she is sensitive. Laryngeal or pulmonary symptoms appearing after such an inadvertent food exposure should be treated immediately with epinephrine or bronchodilator therapy, or both. Patients may exhibit only mild symptoms in the first few minutes after ingestion, followed 10–60 minutes later by hypotension and other severe problems. After self-medication for a systemic reaction, the patient should immediately seek medical attention.

In addition to food allergen elimination for AD, control of pruritus may be attempted with antihistamines and/or a brief application of a topical steroid in severe cases. Maintenance of skin hydration is an important component of therapy. When an infection is suspected, an anti-staphylococcal antibiotic may be prescribed.

Delayed Reactions to Foods

Allergic Eosinophilic Gastroenteritis

Allergic eosinophilic gastroenteritis is a relatively rare chronic disease that is characterized by peripheral eosinophilia and eosinophilic infiltra-

tion of the stomach and/or the small intestine. It is seen more frequently during the third decade of life. Clinical symptoms reflect the extent of eosinophilic infiltration in the bowel wall. When a significant eosinophilic infiltration is present in the mucosa, symptoms correspond to those of malabsorption. The clinical picture resembles that seen with obstruction if the infiltration predominantly occurs in the muscular layer. Serosal involvement produces ascites containing eosinophils.

The immunopathogenic mechanisms involved in allergic eosinophilic gastroenteritis remain unclear. The disease usually appears to be associated with severe food allergies that lead to repeated hypersensitivity reactions in the GI mucosa. Patients often are atopic, have an elevated serum IgE level, and have specific IgE antibodies for 15–40 food antigens (60). T cells from patients with this disease produce high amounts of IL-4 and IL-5, which are Th2-type cytokines (61). γ-interferon messenger RNA could not be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in biopsies from patients with allergic eosinophilic gastroenteritis, but was present in normal biopsies (61).

The current treatment for eosinophilic gastroenteritis is often unsatisfactory. Dietary avoidance of incriminated foods may be tried, but the number of foods involved may preclude the long-term use of such a restrictive diet. Patients who respond poorly to dietary restrictions, and those without evidence of food hypersensitivity, may require oral corticosteroid therapy. Although this course of action usually results in clinical improvement, long-term treatment with corticosteroids is frequently required (60).

Gluten-Sensitive Enteropathy (Celiac Disease)

Gluten-sensitive enteropathy (GSE), also known as celiac disease (CD), celiac sprue, or idiopathic sprue, is a disease of the small intestine that is precipitated by cereal grains that contain gluten. CD is associated with certain HLA genes, including the HLA-A1, -B8, -DR3, and -DQ2 haplotypes (61, 62). The onset of symptoms, which may consist of intermittent diarrhea, abdominal pain, and irritability, typically occurs 6–12 months after introduction of gluten into the diet. Mucosal injury may lead to malabsorption with a clinical picture including steatorrhea, weight loss, peripheral edema from protein loss, anemia, bleeding diathesis, and tetany. A subsequent increase in the incidence of GI lymphoma or carcinoma has been reported (63). The acute reaction of the intestinal mucosa consists of edema, an increase in vascular permeability, and an eosinophil and neutrophil infiltration. The gliadin in gluten induces disease, and consists of proline- and glutamine-rich proteins in the alcohol/water-extractable portion of gluten (64). Peptides containing the tetramer sequence QQQP of PSQQ (where Q is glutamine, P proline, and S serine) are most suspect (65). Blunting of the mucosal surface, villous atrophy, and a dense infiltration of the lamina propria with plasma cells, B cells, and T cells is observed in chronic disease. CD lesions produce large amounts of Th1 cytokines (66).

Diagnosis depends on biopsy evidence of small intestinal mucosa injury upon gluten challenge. IgA antigliadin is quite specific for CD, particularly if present at high titer. Treatment is directed at elimination of gluten from the diet (e.g., elimination of gluten-containing wheat, barley, rye, and oats). Improvement in symptoms is seen as soon as 2 weeks after the institution of a gluten-free diet, although histologic improvement may take 2–3 months. Strict GI rest, and in some instances the use of steroids, is necessary to suppress diarrhea in cases of severe inflammation.

Dermatitis Herpetiformis

Dermatitis herpetiformis is a chronic papulovesicular skin disorder frequently associated with asymptomatic gluten-sensitive enteropathy. The histologic appearance of skin lesions resembles a granulocytic infiltration at the dermoepidermal junction associated with edema and blister formation. Granular IgA deposits with associated J-chains are found in the papillary dermis. Complement-mediated injury is implicated. The histology of intestinal lesions is similar to CD, although it is generally less severe.

Skin lesions are symmetrically distributed on extensor surfaces of elbows, knees, and buttocks. Many patients have little or no GI complaints. The diagnosis rests on the typical appearance of these skin lesions and histologic findings of IgA deposits in the perilesional or uninvolved skin. Fifteen percent of patients will have a normal small intestinal mucosa on histologic evaluation. Treatment consists of the removal of gluten from the diet and the use of dapsone or sulfapyridine.

Natural History of Food Allergies

Some adults (67) may lose their sensitivity if the responsible food allergen is completely eliminated from the diet. Thus, after 1–2 years of allergen avoidance, as many as one-third of children and adults lose their clinical sensitivity (67, 68). Patients with an allergy to peanut, tree nut, fish, or shellfish less commonly lose their clinical reactivity, however (67–69). Loss of sensitivity correlates with allergen avoidance. Reintroduction and repeated exposure to the same allergen will cause the sensitivity to reappear in some individuals.

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Eczema and Food Hypersensitivity

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Atopic dermatitis (AD) is a form of eczema that generally begins in early infancy and is characterized by extreme pruritus, chronically relapsing course, and a distinctive distribution. The rash is generally an erythematous, papulovesicular eruption, frequently with weeping and crusting in early life, and that progresses to a scaly, lichenified rash over time (1). The distribution of the rash typically varies with age (2), involving the cheeks and extensor surfaces of the arms and legs in infancy, the flexor surfaces in the young child, and flexor surfaces, hands, and feet in the teenage patient and young adult. Unlike most dermatoses, AD has no primary skin lesion, but is identified by a constellation of symptoms. The diagnostic criteria of Hanifin and Rajka (3) provide an internationally accepted standard for diagnosing AD, and the SCORAD Index (4) developed by the European Task Force on Atopic Dermatitis provides a standardized method for gauging severity.

Libellus de Aegretudinibus Infantium (*Handbook of Diseases of Children*), a pediatric textbook written in 1472 by the Italian physician Paolo Bagellardo contains the first known scientific discussion of eczema. The chapter on skin provides advice on lubrication of skin and prevention of scratching in children with this skin disorder. In the early 1600s, Helmont discussed the association between a pruritic skin rash and asthma, and in 1884, von Hebra described the flexural distribution of a chronic itchy skin disease (5). Besnier, a French physician, is credited with the first comprehensive description of AD nearly a century ago (6). He emphasized the hereditary nature of this disorder, its chronically recurring course, and its association with hay fever and asthma. Initially the disorder was called "prurigo diathesique," but it later became known as "prurigo Besnier." Wise and Sulzberger (7) further emphasized the relationship between atopic eczema, asthma, and hay fever by coining the term "atopic dermatitis," the term generally used today. The incidence of AD has been increasing over the past 40 years and now is estimated to affect between 10% and 15% of the pediatric population (8, 9).

The pathogenic role of allergy in AD has been debated since Besnier's original description. A number of observations suggested a significant role for IgE-mediated mechanism(s) in AD: 1) Approximately 80% of children have positive immediate skin tests and radioallergosorbent tests (RASTs) to various dietary and environmental allergens (10); 2) Eighty percent to 90% of children have elevated serum IgE concentrations (11); 3) Sixty-five percent to 85% of patients have a positive family history of atopy (12); 4) Fifty percent to 80% of children develop other atopic disorders such as allergic rhinitis and asthma (10); 5) The development of eczema has been noted in recipients of bone marrow transplants from atopic donors (13), and eczema resolves in patients with Wiskott-Aldrich syndrome after successful bone marrow transplantation and engraftment (14). In a recent prospective study of more than 559 Australian infants at "high risk" for atopic disease, Hill and coworkers (15) found a 24% prevalence of AD, and a calculated attributable risk percent of IgE sensitization as a cause of AD of 65% and 64% at 6 months and 12 months of age, respectively. In infants with severe AD, the calculated attributable risk percent of IgE sensitization as a cause of AD was 83% and 65% at these same time points.

Immunopathogenic Role of Allergy in AD

The pathogenic role of IgE-mediated hypersensitivity in AD is further supported by studies delineating the immunopathogenic role of the IgE-mediated cutaneous late-phase response and of the IgE-bearing antigen-presenting cell (APC), especially Langerhans' cells and dendritic cells (DCs), in establishing the Th2 lymphocytic response (12). Before studies utilizing in situ cytokine determination, skin biopsies from patients with AD were thought to be inconsistent with an IgE-mediated mechanism, because they revealed a nonspecific dermatitis characterized by a lymphocytic infiltrate, which appeared indicative of a classic Type IV, cell-mediated response. Acute skin lesions of AD are characterized by spongiosis, epidermal hyperplasia, and ballooning of the keratinocytes secondary to intracellular edema. Mast cell and basophil numbers are normal, and eosinophils are rare (16). Chronic skin lesions have moderate to marked hyperplasia of the epidermis, elongation of the rete ridges, and prominent hyperkeratosis. Spongiosis is variable, and the number of mast cells and Langerhans' cells are significantly increased. Eosinophils are sparse. Demyelination and fibrosis of cutaneous nerves are observed at all levels of the dermis. Capillary numbers are often increased and capillary walls may be thickened. Although this pattern is not considered typical of the Type I, IgE-mediated response, it is consistent with an end-stage cutaneous IgE-dependent "late-phase" reaction and the preferential activation of Th2 cells by IgE-bearing Langerhans' cells (17).

About 85% of patients with AD have elevated serum IgE levels, and about 85% of these have evidence of specific IgE antibodies to food and inhalant allergens (18). The immunopathogenic role of allergen-specific IgE antibodies in AD involves a number of cell types. Receptors for IgE antibodies have been identified on B cells, T cells, monocytes, macrophages, DCs, eosinophils, and platelets (19–21). Langerhans' cells, "professional" APCs in the skin, are more numerous in AD lesions, possess allergen-specific IgE antibodies on their surface (22), and are highly efficient at promoting Th2 responses to allergens (17, 23). Studies utilizing in situ hybridization found that cytokines expressed in lymphocytes that infiltrate skin lesions in AD reflect an allergic milieu. In acute lesions (less than 3 days), infiltrating T lymphocytes express predominantly the Th2 cytokines—interleukin-4 (IL-4), IL-5, and IL-13—whereas T cells in chronic lesions express predominantly IL-5 and IL-13 (24, 25). This is in distinct contrast to classic Type IV cellular responses, such as the tuberculin skin test (PPD) or rhus dermatitis (poison ivy), where cells express primarily mRNA for interferon-gamma (IFN-γ) and IL-2, but not IL-4 and IL-5 (26).

The Th2 cytokines promote chronic allergic inflammation by up-regulating adhesion molecules on vascular endothelial cells, e.g., vascular adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule-1 (ICAM-1) (27); up-regulating high affinity receptors for IgE antibodies on Langerhans' cells and other APCs; recruiting eosinophils and other inflammatory cells to the site; and promoting local synthesis of IgE antibodies. Adhesion molecules are not typically expressed in the skin of non-atopic individuals, but they are expressed in normal-appearing skin of AD patients and are markedly up-regulated in skin lesions or following epicutaneous application of allergen in sensitized, AD patients (28). Keratinocytes do not express MHC class II antigens, a sign of IFN-γ stimulation typically seen in delayed-type cell meditated reactions (29). The high-affinity receptors for IgE on Langerhans' cells, through surface bound IgE antibodies, play a special role as "non-traditional" receptors on these APCs (30). IgE-bearing Langerhans' cells are 100- to 1000-fold more efficient at presenting allergen to T cells (primarily Th2 cells) and activating T cell proliferation (17, 23).

The cutaneous late-phase reaction of IgE-mediated hypersensitivity also may play a pathogenic role in some patients with AD. A number of studies have demonstrated that, within minutes of encountering an allergen, mast cells and basophils become activated by antigen-induced bridging of IgE molecules, which are bound to the cell membrane by high-affinity IgE receptors (FcεRI). An initial flare (vasodilation) and wheal (capillary leakiness) occur when mast cell mediators are released. This response peaks within 15–30 minutes, and then progressively fades. By 90 minutes the lesion is diffuse, and is only slightly erythematous and edematous but largely asymptomatic. Clinically, mild pruritus may begin by 4 hours, but distinctive erythema, tenderness, and warmth of the involved skin develops by 6–12 hours; symptoms then slowly resolve over 24–48 hours.

Histologically, the late-phase lesion begins with an influx of neutrophils and eosinophils within 2–4 hours of the immediate reaction (31). This influx is promoted by cytokines released from mast cells, endothelial cells, and possibly other

cell sources. Once present, the neutrophils and eosinophils release other mediators of inflammation (platelet activating factor [PAF], prostaglandins, leukotrienes, major basic protein [MBP], O_2^-, etc.), which perpetuate the response. After 6–8 hours, primarily mononuclear cells and eosinophils are seen, and lesser numbers of neutrophils, basophils, and mast cells. During the ensuing 24–48 hours the infiltrating cell population again changes, and later biopsies show primarily a mononuclear cell infiltrate, which appear virtually indistinguishable from the classic Type IV cell-mediated response.

Previously, eosinophils were not thought to play a pathogenic role in the skin lesions of AD because they are not abundant in routine hematoxylin-eosin stained histologic sections of eczematous lesions. However, a major role is suggested by the presence of eosinophil MBP, the major protein of eosinophilic granules, in active eczematous lesions. MBP is a cytolytic protein secreted almost exclusively by eosinophils, and is known to damage skin epithelial cells (32) and promote mast cell degranulation (33). Utilizing an antibody specific for MBP and an indirect fluorescein-staining technique, biopsies of eczematous skin lesions from patients with AD were shown to have extensive extracellular MBP deposition in a fibrillar pattern in the superficial dermis (34). MBP was not found in biopsy specimens from uninvolved skin sites in the same patients, indicating that this eosinophil product was the result of specific deposition, and was not due to nonspecific sequestration. Control studies on patients with contact dermatitis revealed no dermal deposition of MBP. This study provided further evidence that the typical mononuclear cell infiltrate in lesions of AD may result from IgE-induced cutaneous late phase reactions, with eosinophil degranulation and dermal deposition of MBP. Infiltrating lymphocytes are capable of releasing a variety of interleukins that may promote mast cell proliferation (IL-3), cell-surface expression of Fcε receptors and IgE synthesis (IL-4), eosinophil proliferation (IL-5), etc.

Food Hypersensitivity and AD

By the turn of the century, physicians were suggesting that reactions to food proteins could cause eczematous skin rashes. Schloss was among the first to present evidence that food allergy could play a pathogenic role in AD (35). He presented several case reports of patients who experienced improvement in their eczema by avoiding specific foods. Shortly thereafter, Talbot (36) and then Blackfan (37) each described a series of patients who were found to have positive skin tests to certain foods and who experienced clearing of their skin when the foods were removed from their diets. Since that time, other reports have appeared in the literature implicating food allergy in the pathogenesis of AD (38, 39). However, after openly challenging eczema patients with foods, other investigators discounted these findings.

In a series of experiments, Walzer and his colleagues (40, 41) unequivocally demonstrated that ingested food antigens readily penetrate the gastrointestinal barrier and are transported in the circulation to mast cells in the skin. To demonstrate this, 65 normal adults were passively sensitized by intracutaneous injection of serum (Prausnitz-Küstner [PK] test) from a patient with severe fish allergy and a normal control (40). On the following day the volunteers were fed fish; 61 subjects (94%) developed a wheal and flare reaction within 90 minutes at the sensitized site but not the control site. Similar results were found in a series of experiments conducted in 66 normal children with serum from an egg-allergic subject (41). Walzer and his colleagues (42) studied the absorption of food antigens from various locations in the gastrointestinal tract and other sites such as the nose, eye, and urinary bladder. They concluded that "the absorption of unaltered protein into the circulation is a normal, physiologic phenomenon occurring in nonatopic as well as atopic individuals, at all ages, and with many allergens" (42). In addition, they were able to show that the intravenous injection of the protein nitrogen equivalent of 1/44,000 of one peanut kernel was sufficient to induce wheal and flare at a passively sensitized skin site. In 1936, Engman et al (43) reported a child with AD and allergy to wheat. Ingestion of a wheat cracker produced intense itching in the child. To demonstrate the role of scratching and rubbing in the development of eczema, they admitted the patient to the hospital when his skin was clear, and bandaged his left arm and leg in a thick, stiff crinoline bandage. The child was given two wheat crackers, and within 2 hours he was itching and scratching. The following morning the boy had typical eczematous lesions where he scratched, but under the bandages the skin remained clear. Engman reportedly repeated this experiment in several other cases. Taken to-

gether, these studies, conducted over 50 years ago, clearly showed that ingested food allergens were readily accessible to cutaneous mast cells and "skin-associated lymphoid tissue," and that in the sensitized host, they could produce intense pruritus, causing the scratching, and rubbing that led to typical eczematous lesions.

In the late 1970s, Hammar reported the induction of eczematous skin lesions in 15 (18%) of 81 hospitalized children less than 5 years of age after 2–3 days of ingesting 100 mL of milk daily (44). The significance of these findings was questioned because the challenges were done openly and a repeat challenge 18 months later produced similar symptoms in only 4 of these 15 patients (i.e., 5% of the original 81) (45), but other investigators have shown that many children "outgrow" milk allergy following milk elimination for 1–2 years (46, 47). Atherton et al (48) reported that two-thirds of children with AD between the ages of 2 and 8 years showed marked improvement during a double-blind cross-over trial of egg and milk exclusion. The trial was conducted over a 12-week period in the patients' homes. In this study, 45% of the patients enrolled dropped out or were excluded from analysis, environmental and other triggers of AD were not controlled, and a significant order effect was found, all raising some question about the authors' conclusions. Utilizing a similar trial design, Neild et al (49) were able to demonstrate improvement in some patients during the milk and egg exclusion phase, but overall no significant difference was seen in 40 patients completing the cross-over trial. Juto et al (50) reported that 7 (35%) of 20 infants with eczema healed, and 12 (60%) of 20 improved on a highly restricted elimination diet. Non-blinded challenges to cow's milk reportedly resulted in increased itching and rash in 12 (60%) of 20 infants. Hill et al (51) treated eight children with severe AD with Vivonex for 2 weeks, followed by the addition of two vegetables and two fruits for 3 months. All patients experienced improvement in their eczema while on the diet but relapsed within weeks of discontinuing it. Although supporting a role for food intolerance in the exacerbation of AD, most of these studies fail to control for other trigger factors, placebo effect, or observer bias. A double-blind study in 23 hospitalized adults of an antigen-free diet (Vivasorb) failed to show any significant difference between the antigen-free and placebo groups (52). However, the study groups were too small to conclude "no significant difference" (Type II statistical error), and some adults with markedly elevated serum IgE concentrations appeared to respond to the antigen-free diet.

In their studies of children primarily with suspected food hypersensitivity and respiratory allergy, May and Bock (53) reported that 4 (57%) of 7 children with a history of eczematous reactions to foods developed skin rashes within 2 hours of administration of a double-blind placebo-controlled oral food challenge (DBPCFC). Using a similar challenge protocol, the author has systematically studied over 500 patients referred for evaluation of severe AD, as reviewed below. Burks et al (54, 55) also have employed the DBPCFC to study children with mild to severe AD presenting to a university dermatology and allergy clinic. Approximately half of their subjects used the university clinics for primary care. As seen in other controlled oral challenge studies, children experienced cutaneous, respiratory, and gastrointestinal symptoms. One third of the children developed symptoms during the blinded food challenges. There was no correlation between the likelihood of having a positive food challenge and the severity of the skin symptoms.

In the past 20 years, our studies have addressed the etiologic role of IgE-mediated food hypersensitivity in AD (52, 56–58). Using DBPCFCs, 470 patients with AD have been evaluated for food hypersensitivity. Subjects ranged in age from 3 months to 24 years with a median age of 4.1 years. The median clinical score for the group was 15 (range 0–30) at the time of the first admission, based on the clinical scoring system depicted in Table 11–1. Family history was positive for atopic disease in 91% of subjects. One hundred fifty-seven patients (39%) had allergic rhinitis and asthma at the time of initial evaluation, and only 94 (20%) had neither allergic rhinitis nor asthma. Serum total IgE concentration was elevated in 376 (80%) patients with a median of 3410 IU/mL and a range of 1.5–45,000 IU/mL.

Any patient with chronic severe eczema fulfilling the criteria of Hanifin and Rajka (3) for the diagnosis of AD was eligible for the study, whether or not clinical history or previous allergy tests suggested a diagnosis of food hypersensitivity. All patients were admitted to the clinical research unit to provide a stable, low-allergen environment. Foods administered during DBPCFCs were selected on the basis of skin test and RAST results and/or a strongly suggestive history of food hypersensitivity. Foods selected for the challenge protocol were excluded from the patient's diet for 7–10 days prior to admission. In addition, the fol-

Table 11–1.
Clinical Scoring System

Characteristics	*Score*
Extension	
Infantile Stage (less than 2 yrs of age)	
Absent	____ 0
Less than 20% involvement	____ 2
20%–50% of skin involved	____ 4
More than 50% of skin involved	____ 6
Childhood and Adult Stages	
Absent	____ 0
Not more than 2 predilection sites involved	____ 2
Involvement more than 2 but less than 6	____ 4
Dermatitis including and extending beyond predilection areas, > 25% involvement	____ 6
Course (remission = eczema not completely absent, but confined to typical areas)	
Absent	____ 0
More than 2 months' remission during quarter	____ 1
Remission less than 2 months	____ 2
Continuous course	____ 3
Scratching	
Intensity–Day Pattern	
Absent	____ 0
Scratches when tired/anxious, easily distracted	____ 2
Evaluated as greater than 2 but less than 6	____ 4
Almost constant, scratching to point of excoriation: school and play concentration impossible	____ 6
Intensity—Night Pattern	
Absent, sleeps through night without attention	____ 0
Occasionally awakens scratching, but back to sleep with minimal attention	____ 2
Awakens scratching 1–2 times each night	____ 4
Requires frequent rubbing during the night to retard scratching, sleeps very little	____ 6
Antihistamine Use	
None or occasional prednisone use	____ 0
Daily bedtime use	____ 1
Daily bedtime use plus 1–2 daytime doses on most days	____ 2
Daily bedtime use plus daytime usage to limit allowed	____ 3
Antibiotic/Prednisone Use (for Skin in Past 3 Months)	
None	____ 0
1 course with noninflamed periods	____ 1
2 courses with noninflamed periods	____ 2
3 or more courses, or no clearing	____ 3
School Missed or Parents Missing Work due to Skin Symptoms	
None	____ 0
Average 1 day missed per quarter	____ 1
Average 2 days missed per quarter	____ 2
Average 3 or more days missed per quarter	____ 3
Total [Possible total = 30]	____

lowing medications were withheld: oral corticosteroids for at least 1 month prior to admission, antihistamines for at least 7–10 days, and inhaled and oral β-adrenergic drugs for 8 hours prior to the challenge. Inhaled cromolyn sodium and oral theophylline were not discontinued.

The initial 220 patients were skin-tested to a battery of 20 food extracts (Greer Laboratories, Lenoir, N.C.) on the day of admission to confirm previous results, standardize skin test data, and determine which foods would be used in the DBPCFC (Table 11–2). In the clinical research unit, patients were treated with an aggressive topical regimen consisting of 2–3 soaking baths or wet wraps per day followed by the application of a lubricating cream to the entire body, and topical corticosteroid (1% hydrocortisone cream for the face and 0.025%–0.1% triamcinolone ointment for the trunk and extremities) to active eczematous areas. Anti-staphylococcal antibiotics were generally administered and chloral hydrate was used for sedation. With this regimen, erythema and pruritus could generally be controlled in 3–4 days. Once a stable baseline was achieved, a venous line was placed prior to initiating challenges to provide an open line in case of a major anaphylactic reaction and to provide access for atraumatic serial blood sampling.

Table 11–2.
Foods Eliciting Positive Prick Skin Tests in 220 Children with Atopic Dermatitis

Food	*No. of Patients*	*% of + Tests*	*Food*	*No. of Patients*	*% of + Tests*
egg	124	20%	beef	42	7%
milk	54	9%	chicken	33	5%
peanut	98	16%	pork	40	6%
soy	58	9%	potato	16	3%
wheat	34	5%	rye	20	3%
gr. pea	34	5%	others	66	11%

During a 1-week admission, 3–4 DBPCFCs were conducted. The clinical research unit dietician determined the order of all challenges using a randomization scheme on the unit's computer. Only the dietician was aware of the contents of the challenge until the code was broken at the end of the study week. Two challenges were performed each day, one containing the test food antigen and one containing placebo. Foods to be administered in the challenge were camouflaged in another food, juice, or formula and administered over a 60–90 minute period. Up to 10 g of dehydrated powdered food was administered. The initial challenge dose was generally 100 mg to 500 mg and was increased in a stepwise fashion at 15-minute intervals until the entire 10 g was consumed or a reaction occurred. A review of the dose at which most children reacted to the food challenge indicated that most children should be started at doses of 100 mg or less (59). Each challenge was evaluated and scored using a previously published symptom sheet (55). All negative DBPCFCs were confirmed by feeding the food openly to the patient before discharge to ensure the accuracy of the blinded challenge and to reassure the patient (and parent) that the food could be ingested safely. In the initial 1000 food challenges performed, there were five reactions during the open feeding following negative DBPCFCs (<1% false-negative results). Three children, two age 4 years and one age 2 years, developed symptoms after drinking milk. One child developed cutaneous, nasal, and respiratory symptoms after consuming approximately 100 mL of milk, one child developed cutaneous symptoms and periocular edema after ingesting about 80 mL, and the third child developed cutaneous, upper respiratory, and gastrointestinal symptoms after ingesting about 10 mL of milk. The fourth child, age 5 years, developed cutaneous, gastrointestinal, nasal, and respiratory symptoms after eating a standard portion of peas. The fifth child, age 3 years, developed scattered urticaria following the ingestion of 1 teaspoon of peanut butter.

In the initial evaluation of 470 children with AD, a total of 1776 DBPCFCs have been conducted (Table 11–3). DBPCFCs were not conducted in 193 instances because clinical history indicated a "convincing" account of a major anaphylactic reaction (mostly to peanuts and tree nuts). The history was considered "convincing" when a patient experienced severe respiratory symptoms (laryngeal edema and/or wheezing) and/or hypotension within minutes of ingesting an isolated food and required emergency care by a physician. In each case where the challenge was not performed, the patients had a markedly positive skin prick test (SPT) to the food in question. No patient experienced a severe anaphylactic reaction during DBPCFC, although about one half the patients required oral diphenhydramine for severe pruritus, and several patients required subcutaneous epinephrine for respiratory symptoms. Of the 1776 total DBPCFCs performed to date, 714 (40%) were positive and 1062 (60%) were interpreted as negative. Cutaneous reactions developed in 529 (74%) of the 714 DBPCFC-positive cases that consisted of a pruritic, erythematous, macular, or morbilliform rash primarily at previous predilection sites. Symptoms confined exclusively to the skin occurred in only 214 (30%) of the positive reactions. Typical urticarial lesions were rarely seen and generally consisted of only a few lesions. Intense pruritus and scratching frequently led to superficial excoriations and occasionally bleeding. Gastrointestinal symptoms were seen in 358 (50%) of the positive reactions even though a history of gastrointestinal symptoms was rarely elicited from the patients. The gastrointestinal symptoms were nausea or abdominal pain, or both, plus vomiting or diarrhea, or both. Respiratory symptoms most frequently involved the upper respiratory tract and were seen in 322 (45%) of the positive DBPCFCs. Respiratory symptoms included nasal congestion, rhinorrhea, sneezing, tightness of the throat, hoarseness, and/or wheezing.

Table 11–3.
Food Challenges in 470 Patients with Atopic Dermatitis

Total number of food challenges	1776
Negative challenges	1062
Positive by history (majority peanut and nut)	193
Positive challenges	714
• Cutaneous symptoms	529 (74%)
Skin only	195 (27%)
• Gastrointestinal symptoms	358 (50%)
• Respiratory symptoms	322 (45%)

Virtually all symptoms secondary to the blinded food challenges developed between 5 minutes and 2 hours of initiating the challenge. Symptoms associated with the immediate response were generally marked, abrupt in onset, and lasted 1–2 hours. Several patients experienced a second episode of increased cutaneous pruritus and transient morbilliform rash 6–10 hours after the initial positive challenge. Symptoms associated with the late response were less prominent

than the immediate symptoms and tended to last for several hours. Only one child (3 years of age) developed an isolated "delayed" reaction; a pruritic, erythematous rash developed about 4 hours after the child ingested egg.

Although many reports have suggested that children with AD are sensitive to a large number of foods, 376 (80%) of the 470 patients evaluated in our study developed symptoms to only one to three foods by DBPCFC. Most of the children had positive SPTs to several foods (mean 3.5; range 0–10], but only about one third of positive skin tests correlated with positive food challenges. Of the 470 children with AD in the study, 169 (36%) reacted to only one food, 122 (26%) reacted to two foods, 85 (18%) reacted to three foods, 47 (10%) reacted to four foods, and 47 subjects (10%) reacted to five or more different foods. Five foods (egg, peanut, milk, wheat, and soy) accounted for about 80% of the positive clinical responses (Table 11–4).

Allergic reactions to foods appear to be very specific. Although patients frequently have positive SPTs and RASTs to several members of a botanical family or animal species, indicating immunologic cross-reactivity, only two patients in one study had symptomatic intra-botanical cross-reactivity, and only two subjects with symptomatic intraspecies cross-reactivity as determined by DBPCFC. Legume cross-reactivity was evaluated in 69 children with AD by SPTs, and by in vitro measurements of specific IgE antibodies by immunodot blot and Western blot analyses (60, 61). Extensive immunologic cross-reactivity was demonstrated in many patients. However, only two patients were symptomatic to more than one legume when challenged orally. Both patients had a history of severe allergic reactions to peanut and mild reactions to a soy challenge. In addition, both "outgrew" their reactivity to soy in 1–2 years. Similar studies with cereal grains showed significant IgE antibody cross-reactivity between grains and grass pollens but little clinical cross-reactivity (62).

Table 11–4.
Five Major Food Allergies in 470 Patients with Atopic Dermatitis

Food	*Challenge +*	*Hx +*	*Total*	*%*
Egg	178	35	213	57
Milk	96	47	143	38
Peanut	28	82	110	29
Soy	55	4	59	16
Wheat	43	0	43	11

Consequently, the practice of avoiding all foods within a botanical family when one member is suspected of provoking allergic symptoms appears to be unwarranted.

As noted above, patients experiencing positive DBPCFCs develop a pruritic, morbilliform rash instead of the classic urticarial lesion. To demonstrate that the ingestion of food antigens led to IgE-mediated reactions, markers of mast cell activation were sought. Thirty-three patients undergoing DBPCFCs were monitored for changes in circulating plasma histamine (63). Histamine concentration was measured before the challenge and after the ingestion of the test antigen. Patients experiencing clinical symptoms following the blinded challenge developed a rise in their plasma histamine (mean 296 ± 80 pg/mL to 1055 ± 356 pg/mL; $P < .001$). As shown in Figure 11–1, subjects ingesting placebo or a food that did not provoke clinical symptoms had no demonstrable rise in their plasma histamine concentration.

Blood samples were also obtained to determine whether basophils had been activated during the challenge and therefore were contributing to the rise in plasma histamine. Samples were obtained before the challenge, immediately after it, and 30 minutes following the development of the first objective symptoms (64). Each sample was evaluated for basophil number and total histamine content of the leukocyte preparation. There was no difference in basophil number or total histamine content of the basophils at any time point, suggesting that circulating basophils do not account for the initial rise in plasma histamine observed.

Circulating food antigen-antibody complexes have been reported by several investigators (65–67). To rule out the possibility that mast cells were being activated by complement, a very sensitive

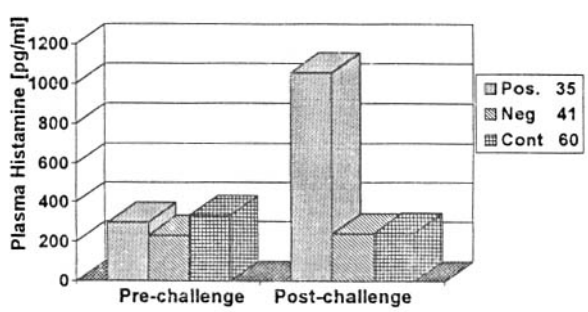

Figure 11–1. Mean plasma histamine levels in patients prior to and following DBPCFCs. Overall, 35 challenges were considered clinically positive and 101 negative. Of the negative DBPCFCs, 41 were negative to food allergens and 60 were negative to placebo.

radioimmunoassay was used to quantitate C3a des-arg and C5a des-arg before DBPCFC and immediately, 15 minutes, and 30 minutes following the development of objective symptoms because measurement of the anaphylatoxins, C3a and C5a, is very sensitive indicator of complement activation. No significant change was observed in plasma C3a des-arg concentrations following positive DBPCFCs in 18 patients. C5a des-arg was undetectable in all samples examined (68). Therefore, there is no evidence that the complement cascade is activated, or in turn activates mast cells, during allergic reactions to food in children experiencing positive challenges.

Studies utilizing the DBPCFC clearly demonstrate an immediate, IgE-mediated food hypersensitivity reaction in some children with atopic dermatitis. Such distinct reactions are rarely seen, because a food is normally not ingested on an empty stomach after prolonged avoidance of that particular food antigen. The second onset of pruritus and rash in some children following DBPCFC is suggestive of a "late-phase" component of an IgE-mediated response. Two food-allergic patients who had experienced clearing of their eczema after maintaining an appropriate food allergen avoidance diet were rechallenged to establish that the ingestion of an allergen during a DBPCFC could induce a late-phase response (68). Both patients developed a pruritic morbilliform skin rash within 30–60 minutes, which cleared in about 45–60 minutes. Skin biopsies were obtained from the involved sites at 4 and 14 hours later. Both revealed an infiltration of eosinophils and deposition of MBP. The dermal infiltrate contained more eosinophils and less-prominent MBP deposition than were seen with the chronic lesions. In three other food allergic patients who had experienced clearing of their eczema after maintaining an appropriate food allergen avoidance diet, we found a change in the "density profiles" of circulating eosinophils from normodense (non-activated) to hypodense (activated) following a positive food challenge. Suomalainen et al (69) found a rise in plasma eosinophil cationic protein (ECP) levels in milk-allergic children who experienced skin symptoms following a milk challenge, but not in children experiencing only gastrointestinal symptoms. These studies indicate that ingestion of a food allergen by an allergic patient leads to activation of circulating eosinophils, which may infiltrate the skin of patients with AD.

The exact pathogenic role of eosinophils in eczematous skin lesions remains to be established. Leiferman et al (34) evaluated skin biopsies from active AD lesions. MBP was found in the dermis of active skin lesions but not in normal-appearing skin. Once recruited, eosinophils may release mediators (e.g., the leukotriene LTC_4), several cationic proteins (e.g., MBP, ECP, and eosinophil-derived neurotoxin [EDN]) that contribute to the pathogenesis of the allergic reaction, and cytokines that may contribute to the inflammatory response (e.g., IL-1β, IL-6, tumor necrosis factor alpha [TNF-α], and macrophage inflammatory protein-1 [MIP-1]) or perpetuate chronic inflammation (e.g., IL-3, IL-5, granulocyte macrophage-colony-stimulating factor [GM-CSF]) (70). MBP is toxic to many cell types and can cause histamine release from mast cells (32). EDN, a powerful neurotoxin, may account for the demyelination of nerves in the dermal layer seen in eczematous skin. These and other mediators such as leukotrienes, prostaglandins, and platelet-activating factor have been reported as prominent in AD and support a pathogenic role for IgE-mediated late phase reaction (68).

Children with AD and newly diagnosed food hypersensitivity have high "spontaneous" basophil histamine release (SBHR) from peripheral blood basophils in vitro, compared to patients with atopic dermatitis and no food allergy, and normal controls (means, respectively: 35.1% ± 3.9%; 1.8% ± 0.2%; and 2.3% ± 0.2%; $P < .001$) (64, 71). When these food-allergic patients were placed on appropriate elimination diets for at least 1 year, their eczema cleared and SBHR fell significantly (Fig. 11–2). Unstimulated peripheral blood mononuclear cells (PBMCs) from food-allergic sub-

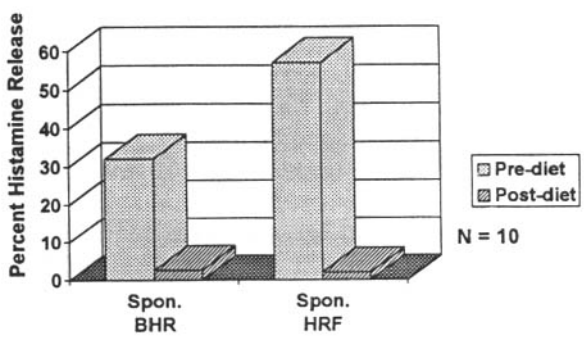

Figure 11–2. The mean percent of "spontaneous" basophil histamine release (Spon. BHR) and "spontaneously" generated histamine-releasing factor (Spon. HRF) in 10 AD patients with food allergy prior to and approximately 1 year after the initiation of an appropriate allergen exclusion diet.

jects with high SBHR produced a histamine-releasing factor (HRF) in vitro that could activate basophils from other food-sensitive individuals, but not from food non-sensitive subjects. When food-allergic patients were maintained on an appropriate food-allergen-free diet for 9–12 months, mononuclear cells no longer spontaneously generated HRF (Fig. 11–2). It was postulated that ingestion of small amounts of food allergens stimulates the production and release of HRF, which could activate mast cells or lower their threshold of activation. Generation of HRF could also account for the increased basophil "releasability" reported in some patients with AD (72), and the high SBHR seen in food-sensitive subjects in vitro. The loss of "spontaneously" generated HRF appeared to correlate directly with the loss of cutaneous hyperreactivity (defined as increased pruritus due to a variety of minor stimuli such as heat, stress, or irritants) and inversely with the improvement in patients' skin.

Several investigators have demonstrated an influx of mononuclear cells in tissue biopsies from eczematous lesions, primarily $CD4^+$ Th2-type lymphocytes (65). It has also been shown that T cells migrating into skin blisters overlying cutaneous delayed-type hypersensitivity reactions were highly enriched for the homing receptor, cutaneous lymphocyte antigen (CLA), whereas T lymphocytes isolated from the lungs of asthmatics were predominantly CLA-negative (66). Thus, the propensity to develop AD may depend on the skin- or lung-seeking characteristics of memory T cells. PBMCs from seven AD patients with milk allergy, 10 with milk-induced gastroenteropathies, and eight normal controls were stimulated in vitro with casein and then evaluated for expression of homing receptors (67). As seen in Figure 11–3, only patients with AD and milk allergy demonstrated a significant increase in CLA-positive T cells, suggesting that the homing receptors expressed on antigen-specific T cells play a role in determining which tissues are involved in allergic responses.

The role of non-IgE-mediated food-induced hypersensitivity in AD remains unclear. However, as discussed below, there has been increasing interest in the use of the atopy patch test to identify foods that provoke eczematous flares in some children (73). Early studies by Mitchell (74) and a more recent study (75) have suggested that this test might be valuable in identifying patients who were reactive to dust mite and other aeroallergens.

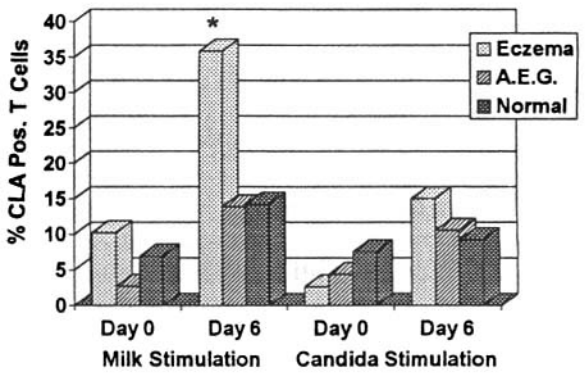

Figure 11–3. Milk-allergic patients with AD have significantly increased numbers of lymphocytes bearing the CLA in vitro compared to patients with gastrointestinal milk allergy and normal controls. There is no similar increase in CLA-positive cells to an irrelevant antigen, *Candida albicans*.

Diagnosing Food Hypersensitivity in Patients with AD

As mentioned in Chapter 9, the DBPCFC is the "gold standard" for diagnosing food hypersensitivity and is essential for clinical research. Alternatively, in an office practice, a 2–3 week trial elimination diet may be implemented if a few foods are suspected after obtaining a careful history and evidence of food antigen-specific IgE antibodies (e.g., by SPTs and RASTs). Symptoms are recorded in a diary during the trial period. If unequivocal improvement is documented, foods thought least likely to be responsible (e.g., foods other than egg, peanut, milk, soy, and wheat) are added back to the diet. However, no food suspected of causing a severe anaphylactic reaction should be administered at home because of the potential for inducing anaphylactic shock (76). If a clear exacerbation of the patient's AD should occur, the food should be removed from the diet. If cause and effect can be established, the patient should remain on the avoidance diet unless it requires elimination of more than one "major" food (e.g., egg, milk, peanut, soy, wheat) and/or two or more "minor" foods (all others). If severe symptoms persist on the elimination diet and food allergy is still in question, a brief trial (e.g., 1–2 weeks) of a severely restricted diet may be undertaken. If elimination diet results are equivocal or several foods are implicated, blinded oral food challenges should be performed once the patient's symptoms have improved sufficiently (which may require hospitalization) to establish the diagnosis.

Multiple dietary restrictions are rarely necessary in children with moderately severe AD, but in recent years an increasing number of infants with severe AD have been identified with multiple food allergies. Other modalities for diagnosis of food hypersensitivity, such as sublingual provocation with drops of antigen extracts, subcutaneous provocation with varying concentrations of food extracts, and measurements of IgG- or IgG4-specific antibody, have not been shown to be useful or effective in controlled studies.

We evaluated 196 children and adolescents with AD and food allergy diagnosed by DBPCFC, and compared the outcome of the food challenge to specific levels of food antigen-specific IgE as determined by the Pharmacia CAP-System FEIA (70). As shown in Table 11–5, we found concentrations of food antigen-specific IgE antibody above which patients were greater than 95% likely to experience a positive food challenge—that is, their positive predictive value (PPV) was 95% or greater. Patients with egg-, milk-, peanut-, or fish-specific IgE concentrations that exceed the 95% PPV may not need to be challenged to diagnose food allergy. However, patients with specific IgE values less than the 95% PPV may still react to the food in question, as indicated by the low sensitivity of the test at this value. Therefore, a food challenge would be necessary to establish the diagnosis. As shown in Table 11–5, even at IgE levels >100 IU/mL, the 95% PPV for wheat and soy are never attained.

Studies using both SPTs and atopy patch testing (APT) with foods have shown value in identifying patients with delayed onset of symptoms (77–81); children with immediate reactions generally had positive SPTs, whereas those with late reactions were more likely to have positive APTs to the relevant foods. Further studies with APT are needed to standardize the reagents and procedures, and to confirm these results, before general recommendations can be made.

Once food hypersensitivity is diagnosed, therapy is straightforward. The patient is placed on a diet that meticulously eliminates all forms of the offending food allergen. Instructing the patient and family to read food labels to avoid hidden sources of the suspect food, as discussed in Chapter 33, is critical. Antihistamines and epinephrine may modify the symptoms of an immediate food hypersensitivity reaction after an accidental ingestion, but they have no prophylactic role in the treatment of food allergy. Although some investigators have advocated prevention of food allergen-induced symptoms with oral cromolyn, a controlled, blinded trial of oral cromolyn in patients with challenge-proven food hypersensitivity demonstrated no benefit (82). Treatment of food hypersensitivity with oral desensitization, immunotherapy, and rotational diets also has not been proven effective in controlled trials.

With the initiation of an appropriate food allergen elimination diet, food-allergic patients generally experience significant improvement in their eczematous symptoms, and in follow-up over 3–4 years have significant improvement in their clinical symptoms compared to patients without food allergy or to those who fail to comply with the allergen elimination diet (47) (Fig. 11–4). In addi-

Table 11–5.
Food-specific IgE Concentrations Predictive of Clinical Reactivity

Allergen	*Decision Point [kU_A/L]*	*Sensitivity*	*Specificity*	*PPV*	*NPV*
Egg	7	61	95	98	38
Infants ≤ 2 yrs+	2			95	
Milk	15	57	94	95	53
Infants ≤ 2 yrs++	5			95	
Peanut	14	57	100	100	36
Fish	20	25	100	100	89
Wheat	26	61	92	74	87
Soybean	30	44	94	73	82
Tree nuts*	~15	—	—	~95	

+Boyano MT, et al. Clin Exp Allergy 2001;31(9):1464–9.
++Garcia-Ara C, et al. J Allergy Clin Immunol 2001; 107(1):185–90.
*tentative values.
Adapted from Sampson HA. J Allergy Clin Immun 107:891–896, 2001.

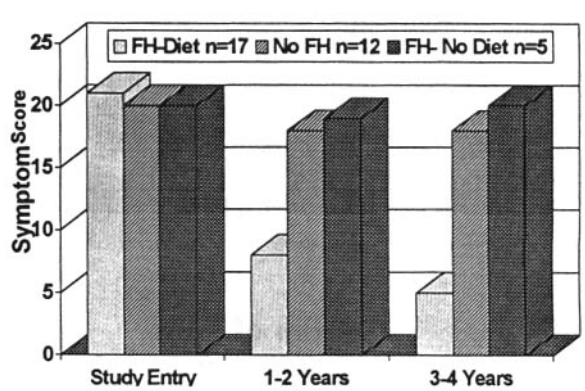

Figure 11–4. Food-allergic AD patients placed on an appropriate food allergen elimination diet [FH-Diet] experienced significant improvement in their eczema over 3–4 years of follow-up compared to atopic dermatitis patients without food allergy [No FH] and food-allergic, atopic dermatitis who did not maintain their food allergen elimination diet [No Diet].

tion, several immunologic parameters normalize, i.e., loss of SBHR, loss of "spontaneous" generation of HRF, normalization of circulating eosinophil and basophil activation (72), and in many cases, loss of clinical food reactivity [see below].

Natural History of Food Hypersensitivity in AD

Approximately one-third of children with AD and food allergy "lost" (or outgrew) their clinical reactivity over 1–3 years (47). Three factors appeared to be most important in determining the likelihood that patients would lose their clinical reactivity: 1) the food to which the patient was allergic, i.e., patients who were allergic to peanuts, nuts, fish, and shellfish were unlikely to lose their clinical reactivity, whereas those allergic to soy, wheat, milk, and egg were much more likely to develop clinical tolerance; 2) the level of specific IgE antibody to a particular food, i.e., the higher the level of food antigen-specific IgE, the less likely it was that clinical tolerance would develop in the subsequent few years (83); and 3) the degree to which the patient adhered to the elimination diet, i.e., patients who ingested small amounts of allergen or had frequent accidental ingestions were less likely to develop clinical tolerance. SPT results did not correlate with loss of clinical reactivity and remained positive for 5 years or more after the food had been reintroduced to the diet. Therefore, patients should be rechallenged intermittently (e.g., for egg, every 2–3 years; for milk, soy, and wheat, every 1–2 years; foods other than peanut, nuts, fish, and shellfish, every 1–2 years) to determine whether their food allergy persists, so that restriction diets may be discontinued as soon as possible. No patient had a recurrence of allergic symptoms or a worsening of eczema once food hypersensitivity was "lost." The immunologic changes associated with loss of symptomatic food hypersensitivity are under intensive study.

Prevention of Food Hypersensitivity and AD

There has been intense interest in prevention of allergic disease by manipulation of an infant's diet and environment. Since Grulee and Sanford (84) reported a decrease in the incidence of AD in breast-fed infants in the 1930s, numerous conflicting reports have been published about the relative benefit of breast-feeding infants to prevent or delay the onset of atopic disease (85–87). However, a recent meta-analysis of studies published between 1966 and 2000 evaluated the association between exclusive breast-feeding for at least 3 months and the development of AD. This analysis found a significantly lower incidence of AD in infants who were from atopic families and were exclusively breast-fed (88). The potential benefit of breast-feeding is complicated by transmission of food antigens in maternal breast milk (89–92). We evaluated 6 infants (ages 2.5 to 6 months) who developed classic infantile AD while being exclusively breast-fed. All infants had positive SPTs to egg and showed complete clearing of their skin when their mothers totally eliminated egg-containing foods from their diets. Four of the 6 infants were challenged on the research unit by first feeding their mothers eggs and then having the mothers breast-feed their babies. Each infant developed an eczematous rash within 4–36 hours after its mother ingested eggs. This evidence, and findings from other studies support the recommendation of placing selected mothers on diets that avoid highly allergenic foods during the period of lactation. Several investigators have evaluated the effect of eliminating certain foods from the maternal diet during lactation (93–96). In two series, infants from atopic families whose mothers excluded egg, milk, and fish from their diets during lactation (i.e., the prophylaxis group) had significantly less AD and food allergy at 18 months compared to infants whose mothers' diets were unrestricted (97, 98). At age 4 years, the prophylaxis group had less AD than the unrestricted group but no differences in food allergy or respiratory allergy (98) (Fig. 11–5). A study by Lindfors and Enocksson (99) has also suggested the possibility of developing high-dose tolerance. These investigators concluded that initial and early regular feedings with a cow's milk formula, followed by a gradual replacement with prolonged breast-feeding, reduced the development of allergic symptoms to 18% in 112 infants in the first 18 months of life, compared to 33% in the 104 exclusively breast-fed infants. Recent studies suggest that the presence of transforming growth factor-beta (TGF-β) in human breast milk plays a critical role in gut maturation and down-regulation of Th2 responses (100), whereas a lack of TNF-α leads to promotion of Th2 responses (101).

In a prospective non-randomized study of 1265 unselected neonates, the effect of solid food introduction was evaluated over a 10-year period

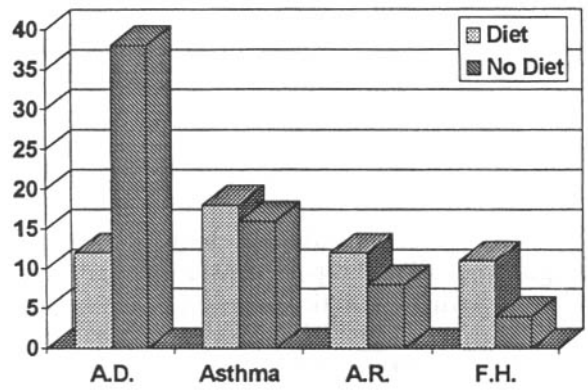

Figure 11–5. Infants from atopic families whose mothers eliminated egg, milk, and fish during lactation has less atopic dermatitis at 4 years compared to similar infants whose mothers had no dietary restrictions during lactation (98). No similar decrease was seen for asthma, allergic rhinitis or food allergy.

(102). A significant linear relationship was found between the number of solid foods introduced into the diet by 4 months of age and subsequent AD, with a three-fold increase in recurrent eczema at 10 years of age in infants receiving four or more solid foods compared to infants receiving no solid foods prior to 4 months of age. No relationship was noted between asthma and the introduction of solid foods. A prospective, non-randomized study comparing breast-fed infants who first received solid foods at 3 months or 6 months of age revealed reduced AD and food allergy at 1 year of age in the group that avoided solids for the 6 month period (103), but no significant difference in these parameters at 5 years. Because neither series randomized patients, these studies must be considered suggestive until an appropriate randomized trial confirms the benefit of delaying solid food introduction.

In the most comprehensive, prospective, randomized allergy-prevention trial, Zeiger et al compared the benefits of maternal and infant food allergen avoidance on the prevention of allergic disease in infants at high risk for allergic disease (94, 95, 104, 105). Breast-feeding was encouraged in both the prophylaxis and control groups. In the prophylaxis group, egg, cow's milk, and peanut were removed from lactating mothers' diets, a casein hydrolysate formula was used for supplementation or weaning, and introduction of solid food to infants was delayed. The control infants received cow's milk formula for supplementation, and the American Academy of Pediatrics (AAP) recommendations for infant feeding were followed (peanuts, nuts, and fish are not recommended in the first 2 years of life). The prevalence of food allergy, cow's milk sensitization, and AD in the prophylaxis group were reduced significantly during the first 2 years compared to the control group, but the period prevalence of AD was not significantly different beyond 3 years. The cumulative prevalence of food allergy remained lower in the prophylaxis group at 4 and 7 years' follow-up. These investigators concluded that maternal and infant food allergen avoidance, in comparison to standard feeding practices, reduced food allergy and AD in the first 3 years, but failed to modify allergic disease after 3 years of age. Consequently, these investigators felt that the benefits of food allergy preventative measures are of limited duration because of the common remission of food allergy in early childhood (95).

Since studies have been inconclusive, it is difficult to make firm recommendations regarding prevention strategies for food allergy and AD. However, in families at high risk for atopic disorders, it would seem prudent to follow the AAP recommendations to avoid exposing young infants to food allergens that can provoke lifelong sensitization (e.g., peanuts, nuts, fish, and shellfish) for the first 2–3 years of life. In highly motivated high-risk families, avoidance of cow's milk for the first year, and egg for the first 2 years, may help prevent some AD and food allergy. Mothers of high-risk infants might also be wise to avoid peanuts, nuts, fish, and shellfish while breast-feeding, and perhaps even during the third trimester of pregnancy, since these foods do not generally comprise a major part of the diet. Whether it is beneficial for all lactating mothers in high-risk families to avoid milk and eggs remains an unanswered question (106).

Other Nutritional Factors Implicated in the Pathogenesis of AD

More than 50 years ago, Hansen (107) reported that essential fatty acid levels were depressed in the blood of patients with AD and that supplementation with corn oil (high in linoleic acid) resulted in improvement of the eczema. Conflicting reports followed, but the advent of topical steroids displaced this form of therapy. Interest was renewed when two studies (108, 109) demonstrated the beneficial effect of evening primrose oil, which is rich in cis-linoleic acid [18:2n6] and gamma-linolenic acid [18:3n6]. Human breast milk is another rich source of linoleic and gamma-

linolenic acids, and claims have been made that the beneficial effect of breast milk on infantile eczema is due to these essential fatty acids. The purported beneficial effect is secondary to changes in arachidonic metabolism brought about by normalization of fatty acid levels. However, studies disagree on whether these levels differ in patients with AD compared to normal controls (110, 111). A large-scale study conducted in the US demonstrated no beneficial effect for evening primrose oil (112). A study with fish oil supplementation (113) claimed mild beneficial effect, but overall the improvement noted was not clinically impressive.

Several investigators have reported abnormal absorption of non-metabolizable sugars in some children with AD, indicating abnormal gut permeability in these patients (114, 115). In children with AD, all subjects with food hypersensitivity confirmed by DBPCFC were found to have abnormal lactulose absorption (116), which normalized when the responsible allergen was eliminated from the diet. Similarly, Dupont and coworkers (117) demonstrated a three-fold rise in the lactulose/mannitol urinary ratio in AD patients following a positive oral allergen provocation test. These studies indicate that gastrointestinal changes (often subclinical) are present in most children with AD and food hypersensitivity and may account for the low percentile weights seen in many children with AD.

Despite years of debate, it is now apparent that food hypersensitivity plays a significant pathogenic role in AD in up to 40% of children with moderate to severe AD (118). In a recent search of the literature, Hoare identified 1165 possible randomized controlled therapeutic trials for the treatment of AD (119). Of these, however, only 272 had sufficient data for analysis. These trials covered at least 47 different interventions, which were broadly categorized into 10 main groups. Although the quality of reporting was generally poor and only limited statistical pooling was possible, the authors concluded that there was reasonable evidence from these randomized controlled trials to support the use of oral cyclosporin, topical corticosteroids, psychological approaches, and ultraviolet light therapy for treatment of AD. They concluded that there was insufficient evidence to make recommendations on maternal allergen avoidance for disease prevention, use of oral antihistamines or Chinese herbs, dietary restriction in established atopic eczema, homeopathy, house dust mite reduction, massage therapy, hypnotherapy, evening primrose oil, emollients, topical coal tar, and topical doxepin.

Although the pathogenic role of food hypersensitivity appears well established in some children with AD, the significance of food allergy in adults remains unknown. Given the current literature, it is apparent that further studies of this subset of AD patients with food-induced inflammation are necessary, and they should provide an excellent human model to study the IgE-mediated hypersensitivity reaction.

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12

Food-Induced Urticaria and Angioedema

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Urticaria and angioedema constitute a heterogeneous group of disorders that may be classified by duration and trigger factors. A classification based on clinical grounds and by trigger factors is convenient, but has inherent inconsistencies (Fig. 12–1). The distinction between acute and chronic urticaria is arbitrarily chosen and the duration of acute urticaria is normally limited to 6 weeks (1); classification of the many cases of recurrent acute attacks is difficult in cases of food-associated urticaria. Elicitation of wheals by direct contact between immunologic or non-immunologic stimuli, known as contact urticaria, is an important disease entity from an allergological point of view and is characterized by wheals confined to the area of contact. In contrast, wheals may erupt anywhere on the skin in the other types of acute urticaria (2).

Chronic urticaria can be further subdivided into primary urticaria and urticaria associated with other diseases (thyroid diseases, infection, or syndromes such as Schnitzler's or Muckle-Wells [3, 4]). Within the primary chronic urticarias, further classification into physical urticarias, which are elicited by cold, pressure, heat, ultraviolet light, and other such factors; and autoimmune urticaria, in which antibodies against IgE or against the FcεRI receptor on the mast cell (MC) are present (5); and the remaining types, usually called chronic idiopathic urticarias (CIU) (Fig. 12–1). This classification is suitable from a clinical point of view, because the physical urticarias are rarely associated with any other disease (including food allergy), thus extensive investigations are rarely needed (1, 6). It is, however, important to emphasize the frequent combination of physical urticaria and CIU in the majority of patients (7–9).

The single urticarial wheal is present on the skin for less than 24 hours; if it persists longer, urticarial vasculitis, which rarely has an allergic etiology, must be suspected (8). Diseases such as mastocytosis and urticaria pigmentosa are not associated with allergy, and will not be discussed here.

Angioedema is a variant of urticaria in which mainly the subcutaneous tissues, rather than the dermis, are involved. The same multiple etiology and lack of precise diagnosis that applies to chronic urticaria also applies to angioedema (10), with the exception of a hereditary form that accounts for about 1% of all angioedema cases without concomitant urticaria. In this form, a deficiency of the complement C1 esterase inhibitor in serum is found. This disease is not associated with allergy (11).

Pathophysiology

Skin biopsies of urticarial wheals reveal only sparse pathologic findings—the number of MCs are within the normal range, and by light microscopy usually only vascular and lymphatic dilatation are found, together with a variable perivascular cellular infiltrate consisting of lymphocytes, monocytes, neutrophils, and eosinophils.

The cell that is central to the pathophysiology of urticaria and angioedema is the cutaneous MC, which may be activated by immunologic or non-immunologic stimuli. Interestingly, by microdialysis technique, released histamine has been found to be confined to the wheal area only; no histamine is found in the surrounding flare area (12).

Acute Urticaria

The most common cause of acute urticaria is infection, especially in infants and children (13). In food allergy, acute urticaria is normally present

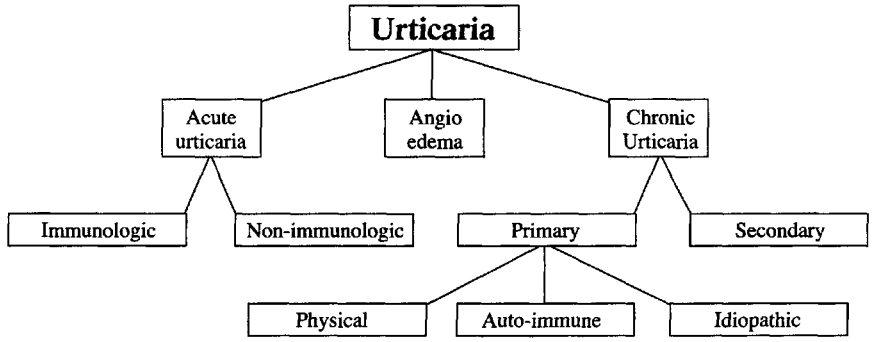

Figure 12–1. Classification of urticarias and angioedema.

together with symptoms and signs from other organ systems, such as the respiratory or gastrointestinal (GI) systems (14). As can be seen in Table 12–1, urticaria is elicited by challenge in about 12% of the challenges that have been reported. Although data on the exact incidence of type 1 food allergy in the population are not available, a reported prevalence of 7%–10% of children (14) suggests that the incidence of acute food-dependent urticaria is about 1%–2% in children.

Food additives can elicit acute urticaria in children, as was demonstrated by double-blind challenge with colorants and other additives in two trials where the incidence of acute urticaria in children attending a pediatric allergy clinic was found to be 1%–2% (15, 16).

Acute urticaria as the only sign of a food allergy has been reported rarely. In rare cases, monosymptomatic acute urticaria can be elicited by skin prick test (SPT) in highly sensitive patients, especially with unstandardized extracts (Fig. 12–2a and Fig. 12–2b). A 31-year-old female with known allergy to Brazil nut (and no other history of urticaria) experienced generalized urticaria requiring treatment with antihistamines and glucocorticoid during skin testing with Brazil nut and other nuts. From a stochiometric point of view, the total dose of absorbed allergens in this case would be <1 ng in total.

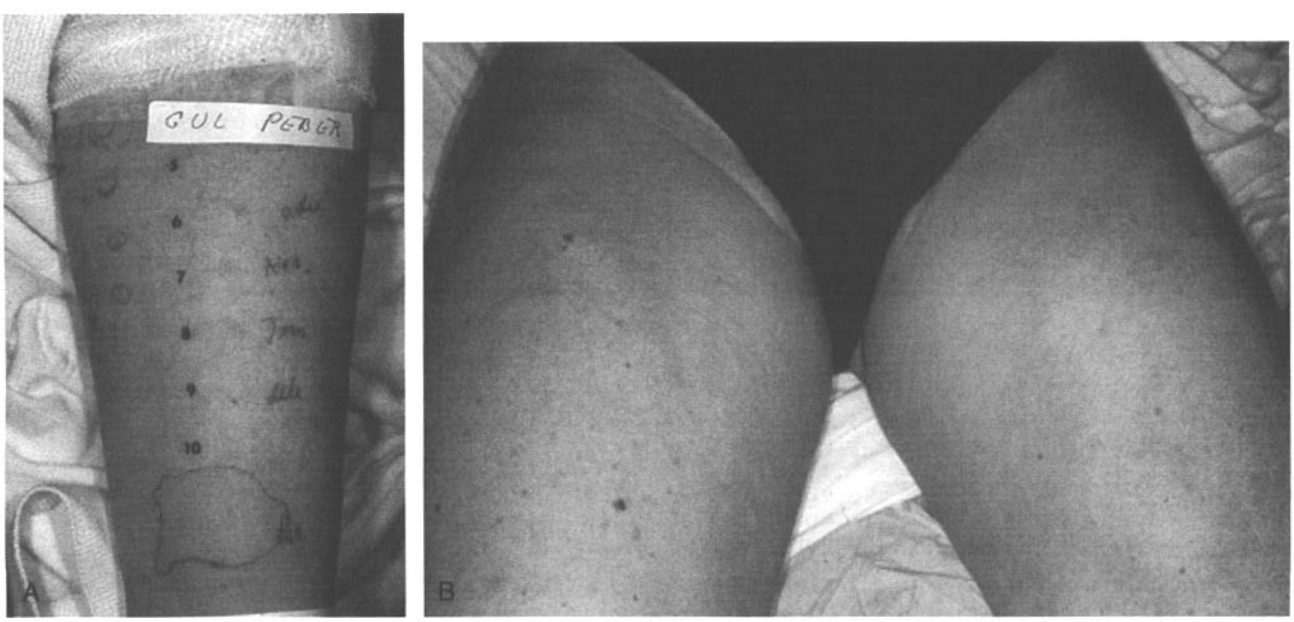

Figure 12–2a. SPT with fresh foods. Wheal elicited by Brazil nut is presented below number 10; it measures 32 mm by 49 mm.

Figure 12–2b. Generalized acute urticaria elicited by SPT in the same patient depicted in Figure 12–2a, 20 minutes after administration of SPT. Note typical wheal and flare on right thigh and confluent wheals proximally.

Table 12–1.
Incidence of Acute Urticaria in Food Allergy; Urticaria Reactions to Food and Additives

Food	*Number of studies (OFC/SBFC/ DBPCFC)*	*No. of patients reacting with urticaria*	*References*
Cow's milk	16 / 1 / 51	240 of 1634	29–89
Egg	7 / 1 / 40	183 of 1422	32, 33, 41–43, 47–52, 54–59, 61, 63, 65, 66, 68–73, 75, 77–80, 85, 86, 89–100
Peanut	14 / 1 / 18	146 of 611	33, 41, 57–59, 63, 68, 70–72, 77–79, 85, 92, 97, 100–110
Additives	4 / 2 / 1	46 of 179	15, 111–121
Mustard	0 / 2 / 0	35 of 64	63, 122
Cod	0 / 1 / 6	20 of 187	51, 52, 55, 57–59, 63, 68, 69, 71, 72, 77, 80, 85, 86, 92, 93, 123–126
Goat's milk	0 / 1 / 1	15 of 27	37, 63
Wheat	6 / 1 / 30	9 of 261	32, 43, 48–50, 52, 54–59, 63, 68, 69, 71–73, 75, 77, 78, 80, 85, 86, 89, 93, 96, 100, 109, 110, 127–131
Soy	6 / 1 / 23	5 of 281	31, 33, 43, 51, 52, 54–59, 63, 68, 70, 72, 73, 75, 77, 78, 85, 86, 89, 92, 93, 96, 99, 132–134
Celery	4 / 0 / 6	2 of 33	92, 100, 104, 110, 135, 136
Potato	4 / 0 / 3	2 of 16	32, 72, 73, 86, 104, 137
Hazelnut	3 / 1 / 3	1 of 137	32, 63, 69, 77, 92, 104, 138, 139
Shrimp	0 / 1 / 6	1 of 30	46, 52, 63, 77, 97, 100, 140
Apple	7 / 1 / 3	1 of 159	63, 78, 104, 109, 141–146
Cashew	0 / 0 / 2	1 of 6	33, 52
Garlic	2 / 0 / 1	1 of 3	32, 92, 110
Orange	3 / 0 / 3	1 of 47	68, 73, 78, 89, 104, 109
Pea	2 / 1 / 3	1 of 46	63, 71, 77, 99, 137
Almond	2 / 1 / 1	0 of 36	63, 109, 110, 145
Apricot	2 / 0 / 1	0 of 12	110, 145, 147
Avocado	1 / 0 / 0	0 of 1	137
Banana	2 / 1 / 1	0 of 6	48, 63, 77, 137
Barley	1 / 0 / 2	0 of 9	89, 93, 131
Bean	2 / 0 / 0	0 of 2	110, 137
Beef	2 / 1 / 4	0 of 30	52, 59, 71, 86, 89, 148
Beer	0 / 0 / 1	0 of 1	97
Carrot	3 / 0 / 2	0 of 14	97, 104, 109, 110, 137
Citrus	1 / 0 / 0	0 of 10	126
Coconut	0 / 0 / 1	0 of 1	46
Corn	3 / 0 / 3	0 of 26	78, 80, 131, 137, 149
Fennel	2 / 0 / 0	0 of 5	104, 110
Fenugreek	2 / 0 / 0	0 of 5	150
Kiwi	1 / 1 / 1	0 of 15	63, 110, 151
Lamb	2 / 0 / 0	0 of 7	137
Lentil	0 / 1 / 1	0 of 7	63
Lettuce	1 / 0 / 0	0 of 1	136
Lupinflour	0 / 1 / 0	0 of 7	63
Melon	1 / 0 / 1	0 of 18	104, 152
Oat	0 / 0 / 3	0 of 8	46, 93, 131
Onion	1 / 0 / 0	0 of 1	110
Peach	3 / 0 / 2	0 of 101	104, 145
Plum	1 / 0 / 1	0 of 12	145, 147
Pork	3 / 1 / 1	0 of 25	46, 63, 86
Rabbit	1 / 0 / 1	0 of 1	137
Rice	2 / 1 / 2	0 of 11	32, 89, 131, 137, 153
Rye	1 / 0 / 5	0 of 9	49, 71, 77, 92, 131
Sesame seeds	0 / 1 / 1	0 of 6	63, 154
Strawberry	1 / 0 /1	0 of 23	110, 145
Sunflower	1 / 0 / 0	0 of 25	109
Tomato	5 / 0 / 3	0 of 25	32, 46, 73, 89, 104, 109, 110, 155
Turkey	0 / 0 / 0	0 of 1	77
Vanilla	0 / 1 / 0	0 of 4	63
Walnut	2 / 0 / 0	0 of 5	104, 110
Yeast	0 / 0 / 1	0 of 1	67
Zucchini	0 / 0 / 2	0 of 5	77, 156
Total	127 / 25 / 242	710 of 5622	

The average number of patients demonstrating urticaria upon challenge is 12%.

The mechanisms underlying elicitation of non-localized urticarial wheals on the skin immediately after oral challenge with non-tolerated foods remain obscure. Wheals often develop within less than 1–2 minutes after ingestion of the food; thus, direct contact between absorbed proteins (via the blood stream) from the food and the MC in the skin seems unlikely.

As mentioned previously, urticaria may develop anywhere on the skin, but special attention should be paid to itching of the palms and soles, where wheals are often difficult to see because of the tightly bound epidermis; this sign may be a special warning signal for subsequent development of systemic anaphylaxis (17).

Contact Urticaria

In contact urticaria an immediate wheal and flare response develops upon topical application of a substance to the skin. The substances involved are numerous and may be chemically defined molecules such as cinnamic acid, benzoic acid, or parabens, or chemically undefined, as are found in arthropods, plants, spices, fruits, or fish (18).

Contact urticaria can be subdivided into immunologic and non-immunologic contact urticaria (2, 18). In immunologic contact urticaria, wheals are elicited by direct contact with the proteins to which the patients are sensitized, for example, on the hand of a latex-sensitive patient wearing latex gloves or periorally in a food-allergic infant. This condition should not be confused with non-immunologic perioral contact urticaria elicited by the sorbic and ascorbic acids in tomatoes and citrus fruits (2). This harmless phenomenon, which reportedly rarely if ever is followed by a systemic reaction, is often misinterpreted by parents and physicians as an allergic reaction, and unnecessary avoidance of the offending food can result. True allergic contact urticaria can proceed to a systemic reaction. Therefore, a thorough diagnostic workup to rule out or demonstrate involvement of the immune system is important so that the patient (or most often the parents) is properly informed.

Contact urticaria to foods is also common in cooks and food handlers (19). A characteristic feature in these patients is that, although skin contact with foods such as fish or meat may cause wheals, oral intake of the same food is often tolerated (20).

In the vast majority of patients, contact dermatitis (an immunologic type IV reaction in the skin) is due to sensitization to small molecules such as nickel, but protein contact dermatitis is seen especially in food handlers, where an allergic hand eczema develops over 2–3 days of contact between the skin and the food in question (19).

Treatment of immunologic contact urticaria is avoidance, because a systemic reaction may follow the localized reaction, whereas non-immunological contact urticaria normally is harmless and may be prevented by application of an ointment around the mouth of the infant prior to feeding.

Chronic Urticaria

Although there is little doubt that acute urticaria in food-allergic patients belongs to the Th2-related diseases, new data point toward chronic urticarias belonging to the Th0 diseases (6).

According to Greaves (5), food additives are causative in less than 5% of the cases seen in his clinic; therefore, most of the chronic urticarias (and all of the purely physical urticarias) seem not to be associated with hypersensitivity to foods or additives. In a study in children, foods were incriminated in 4% of the cases, whereas additives were thought to be involved in only 2.6% of the cases (21). In contrast, Henz and Zuberbier (22) find most chronic urticaria to be food-dependent and not idiopathic. On a diet eliminating preservatives, dyes, and natural pseudoallergens, 73% of their adult patients experienced remission over a period of 6 months, compared to 24% who had spontaneous remission. Subsequent double-blind challenges revealed that 18% of the patients reacted to dyes and preservatives, whereas 71% reacted to pureed tomatoes. In a subsequent study, Zuberbier (23) demonstrated that low molecular weight substances (salicylate, histamine, aldehydes, and ketones) were responsible for the reactions. Abnormal histamine metabolism has been described in chronic urticaria, but the nature of the involvement remains to be elucidated (24). Ehlers (25) reported about the same percentage (75%) of reactors to additives in children.

The discrepancies between the reported incidences are too large to be attributed to differences in patient populations, although at present no epidemiological studies on the actual incidence of urticarias in different populations exist. Differences in patient selection criteria may play a central role.

More well-controlled epidemiologic trials focusing on food additives and chronic urticaria are

needed to establish their role in disease. Currently the question is unresolved; therefore, a diet omitting additives may be worth trying in severe cases of chronic urticaria unresponsive to conventional antihistamine therapy. No data exist on a possible relationship between additive-dependent and autoimmune urticaria.

Although aspirin is degraded to salicylate in plasma and augments food allergy (26), no conclusive data exist on a possible role of salicylate in the same mechanism; aspirin-intolerant asthmatics tolerate salicylate in high amounts, so at least in these patients, it is not likely that the salicylate component is involved (27).

Treatment

Once the diagnosis of a food dependent urticaria is established, the only available treatment is avoidance of the food or additive in question. Food-dependent acute urticaria can often be effectively treated with antihistamines, but these drugs should be used with caution in food-allergic patients because they also block the warning signs preceding a systemic reaction. The oral allergy syndrome (OAS), which most often is the initial warning sign, is prevented by prior intake of antihistamines (28) and careful instruction of the patient is necessary.

Conclusions

Acute urticaria is a frequent part of the symptoms and signs elicited in food-allergic patients. Contact urticaria can also be attributed to direct contact with foods, but the distinction between an immunologic and a non-immunologic contact urticaria is important.

The role of food hypersensitivity in chronic urticarias remains unsettled. In severe cases, a trial diet avoiding additives may be considered, but it is our view that, although we frequently use such a diet in the diagnostic workup of our patients, we rarely see a clear-cut response to an additive-free diet.

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13

Oral Allergy Syndrome

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Definition

Oral allergy syndrome (OAS) is the clinical term used to refer to food allergy symptoms involving the mouth and the pharynx (1, 2). The name of the syndrome focuses on the need for direct contact of the oral mucosa with the offending food to trigger local symptoms, usually in the form of oral itching, lip swelling, and labial angioedema, but occasionally also glottal edema. OAS symptoms arise immediately (within 1–5 min) after the culprit food(s) comes in contact with the oral mucosa. Symptoms recur regularly after each new exposure to the culprit food(s) (2). OAS is an IgE-mediated food allergy that can be diagnosed in patients by positive allergy tests such as specific IgEs, skin tests, etc. During the last few years, OAS has been defined as a distinct condition by clinical studies using double-blind placebo-controlled oral food challenges (DBPCFCs), and by studies that identified several allergens involved in this syndrome.

The first reports of oral symptoms associated with food allergy date back more than 50 years (3, 4). Today OAS constitutes a true clinical syndrome with complex characteristics. OAS occurs primarily in atopic subjects—with or without pollenosis—who are sensitized to fruits and vegetables, especially when consumed raw. In many cases oral symptoms appear first and are then followed by more complex symptoms that may include other organs or systems or are generalized (5). Occasionally, more severe conditions such as anaphylactic shock or glottal edema may be associated with oral symptoms (5–9). For these reasons, OAS should not be considered a minor clinical syndrome localized to the oropharynx, but rather as a condition that could involve more severe and even life-threatening symptoms.

OAS is used as a synonym for the association between fruit-and-vegetable allergy and tree-pollen allergy, especially in the case of birch-tree allergy, i.e., the pollen-food allergy syndrome (10). However, OAS is often present in subjects who are not allergic to pollens; for example, it is found in subjects allergic to Prunoideae, particularly in the Mediterranean area (6, 11), and in subjects allergic to latex (*Hevea brasiliensis*) in the so-called latex-fruit allergy syndrome, which in turn may or may not be associated with allergy to pollens (12–14).

Oral allergy symptoms provoked by foods of animal origin (such as milk, eggs, shrimp, etc.) (1, 15–17) occur less frequently. In contrast, oral symptoms provoked by plant-origin foods almost always involve OAS, to the point that it is known as the most characteristic sign of plant-origin food allergy. One important question is whether the term oral allergy syndrome should be used only to refer to a clinical entity characterized by an IgE-mediated allergic sensitization to plant-origin foods, or should it include all the oropharyngeal manifestations of food allergy? Although many authors seem to share the former position, many observations of OAS associate the allergy to animal-origin foods, suggesting caution when considering the culprit food to diagnose OAS.

Epidemiology

Few studies have addressed the prevalence of OAS, and all of them concerned plant-origin food OAS. A recent study evaluated food allergy and intolerance data gathered by questionnaire from 1537 subjects who were randomly sampled

and cross-sectionally stratified for age and sex (17). Of the reported allergic reactions, 659 (42.9%) were thought to be caused by OAS. The frequency of occurrence of OAS varied according to the culprit food: 72.3% for walnuts, 68% for apple and related fruits, 62.9% for Prunoideae, 94.5% for tomato, 43.6% for other vegetables, 24.4% for citrus fruits, 37.7% for other fruits, 30.3% for peanuts, 28.6% for soy, 20% for milk, 13.6% for fish, 8.3% for spices, and 25% for wine. OAS was caused most frequently by fruits and nuts; moreover, ingestion of these foods was most often associated with systemic reactions.

Adults develop OAS to fresh fruits and vegetables more frequently than children. A study from Israel found that fruits and vegetables were the most common sources of food allergy for patients over the age of 10 (18). The association between OAS and pollen allergy has been widely described. Two studies determined OAS prevalence in subjects allergic to pollens. A study from Switzerland (19) reported that approximately 35% of patients allergic to pollens shared allergic symptoms and positive skin prick tests (SPT) to fresh fruits and vegetables. Pastorello et al (20) observed a prevalence of 25% of OAS among 300 patients allergic to pollens. Allergy to fruits and vegetables occurred most frequently in subjects with hay fever from birch allergy. Studies conducted in subjects allergic to birch pollen found different prevalences of OAS in different European countries: 35% in Finland, 63% in Sweden, 75% in Austria, and 59% in Italy (20–23).

Fruit allergy also often occurs in subjects allergic to rubber latex. In one study, 27% of latex-allergic subjects had positive skin tests for latex, and 14% of these showed local oral symptoms (24). Another study found that 13 (52%) of 25 patients allergic to latex showed symptoms after eating some fruits (13). A third study reported that 69.1% of latex-allergic subjects had positive IgEs for some associated fruits, and that 42.5% had allergic reactions after eating these fruits (14).

Allergy to fruits and vegetables can occur in the absence of pollen allergy, albeit less commonly. Ortolani et al (2) found that 21% of subjects allergic to fruits and vegetables were not allergic to pollens. Fernandez-Rivas et al (6) found 15% of subjects allergic to fruits and vegetables were not allergic to pollens. Cuesta-Harranz et al (25) reported that 17% of subjects allergic to peach did not have any associated allergy to pollen.

Symptoms

Typically, symptoms occur immediately after the culprit food comes into contact with the oral mucosa. The rapidity of the reaction is one of the peculiar characteristics of OAS, and it is one of the most valuable diagnostic elements in determining a given food's role in provoking symptoms. A study of the time of occurrence of symptoms after food ingestion showed that most patients had symptoms within 5 minutes of food contact, and in only three (7%) of 43 patients did symptoms appear after 30 minutes (2). DBPCFC studies further confirmed the rapidity of onset of symptoms for two other foods: a few minutes for hazelnut (26) and within 30 minutes for cantaloupe (9). Oral symptoms are immediate and arise in the lining of the lips, the oropharynx, and the gastrointestinal tract, which comes into direct contact with the offending food (Table 13–1). Symptoms consist of intra-oral and lip irritation, angioedema, papulae, and, more rarely, blisters, which appear within a few minutes after contact with the culprit food. Systemic symptoms such as urticaria, rhinitis, asthma, and occasionally even anaphylactic shock, may appear after contact with the culprit food associated to the local symptoms (Table 13–2).

It seems appropriate to classify OAS symptoms into four levels of increasing severity: level 1, oral mucosa symptoms only; level 2, oral mucosa plus gastrointestinal symptoms; level 3, oral mucosa symptoms plus systemic symptoms (urticaria, rhinoconjunctivitis, or asthma); and level 4, oral mucosa symptoms plus life-threatening problems (glottal edema, anaphylactic shock) (27). This classification of symptoms shows the evolving pathway of this syndrome. Local symptoms clearly prevail, as has been well documented by studies on patients with allergic reactions to fresh fruits and vegetables (5, 28–30). In a study of 90 patients suffering from ragweed allergic rhinitis and allergy to melon and banana, Anderson et al (28) found that all the subjects experienced oropharyngeal symptoms. Similarly, Eriksson et al (29) reported that

Table 13–1.
Skin-Mucosal Contact Provoked Symptoms Observed in 706 OAS Patients (5)

Symptoms	*Number of Patients*	*%*
Oral only	596	84.4
Oral + gastrointestinal	67	9.5
Gastrointestinal only	29	4.1

Table 13–2.
Systemic Symptoms Associated with Oral/Gastrointestinal Contact Symptoms Observed in 706 OAS Patients (5)

Symptoms	*Number of Patients*	%
Urticaria/angioedema	191	27.0
Rhinitis	63	8.9
Asthma	50	7.1
Conjunctivitis	25	3.5
Anaphylactic shock	15	2.1

199 (78%) of 255 patients allergic to birch and related foods (e.g., apple, peach, cherry, pear, and carrot) complained of symptoms localized in the oral mucosa. Ortolani et al reported that local symptoms occurred in 219 (83.6%) of 262 patients allergic to fresh fruits and vegetables (2). In a subsequent study on a larger population, the same authors found that 663 (93.9%) of 706 patients had local oral symptoms (5) (Table 13–1).

The clinical features of OAS have emerged from a series of studies published in the last few years in Europe. These studies applied DBPCFC to diagnose allergy to fruits and vegetables (9, 26, 31, 32). These studies, carried out on adults, showed that oral symptoms (level 1) were the most common clinical manifestation elicited by the following plant-origin culprit foods: carrot, celery, hazelnut, melon (Table 13–3). In a small percentage of subjects, OAS appeared to be associated with gastrointestinal symptoms (level 2); in 21% of cases it was associated with the following systemic symptoms: cutaneous (9.5%), rhino-conjunctival (6.3%) and asthmatic (3.2%). These extra-oral symptoms observed in these 126 DBPCFC positive patients were self-limiting and slight, probably because of a patient selection regime that excluded severe cases.

The most severe local symptom of OAS is glottal edema. This symptom appears particularly frequently in allergy to celery, a vegetable known to induce severe allergic reactions (33). In a study of 262 patients with OAS from fresh fruit and vegetables, Ortolani et al observed 62 cases (26%) of glottal edema after ingestion of several fresh foods (2). In a subsequent study, the same authors reported that 98 (13.9%) of 706 OAS patients presented at least one well-documented episode of glottal edema (5).

In some cases, OAS may rapidly evolve to a generalized anaphylactic reaction with respiratory difficulty, generalized urticaria, angioedema, and hypotension. Ortolani et al (5) found that 15 (2.1%) of 706 patients with OAS had anaphylactic shock after ingesting one of the following foods: peach, apricot, walnut, cherry, tomato, apple, hazelnut, or pear. One study examined the prevalence of severe reactions in OAS and reported six (23%) severe anaphylactic reactions occurring after oral symptoms in 26 patients (7). The foods responsible for these severe reactions were banana, apple, plum, nectarine, cherry, apricot, strawberry, grape, carrot, and peanut. Two (10.5%) anaphylactic shock reactions were also reported in 19 patients allergic to cantaloupe (9). Subjects allergic only to peach but who had no pollen allergy appeared to have a higher frequency of severe allergic reactions, compared to subjects who were allergic to peach and pollen both. One study showed that 36% of subjects allergic to peach but without pollenosis had at least one anaphylactic shock episode, in contrast to only 9% of subjects allergic to both peach and pollen (6). It seems that sensitization to lipid transfer protein (LTP) allergens is responsible for the severity of symptoms occurring in these subjects. LTP allergen sensitivity is also associated with severe reactions reported in corn and hazelnut allergy (34, 35). A high association between OAS and systemic anaphylaxis has also been reported in children (e.g., 3 [38%] of 8 children with OAS had systemic anaphylaxis) (8).

Table 13–3.
Symptom Distribution in 126 DBPCFC-positive Patients (9, 26, 31, 32)

Symptoms	*Number of patients*	%
OAS alone (grade 1)	100	79
OAS+ gastrointestinal (grade 2)	7	5
OAS+ systemic (grade 3)	21	16
skin	12	9.5
rhino-conjunctive	8	6.3
asthma	4	3.2
OAS + life threatening*	0	0

* exclusion criterion for DBPCFC.

Clusters of Hypersensitivity

Sensitization to some fruits or vegetables may be significantly associated with sensitization to other foods belonging to the same botanical family, as well as with sensitization to botanically unrelated foods. Clinically this phenomenon has been defined as "cluster of hypersensitivity" (36).

Several clusters have been observed since the first reports of this disease. For example, in 1984 Eriksson (36) reported the following clusters based on a long list of case studies:

1. Hazelnut, walnut, brazil nut, almond, with desert almond, as well as nuts combined with apple and stone fruits.
2. Stone fruits in combination with apple and pear.
3. Apple and pear.
4. Kiwi fruit and avocado.
5. Potato and carrot.
6. Parsley and celery.

Other "clusters" have also been described: celery, carrot, mugwort, and spices (37); apple, carrot, and potato (38); fennel and celery (2); cherry and apple (2); melon, watermelon, and tomato (2); fennel, celery, and carrot (39); lettuce and carrot (40); tomato and peanut (41); and, celery, cucumber, carrot, and watermelon (42). Moreover, Pastorello et al (11) performed oral open food challenges to check for clinical cross-reactivity in members of the Prunoideae subfamily such as peach, apricot, plum, and cherry, and found high cross-reactivity between these fruits.

During recent years, it has become increasingly evident that the presence of common allergens, or allergens with a similar molecular structure but belonging to different foods, may influence allergic cross-reactivity. This finding might help explain the clustering of allergy-provoking foods. The most common clusters in OAS are: 1) birch-fruit syndrome due to cross-reactivity between Bet v 1 homologous proteins (PR-10); 2) latex-fruit syndrome due to PR-2, β-1,3-glucanase, and PR-3 class 1 chitinase sensitization; and 3) LTP-PR 14 sensitization.

Many past observations support the existence of three syndromes and can be encompassed by them. Birch-fruit syndrome is characterized by allergy to birch and hazel pollen associated with food allergy toward apple, pear, celery, carrot, parsley, potato, hazelnut, and less frequently, cherry and apricot (10, 21, 29). Latex-fruit syndrome is characterized by allergy to latex and to avocado, banana, chestnut, fig, kiwi (13, 14), tomato, and potato (43). LTP syndrome is characterized by allergy to peach with cross-reactivity extended to other Prunoideae such as cherry, apricot, plum, apple (Mal d 3), and corn (11, 27, 34, 44).

Association With Rhinitis or Asthma Due to Pollen Allergy

In many cases, OAS to fresh fruits and vegetables is associated with allergy to pollens. The association with birch pollen allergy has been confirmed, and other associations have been described involving pollens from grasses, ragweed, and mugwort (Table 13–4) (45, 46). Hay fever often occurs before OAS with a significant difference in the timing of occurrence (2). One study using immunoblot inhibition with the major allergen of birch, Bet v 1, showed that sensitization to pollen causes sensitization to fruits and vegetables. Sensitization to birch pollen is certainly the main reason that OAS develops toward birch-related foods (e.g., apple, hazelnut, carrot, celery, etc.). Mugwort's pollen allergy may be associated with food allergy toward celery, carrot, and spices (47). Grass pollen allergy was found to be related to food allergy to tomato, melon, watermelon, and orange (2, 41). The association between kiwi fruit allergy and grass pollen allergy was reported in Italy (5), whereas kiwi fruit allergy has been described in association with birch pollen allergy only in Scandinavia and the US (48, 49). In the US, an association between ragweed allergy and allergy to melon and banana has been re-

Table 13–4.
Associations Between Pollinosis and Allergy to Fresh Fruits and Vegetables

Author	*Year*	*Pollen*	*Fruit/Vegetable*
Tuft, Blumstein (3)	1942	Birch	Apple
Juhlin-Danfelt (4)	1948	Birch	Apple, hazelnut
Anderson et al (28)	1970	Ragweed	Melon, banana
Eriksson (29)	1978	Birch	Apple, hazelnut, carrot, potato
Wüthrich (45)	1981	Mugwort	Celery
Pauli et al (46)	1982	Mugwort	Celery
Wüthrich (37)	1985	Mugwort	Celery, carrot, spices
Pauli et al (47)	1985	Birch, mugwort	Celery
Enberg et al (30)	1987	Ragweed	Watermelon, gourd family
Ortolani et al (5)	1988	Grass	Tomato, melon, watermelon
De Martino et al (41)	1988	Grass	Tomato, peanut
Ebner et al (21)	1991	Birch	Apple
Ortolani et al (5)	1992	Birch	Celery, fennel

ported (28). Ragweed allergy has also been found to be associated with allergy to members of the gourd family (i.e., watermelon, cantaloupe, honeydew, zucchini, and cucumber) (30). A common finding of all these studies is a statistically significant relationship between the presence of allergy symptoms to fresh fruits and vegetables and high levels of specific IgE to related pollens. In a study by Enberg et al (30), only those patients with the highest radioallergosorbent test (RAST) levels to ragweed presented symptoms to fruits of the gourd family. Similarly, Eriksson et al (50) found that high levels of birch-specific IgE antibodies in serum were closely related to the occurrence of allergy to fruits and vegetables. Finally, Ebner et al (21) confirmed a higher incidence of apple allergy in subjects with high levels of birch-specific IgE compared to subjects with lower IgE values.

Etio-pathogenesis

OAS is a true IgE-mediated food allergy. When this syndrome is suspected, diagnosis is based on specific tests that demonstrate the presence of IgE mechanisms. If these tests are negative, an irritant mechanism due to enzymatic components or the acidic nature of certain foods may be involved instead. The route of sensitization to plant-origin foods has not yet been determined. Only in OAS associated with pollen allergy are we almost certain that the primary sensitization is toward pollens, and that food allergy is a consequence. Kazemi-Shirazi et al (51) demonstrated that in subjects with birch pollen allergy and OAS to apple, all the allergenic epitopes are on Bet v 1, the major pollen allergen, whereas only a few of them are represented on its homologous counterpart in apple, Mal d 1. Moreover, the cross-reactivity between apple and birch pollen, which causes OAS, is not only serologic but also at the level of allergen-specific T helper cells (52). On the basis of this observation the authors hypothesized that, in early infancy, contact with the implicated foods could prime T cells that could then react with pollens. In latex-fruit syndrome, the primary sensitization is still unknown, and in LTP syndrome it seems to be due to peach LTP, because in all crosswise inhibition experiments performed with pollen, peach LTP was the strongest inhibitor (53).

Localization of symptoms to the oral mucosa is another unsolved issue. Amlot et al (1) suggested that local oral symptoms are caused by a high concentration of mast cells in the oropharyngeal mucosa. This condition would lead to a stronger interaction between the allergens that are rapidly released from the fruit or vegetable and specific IgE on the cell surface. This interaction, in turn, might explain the early onset of OAS symptoms. Local oral symptoms are also caused by a high concentration of allergens on the oral mucosa that are rapidly released from the culprit fruit or vegetable as they come in contact with the saliva of the allergic subject. This kind of reaction resembles that seen with pollens, which react in their intact form with IgE antibodies bound to mast cells in the mucosa of the upper and lower airways. An alternative hypothesis is that the high concentration of T cells in the oropharyngeal lymphoid tissue might have a food-specific T cell response. For example, in birch-fruit syndrome, a positive birch pollen-specific T cell response was found only in the injured skin of patients reacting with atopic eczema following ingestion of birch-related foods in DBPCFC experiments (54).

Allergens

During recent decades, a number of allergenic proteins of plant-origin foods have been characterized. In several cases, descriptions of allergens with homologous sequences in different allergenic sources were the key to understanding the molecular basis of the cross-reactivity so common in OAS (55). The presence of such similar components in pollens and foods is the main cause of the three previously mentioned clinical syndromes. The allergens responsible for these syndromes will be described below.

Birch-Fruit Syndrome

The major allergens of birch, Bet v 1 and Bet v 2, are proteins that share significant amino acid sequences with other proteins that are widespread in the vegetable kingdom, especially in apple, pear, hazelnut, carrot, celery, potatoes, parsley, and beans. The association between OAS to two or more of these foods and birch hay fever, now known as "birch-fruit syndrome," is due to the cosensitization to these proteins in different sources (56, 57). Bet v 1 and its apple homolog, Mal d 1, are proteins with a molecular mass of 17 kilodaltons (kDa) (58). These proteins share 64.5% of their amino acid sequence identity, and this homology explains why

about 70% of birch-allergic patients are also allergic to apple. Other Bet v 1-homologous allergens are Api g 1 in celery, Pyr c 1 in pear, Dau c 1 in carrot, Pru ar 1 in apricot, Pru a 1 in cherry, and Cor a 1 in hazelnut (10). The involvement of allergens such as Mal d 1, Api g 1, and Dau c 1 has been confirmed by the IgE reactivity of the sera of subjects positive at DBPCFC with the relevant food items (31, 32, 59). Pastorello et al (35) demonstrated the clinical importance of hazelnut's major allergen, which is an allergen with 72% amino acid sequence identity to Bet v 1 (60). In a multi-center study within the framework of an EU project, we found that in 67 subjects with a positive DBPCFC result for hazelnut, the most important allergen was an 18 kDa protein entirely cross-reacting with Bet v 1 (35). All these patients developed OAS, showing that this allergen was the basis of their symptoms (26). In inhibition experiments with sera from the same patients, we demonstrated that this allergen is destroyed by roasting the hazelnuts and is thus quite labile (35). All these Bet v1 homologous allergens are proteins belonging to group n.10 of the pathogenesis-related (PR) protein family (61), being thus named PR-10. These are plant-defense proteins that plants express (through regulation of their mRNAs) in response to different environmental, chemical, or biological attacks. As shown by Son et al (62) the amino acid serine-112 in Mal d 1 and Bet v 1 is essential for both IgE binding and cross-reactivity between them. It is interesting to observe that this SER 112 is conserved in all reported sequences of PR-10.

Another important cross-reacting allergen of birch with foods is Bet v 2, which belongs to the profilin family. These actin-binding proteins regulate cellular movement and are ubiquitous in nature (63). Profilin is the 12 kDa actin-binding protein first identified as an allergen in birch pollen and now found in several important allergenic sources. It is particularly important in celery, where it is also involved in the celery-mugwort-spices syndrome (64). Bet v 2 homologs are present in apple, pear, celery, carrot, and potatoes. Although the in vitro cross-reactivity of the various profilins is well recognized, their clinical role has never been satisfactorily demonstrated.

Other allergens also seem to be important in the cross-reactivity between birch and related foods. For example Karamloo et al (65) described a 33 kDa protein in birch, Bet v 5, where it behaves as a minor allergen belonging to a family of isoflavone reductase-related proteins. Bet v 5 showed a high degree of cross-reactivity with pear, a well-known birch-related food (65). In our study of hazelnut allergens (35) we found three other major allergens at 32 kDa, 35 kDa, and 47 kDa (represented respectively by a 2 S albumin, a legumin, and a sucrose-binding protein) that were totally inhibited by preincubation with birch pollen extract; this showed indirectly the presence of cross-reactive structures in birch that could be either cross-reacting carbohydrate determinants or new cross-reacting allergens.

Lipid Transfer Protein Syndrome

In the Mediterranean area, IgE-mediated allergic reactions to several plant-origin foods are not associated with pollen hypersensitivity but are due to sensitization to molecules belonging to the LTP family. These ubiquitous proteins (66) are present in homologous forms in many fruits and vegetables and cause a specific sensitization that is at the basis of some severe systemic reactions. LTPs are defense proteins up-regulated in some plants in response to infection by various fungal pathogens (67). For this reason they have been included in the PR protein family, forming group n. 14 that is thus named PR-14 (10). We first identified an LTP as the major allergen of peach, Pru p 3 (Fig. 13–1 and Fig. 13–2). This is a 91 amino acid molecule with a mass of 9.2 kDa characterized by a structure typical for all known LTPs containing eight strictly conserved cysteine residues (27). It was especially resistant to heating and acid treatment, which may explain the in vivo stability demonstrated by this allergen. In fact, the only technological treatments found to decrease the amount of peach major allergen were chemical (lye) peeling of fruits and juice ultrafiltration (68). By studying peach-allergic patients we found that the LTP major allergen was the only one recognized by the IgE antibodies of subjects allergic to peach but not allergic to pollen (27). Furthermore, as in a previous study (11) we confirmed an in vivo and in vitro IgE cross-reactivity between peach, apricot, plum, and cherry. After we discovered the important role played by LTP allergen in peach, we tested IgE-binding LTP proteins in these other allergenic sources, and found that LTPs were also the major allergens in plum (Pru s 3) and apricot (Pru ar 3), and that both were highly homologous to peach LTP major allergen (69, 70) (Table 13–5 and Table 13–6). From apricot we purified a second LTP with a lower molecular weight (7 kDa), a sequence homologous to Pru ar 3 but

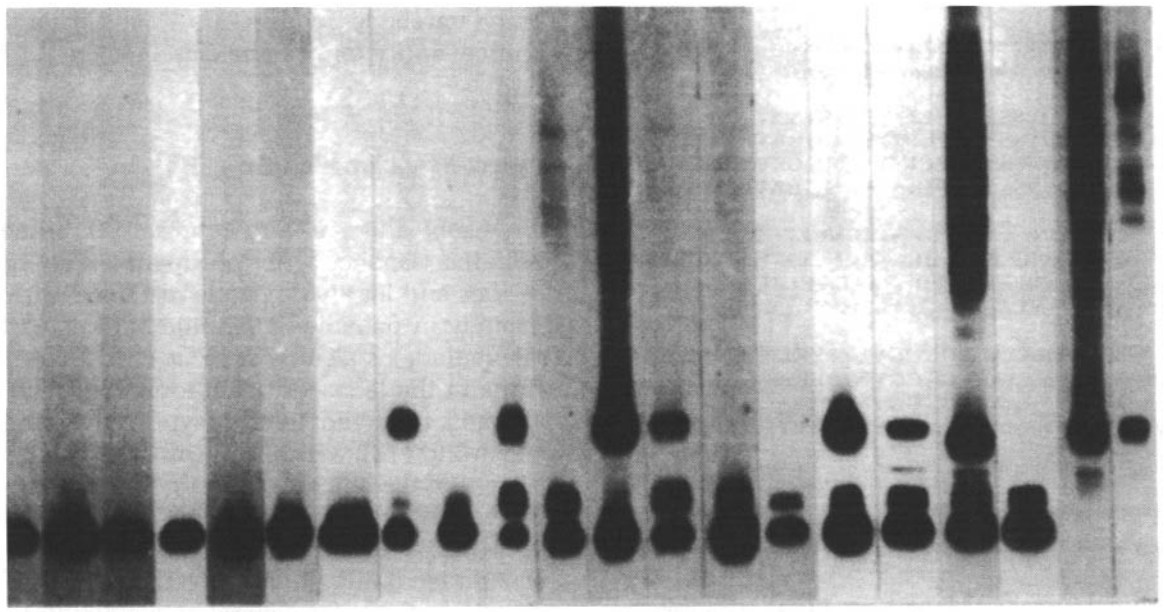

Figure 13–1. Autoradiographs of IgE-immunoloblot analysis of peach extract with sera from 21 patients, presenting specific IgE to peach. The molecular weight of some of the allergens are given. Patients 1 to 19 presented OAS to peach. (Reprinted from Pastorello EA, et al. *J Allergy Clin Immunol 1994;94:699–707.*)

with no IgE binding activity (71). As we found for peach, the LTP major allergens in apricot and plum were the only allergens recognized by IgE antibodies of subjects not allergic to pollen. Another LTP was also found to be a major allergen of cherry (72). After comparing IgE binding to cherry in Italian and German patients, we found that only Italian patients were sensitized to this allergen (72). Similarly, in a previous study we showed that Italian patients allergic to apple but not to birch were sensitized only to an LTP allergen having a high percentage of homology with peach LTP (44). Almost all of these patients were allergic to peach and to other fruits of the Rosaceae family. It is interesting that patients allergic to apple but not to birch have never been described in northern and middle Europe, whereas they are usually observed in Spain, where a specific reactivity only to

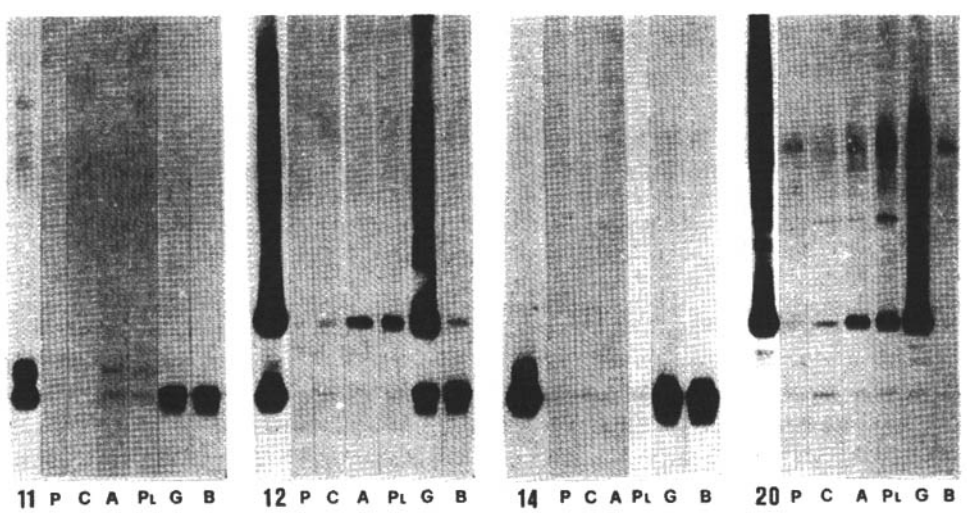

Figure 13–2. Immunoblotting inhibition in the sera of four patients. P, peach; C, cherry; A, apple; PL, plum; G, grass; B, birch. (Reprinted from Pastorello EA, et al. *J Allergy Clin Immunol 1994;94: 699–707.*)

Table 13–5.
Amino Acid Sequence Alignment of Peach, Apricot, Plum, and Cherry 9 kDa Major Allergens

Peach	ITCGQVSSALAPCIPYVRGGGAVPPACCNG
Apricot	ITCGQVSSSLAPCIGYVRGGGAVPPACCNG
Plum	ITCGQVSSNLAPCINYVKGGGAVPPACCNG
Cherry	LTCGQVSSNLAPCIPYVRGGGAVPPACCNG
Peach	IRNVNNLARTTPDRQAACNCLKQLSASVPG
Apricot	IRNVNNLARTTPDRRTACNCLKQLSGSISG
Plum	IRNNNNLARTTADRRAACNCLKQLSGSIPG
Cherry	IRNINNLAKTTASRQAACNCLKQLSASVPG
Peach	VNPNNAAALPGKCGVHIPYKISSATNCATVK
Apricot	VNPNNAAALPGKCGVNIPYKISASTNCATVK
Plum	VNPNNAAALPGKCGVNVPYKISASTNCATVK
Cherry	VNANNAAALPGKCGVNVPYKISPSTNCATVK

Major allergen molecules in these fruits are: peach (prunus persica), Pru p 3; apricot (prunus armeniaca), Pru ar 3; plum (prunus domestica), Pru d 3; and cherry (prunus avium), Pru a 3.

the LTP allergen is also reported (73). The reason for this specific sensitization in Italian and Spanish patients needs to be elucidated. Table 13–5 depicts the alignment of LTP amino acid sequences for the most relevant members of the Rosaceae family, and Table 13–6 shows the degree of homology with peach LTP (74). Spanish authors recently confirmed the cross-reactivity among fruits of Rosaceae family by DBPCFC (75).

An LTP is also the major allergen of maize, as we demonstrated in a population of subjects with anaphylactic reactions to maize that were or were not associated with OAS to peach (34) (Fig. 13–3). It was interesting to observe that the cross-reactivity between maize and peach LTPs was higher than that between maize and wheat LTPs (Fig. 13–4 and Fig. 13–5); this cross-reactivity may explain the higher observed frequency of allergic reactions to maize with peach than to maize with wheat. These results strongly support the clinical role of LTPs as allergens that cause both localized and severe systemic reactions to multiple, apparently unrelated foods. This seems a particular clinical entity that can be classified as "LTP syndrome."

Latex-Fruit Syndrome

Latex allergens are represented by several proteins responsible for occupational allergic reactions and for anaphylactic reactions arising especially in patients with spina bifida (76). Some latex allergens also cross-react with a number of fruits in the latex-fruit syndrome (14). The main allergen involved in this syndrome is hevein, Hev b 6.02 (77), which is the most allergenic component of the latex protein prohevein. Prohevein is also a latex allergen called Hev b 6.01 (78), and is the latex-allergenic component implicated in occupational allergic reactions, as shown by the demonstration of a general sensitization to it in health care workers with allergies. The latex component implicated in sensitization of patients with spina bifida is Hev b 1 (79). Prohevein, an important defense protein of *Hevea brasiliensis* (80), is a protein of 187 amino acid residues with a molecular weight of 20 kDa. It has two domains that may be processed by post-translational modifications into an amino-terminal domain, i.e., the previously mentioned Hev b 6.02, the allergenic hevein of 4.7 kDa; and a carboxy-terminal domain of 14 kDa, named Hev b 6.03 (79). Hev b 6.02 is much more allergenic than Hev b 6.03 because of its higher chemical stability due to seven disulfide bridges (78). Several food allergenic sources, such as avocado, chestnut, and banana, contain proteins homologous to Hev b 6.02 (81–85) (Table 13–7).

Hevein and its homologous allergens belong to class 1 of the family of plant chitinases (86), defense proteins widely distributed throughout the plant kingdom (87). These are basic proteins with

Table 13–6.
Degree of Homology of 9 kDa Major Allergens of Apricot, Plum, Cherry, and Apple with Peach 9 kDa Major Allergen

Organism	*Taxonomy*			*Percent Identity with Peach Major Allergen*
APRICOT NLT1	Dicotyledoneae	Rosales	Rosaceae	89%
(*Prunus armeniaca*)	Dicotyledoneae	Rosales	Rosaceae	87%
PLUM NLT1	Dicotyledoneae	Rosales	Rosaceae	87.9%
(*Prunus domestica*)				
CHERRY	Dicotyledoneae	Rosales	Rosaceae	75.8%
(*Prunus avium*)				
APPLE	Dicotyledoneae	Rosales	Rosaceae	84%
(*Malus domestica*)				

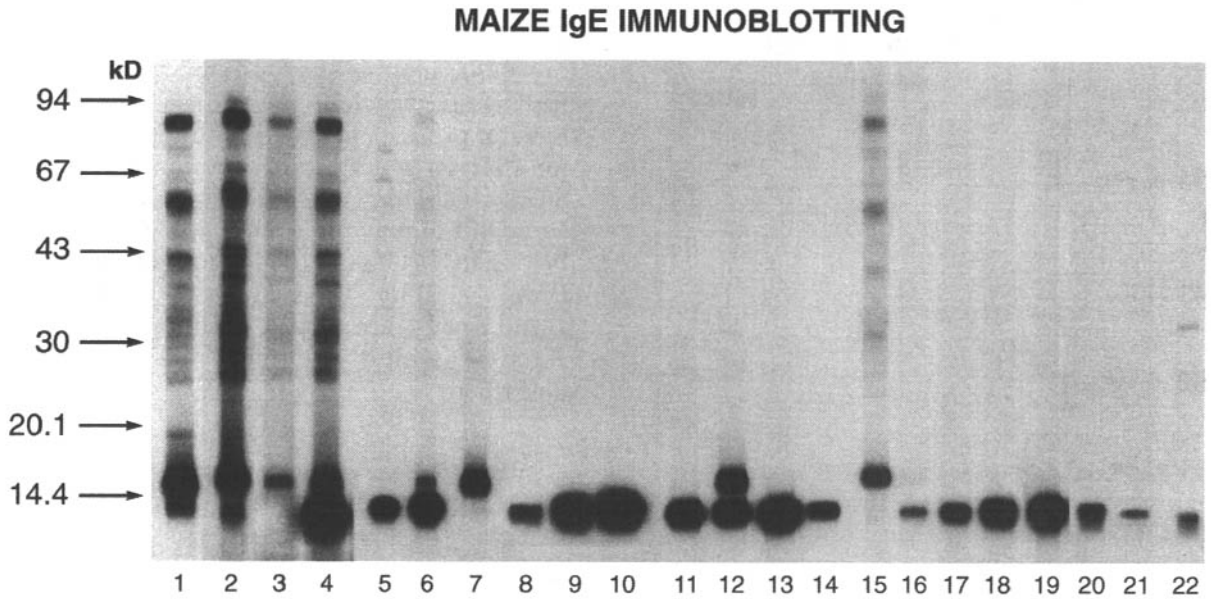

Figure 13–3. IgE immunoblotting of maize extract with the sera from 22 patients with severe systemic reactions upon ingestion of maize. (Reprinted from Pastorello EA, et al. *J Allergy Clin Immunol* 2000;106:744–51.)

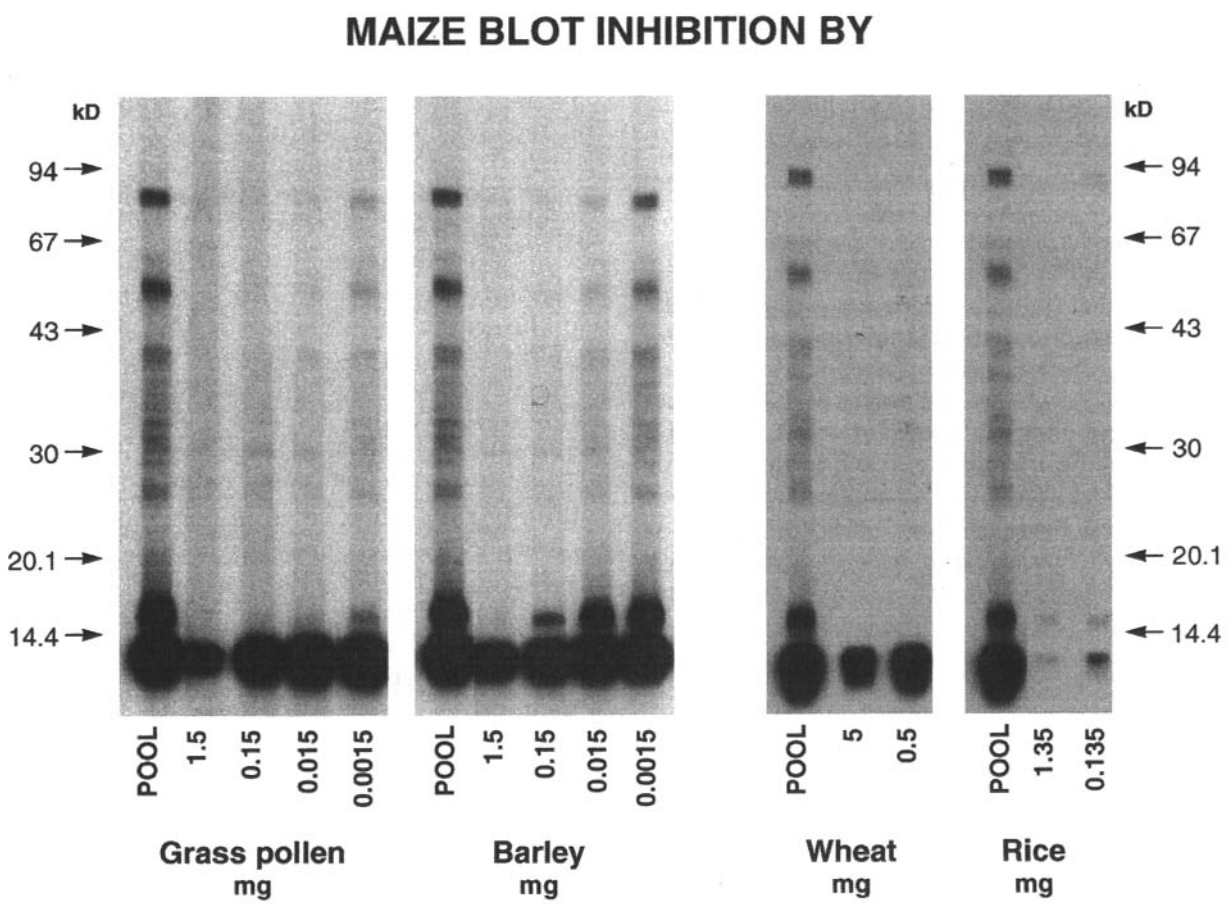

Figure 13–4. Inhibition of maize IgE immunoblotting (pool) by different amounts of grass pollen, barley wheat, and rice. (Reprinted from Pastorello EA, et al. *J Allergy Clin Immunol* 2000;106:744–51.)

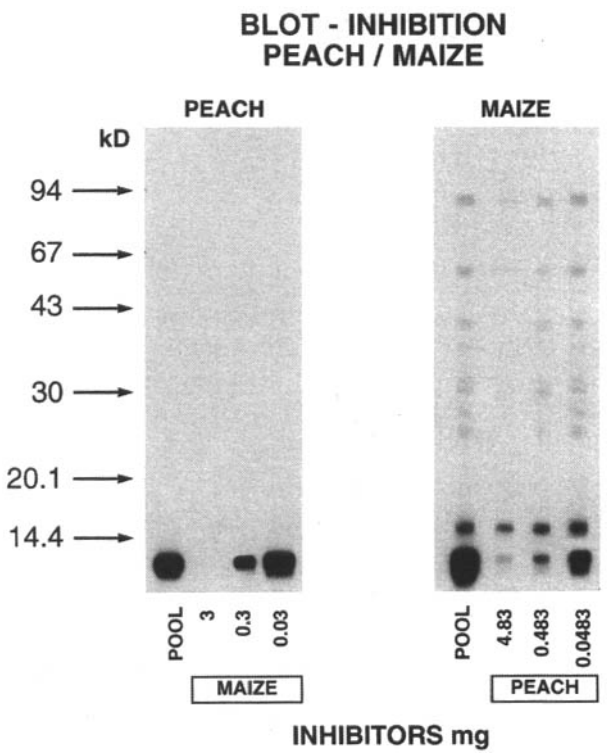

Figure 13–5. Inhibition of IgE immunoblotting of the purified maize 9 kDa protein by peach row extract and inhibition of peach row extract IgE immunoblotting by maize crude extract. The protein at 9 kDa was also completely inhibited in both experiments. (Reprinted from Pastorello EA, et al. *J Allergy Clin Immunol* 2000;106: 744–51.)

a cysteine-rich domain that is responsible for the chitin binding function. It is interesting that other plant foods, such as green beans, contain class 1 chitinases, but they are not cross-reactive with latex, probably because they are consumed after cooking and the allergenic activity is destroyed by heating (81). Allergenic cross-reactivity has been demonstrated between latex and fruits such as kiwi, papaya, mango, and passion fruit; class 1 chitinases may be the relevant cross-reactive components, but this has not been demonstrated definitively (86).

Other plant-origin foods may cause OAS; kiwi fruit is an especially common allergenic source. Several proteins of kiwi are allergenic but its major allergen is actinidin, a proteolytic enzyme of 30 kDa belonging to the thiol protease family (89). Bromelin of pineapple and papain of papaya also belong to this family, which may cause allergenic cross-reactivity. The major mite allergens Der p 1 and Der f 1 are also thiol proteases, but cross-reactivity with plant-origin foods has never been described.

Table 13–7.
Allergens Homologous to Hev b 6.02

Allergens	*Fruits*	*Molecular Weight*	*% Homology*
Pers a 1 (82)	Avocado	32 kDa	70%
Cas s 1 (83)	Chestnut	32 kDa	71%
Bra r 1 (84)	*Brassica rapa*	18.7 kDa	70%
Major allergen (85)	Banana	32 kDa 34 kDa	—

Diagnosis

Diagnosis of OAS is based on the generally accepted procedure for the diagnosis of IgE-mediated food allergy (90–93). Because of its particular features, however, OAS requires a slightly different diagnostic approach.

The clinical history plays a substantial role in the diagnosis. In most cases, an association is seen between contact of the food with the oral mucosa and the occurrence of symptoms. The rapid appearance of symptoms (within 30 minutes) after oral contact pinpoints the food as the causal agent. The diagnosis becomes especially clear when symptoms are always manifested after each contact with a particular food.

Other elements of the clinical history may support a diagnosis of OAS, such as localization of the symptoms to the mouth, lips, pharynx, and glottis, and the coexistence of allergic rhinitis. The diagnosis is strengthened by known associations between food and pollen allergy (e.g., birch and apple, or mugwort and celery). Skin tests and allergen-specific IgE titers can be used to confirm the clinical history; however, their low overall accuracy makes them unsatisfactory for formulating a definitive diagnosis by themselves.

Recent studies have reported the accuracy of skin tests and antigen-specific IgE in serum compared to the gold standard, i.e., DBPCFC, in patients who have OAS to plant-origin foods (Table 13–8). SPTs with commercial extracts for carrot have low sensitivity and, consequently, high specificity; in contrast, SPTs with natural foods show 100% sensitivity and 0 specificity. Similarly, high sensitivity and low specificity was seen for skin tests for both commercial and natural hazelnut, and for celery when an Allergopharma extract was used. When a Stallèrgenes extract was

Table 13–8.
Performance of SPT and CAP

	Sensitivity	*Specificity*	*PPV*	*NPV*
Carrot (31)				
Commercial SPT	0.26	1.00		
Raw SPT	0.1	0.00		
CAP	0.9	0.5		
Hazelnut (26)				
Commercial SPT	1.00	0.05	0.93	0.04
Raw SPT	0.88	0.27	0.94	0.15
CAP	0.75	0.18	0.92	0.05
Celery (32)				
Commercial SPT				
Stallergenes	0.48	0.88	0.96	0.19
Allergopharma	0.86	0.13	0.87	0.11
Raw SPT	0.96	0.0		
CAP	0.73	0.38	0.88	0
Melon (9)				
Raw SPT	0.79	0.38	0.42	0.77

used for celery, however, sensitivity was insufficient and the specificity was good. In general, clinical assay Pharmacia (CAP) measurements have been unsatisfactory for the foods reported in the table and for the studies in question; good sensitivity was reported only for carrot. Diagnostic sensitivity and positive predictive value (PPV) of SPTs and specific IgE determinations are sufficient, but these tests produce poor specificity and negative predictive value (NPV).

Cross-reactivity between allergenic molecules that share common epitopes in different foods is common in patients who are allergic to plant-origin foods, making the results of the skin tests and in vitro tests unreliable. These subjects are allergic to a certain food, but can test positive to many other foods even if they tolerate them (i.e., false-positive results). The large number of false-positive results obtained with conventional diagnostic procedures such as SPT and/or in vitro tests, makes these tests useless for the diagnosis of OAS. Lack of standardization of the allergenic extracts used in these methods is at fault. Moreover, lectins in vegetables are theoretically capable of giving false-positive results in vitro because of specific bonds with the solid phase (94).

Diagnostic sensitivity of SPT and specific IgE determinations are sometimes also poor because they yield many false negative results. This outcome has become evident in clinical studies where patient selection for DBPCFC was based exclusively on histories, not on SPT and specific IgE results. With these "inclusive criteria," patients with negative conventional tests were not excluded by the DBPCFC. Two studies listed in Table 13–8 (celery [28] and hazelnut [29]) adhered to these "inclusive criteria"; the diagnostic sensitivity of CAP for celery was 73% and for hazelnut 75%, while NPV for celery was 0% and for hazelnut 0.05%.

This result is partly due to insufficient knowledge of the chemical structures or components of the main allergens of plant-origin foods. The above study on hazelnut allergy (26) described four new hazelnut allergens—three major and one minor—all of which are important in sustaining symptoms. These allergens had not been known previously, and hazelnut diagnostic extracts had not considered them in the standardization.

Another cause of false-negative responses is the lability of some food allergens. These allergens lose their allergenicity during the preparation of the extract. Many patients suffering from severe OAS can eat a cooked version of the offending food without developing any symptoms. In a study of 70 patients with positive SPTs to birch and/or mugwort pollens and celery, 66 (94%) patients gave a positive SPT to raw celery but only 25 (36%) reacted to the cooked vegetable (95). In patients with a DBPCFC positive to cooked celery, this vegetable remains allergenic even after extended thermal treatment (76.07 minutes at 100°C) (96). Loss of allergenicity can occur during the preparation of commercial extracts (97–99). Fresh foods—particularly fresh apple—have been proposed as coating material for the RAST disk (100). RAST prepared by this technique showed concordance with both clinical history and skin tests. Björkstén et al (99) increased apple RAST diagnostic sensitivity to 90% by inhibiting reactions with phenolic compounds during apple extract preparation.

Another factor influencing the sensitivity of SPT with fresh fruits and vegetables is ripeness. Allergenic potency may increase during maturation of the fruit or vegetable, as shown in Golden Delicious apples by Vieths et al (101). This increase in the allergenic properties of the mature apples is due to an 18 kDa allergen. In another study, Vieths et al showed that the different allergenic potencies of 16 apple strains were related to the occurrence of this 18 kDa allergen (102).

The role of some single allergenic proteins is further highlighted by studies on hazelnut and brazil nuts (26, 103) showing that some allergens are related to the presence of symptoms.

In conclusion, are the SPT and/or antigen-specific IgE levels useless or do they play a role? On the contrary—we suggest that they be per-

formed as part of the diagnostic workup. In fact, positive SPT, in vitro, and antigen-specific IgE results are useful to confirm a diagnostic suspicion of IgE-mediated food allergy, especially when the history is clear, e.g., when symptoms occur regularly after the contact with a certain food. Although the SPT with fresh foods is impractical and has a low diagnostic specificity that generates many false positive results, it can be useful to consolidate the history-based possibility of food allergy. A positive test can suffice for concluding the diagnostic procedure in these cases. However, a negative SPT with fresh foods, especially if it is negative for all the tested foods, forces one to reconsider the correct diagnosis and re-evaluate the etiology of the symptoms.

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14

The Respiratory Tract and Food Hypersensitivity

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Introduction

The most common clinical manifestations of food allergy involve the skin and gastrointestinal tract (1). In addition, respiratory tract symptoms may be observed following an allergic reaction to food, but these symptoms are not common and typically do not occur in isolation. A wide spectrum of respiratory symptoms has been attributed to food allergy, including nasal congestion, rhinorrhea, sneezing, itching of the nose and throat, coughing, and/or wheezing. Asthmatic reactions caused by food allergy constitute a more worrisome group of clinical manifestations because they are common during fatal and near-fatal allergic reactions (2, 3).

The main objective of this chapter will be to summarize scientific information implicating food hypersensitivity as an etiology for respiratory tract symptoms. A specific focus will be placed on the role of food allergy in asthma and anaphylactic reactions. This information should provide a practical approach to the identification of patients presenting with respiratory tract complaints and food allergy. Extensive details related to reliable diagnostic methods, appropriate management, natural history, and prevention of food allergy have been addressed in other chapters of this text.

Epidemiology

Adverse food reactions consist of any abnormal clinical responses to the ingestion of a food or food additive (1). These reactions can be further divided into two major categories. Food allergy is an immunologically mediated food reaction unrelated to any physiologic effect of the food or additive (e.g., an IgE-mediated reaction to an ingested peanut resulting in laryngeal edema, coughing, and/or wheezing). In contrast, food intolerance consists of an abnormal physiologic response to a food that is not immunologically mediated (e.g., an exaggerated physiologic reaction following the ingestion of monosodium glutamate (MSG) including headache, flushing, muscle tightness, and generalized weakness). Understanding this terminology and the basic classification of adverse food reactions will aid in the interpretation of scientific studies implicating food allergy in respiratory tract symptoms.

The real prevalence of respiratory tract symptoms induced by food allergy has been difficult to ascertain. For example, public perception of food allergy-induced asthma is great (4), but these perceptions have not always been substantiated when food challenges have been used to confirm patient histories (5, 6). When the specific focus was on the role of allergic reactions to food and respiratory tract manifestations, the prevalence was estimated between 2% and 8% in children and adults with asthma (7, 8).

Recently, a population-based study of food allergy was completed in France to determine the prevalence, clinical features, specific allergens, and risk factors of food allergy (9). This investigation was conducted on 33,110 persons who completed a questionnaire addressing these issues. Overall, the prevalence of food allergy was estimated to be 3.24%. Of the respiratory reactions reported, rhinitis and asthma were noted in 6.5% and 5.7% of cases, respectively. In addition, the clinical ex-

pression of food allergy depended on sensitization to pollens and was typically expressed as asthma, rhinitis, and angioedema. In contrast, an Australian survey noted that 114 (17%) of 669 adult respondents reported food-induced respiratory symptoms (10). Although the patients with asthma did not report food-related illness more frequently than the respondents without asthma, those reporting respiratory symptoms following food ingestion were more likely to be atopic. In addition, a high percentage of patients with asthma believed that food additives worsened their respiratory symptoms (11). Well-controlled investigations of food additives, however, have reported a prevalence rate of well below 5% (7, 12).

Investigators from the Isle of Wight recently reported that egg allergy in infancy predicts allergic respiratory disease by 4 years of age (13). A cohort of 1218 consecutive births was recruited and followed until 4 years of age. Of these, 29 (2.4%) developed egg allergy by 4 years of age. Increased respiratory allergy (e.g., rhinitis, asthma) was associated with egg allergy (odds ratio [OR] 5.0; 95% confidence interval [CI] 1.1–22.3; $P < .05$) with a positive predictive value (PPV) of 55%. Furthermore, the addition of the diagnosis of eczema to egg allergy increased the PPV to 80%. The investigators concluded that egg allergy in infancy, especially when associated with eczema, increases the likelihood of respiratory allergic symptoms in early childhood. In addition, Rhodes et al (14) conducted a prospective cohort study of subjects at risk for asthma and atopy in England. Of the 100 babies of atopic parents who were recruited at birth, 73 (73%) were followed up at 5 years, 67 (67%) at 11 years, and 63 (63%) at 22 years. Skin sensitivity to hen's egg, cow's milk, or both in the first 5 years of life was predictive of asthma (OR 10.7; 95% CI 2.1–55.1; $P = .001$; sensitivity 57%; specificity 89%).

The German Multicenter Allergy Study followed 1314 children from birth to the age of 7 years with the aim to prospectively investigate the pattern of atopic sensitization typically associated with the development of asthma in childhood (15). Parental questionnaires on asthma and asthmatic symptoms were completed six times during the first 2 years of life and thereafter yearly. Specific IgE levels to nine food and inhalant allergens were evaluated yearly, and at the age of 7 years a bronchial histamine challenge was conducted. Onset of allergen sensitization in atopic children with active asthma at the age of 7 years was significantly earlier than in atopic children without asthma at age 7 (39.4% before age 1 year vs 21.0% at age 7, $P = .015$). Early allergen sensitization without any sensitization to inhalant allergens at the age of 7 years conferred no increased risk for asthma at this age. Only those children sensitized to any allergen early in life and sensitized to inhalant allergens by the age of 7 years were at a significantly increased risk of being asthmatic at this age (OR 10.12; 95% CI 3.81–26.88). However, even in this group of persistently sensitized children, the risk of asthma at the age of 7 years was increased only with a positive parental history of asthma or atopy (OR 15.56; 95% C 5.78–41.83), with the effect strongest for maternal asthma. These data indicate that an underlying factor in asthma and maternal transmission may determine both a certain pattern of sensitization and the expression of asthma.

Pathogenesis

Immune responses mediated by specific IgE antibodies to food allergens are the most widely recognized mechanism for food-induced respiratory tract symptoms (16). Atopic patients produce IgE antibodies to specific epitopes in the food allergen. These antibodies bind to high affinity IgE receptors (FcεRI) on basophils and tissue mast cells (MCs) throughout the body, including the upper and lower respiratory tract. The establishment of IgE-bearing cells in the nasal or bronchial mucosa during allergic sensitization sets the stage for their activation during subsequent allergen exposure (17). When antigen binds to multiple adjacent IgE antibodies on an MC or basophil, these cells are activated, which leads to degranulation and release of proinflammatory mediators such as histamine, tryptase, leukotrienes, and prostaglandins. These mediators are responsible for the immediate allergic reaction, which is characterized by vasodilatation, smooth muscle contraction, and mucus secretion, which in turn lead to the different clinical symptoms observed in the respiratory tract.

These specific mediators can also contribute to late-phase allergic reactions that occur 4–8 hours after an immediate allergic response. MC-derived mediators can induce endothelial cells to increase expression of adhesion molecules for eosinophils, basophils, and lymphocytes. In addition, tryptase may activate endothelial cells, increasing vascular permeability. Leukocytes are then drawn to the airways during a relatively symptom-free recruit-

ment phase, where they release cytokines and tissue-damaging proteases that contribute to the late-phase response, including congestion in allergic rhinitis and bronchoconstriction in asthma. Chronic inflammation may eventually cause an increase in airway hyperresponsiveness. Specific T cells also develop a memory response, which may contribute to the exacerbation of asthma symptoms on re-exposure to relevant stimuli.

Allergens

Chicken's egg, cow's milk, peanut, wheat, fish, shellfish, and tree nuts are common examples of foods that have been implicated in respiratory reactions and subsequently confirmed in well controlled, blinded food challenges (5, 12, 18, 19). A recent investigation summarized data from a voluntary registry of 5149 individuals with peanut and/or tree nut allergy (20). The aim was to characterize clinical features, including respiratory reactions, in the registrants (median age 5 years). Respiratory reactions, including wheezing, throat tightness, and nasal congestion, were reported in 2163 (42%) and 2883 (56%) of respondents as part of their initial reactions to peanuts and tree nuts, respectively. One half of the patients had reactions involving more than one system, and more than 3862 (75%) required some form of medical treatment. Interestingly, registrants with asthma were significantly more likely than those without asthma to have severe reactions (33% vs 21%; $P < .0001$). Likewise, investigations of patients experiencing near-fatal and/or fatal anaphylactic reactions following food ingestion have mainly been secondary to peanut, tree nuts, and shellfish (2, 3, 21). In contrast, several food additives, including MSG, sulfites, and aspartame, have been implicated in adverse respiratory reactions (22), although such reactions are extremely rare. For example, a large population survey in the United Kingdom found the prevalence of adverse reactions to food additives in 0.01%–0.23% of adults (23).

Route of Exposure

Oral ingestion is the primary route of exposure to foods that can cause or exacerbate respiratory symptoms (e.g., asthma). In addition, asthmatic responses may also occur from direct inhalation of aerosolized particles containing allergenic food proteins. For example, highly allergic individuals may react when exposed to clinically relevant levels of allergenic food particles in a seafood restaurant, or when fish, shellfish, or eggs are cooked in a confined area (16). Moreover, patients with peanut allergy may experience similar reactions when they are exposed to peanut dust on airline flights serving peanut snacks (24). Occupational exposures to airborne food allergens can also result in chronic asthma. For example, baker's asthma is caused by occupational exposure to airborne cereal grain dust (25). The inhalation of lupine seed flour has been reported to be an important cause of allergic sensitization in exposed workers and may actually give rise to occupational asthma and food allergy (26).

Individuals sensitized by occupational exposure to psyllium dust have been reported to be at high risk for allergic reactions to ingested psyllium-containing products (27). Historical data were obtained by questionnaire and telephone survey of 20 of 24 women with reported allergic reactions to a psyllium-containing cereal. Eighteen (90%) of the 20 women had historical and/or laboratory evidence of atopy. Exposures included ingestion or dispensing of psyllium-containing products. Symptoms developed shortly after small amounts of the cereal were ingested and most commonly included moderate to severe wheezing, throat and chest tightness, and urticaria. All the women required medical therapy, 11 (55%) in an emergency room. Specific IgE and IgG antibodies to various psyllium protein fractions were documented in all the subjects.

Clinical Background

In the overall evaluation of patients with respiratory tract symptoms, one must consider numerous etiologies in the differential diagnosis (Table 14–1). As compared to viral upper respiratory tract infections, allergic rhinitis, and sinusitis, food allergy as a specific etiology for respiratory tract symptoms has been less well defined.

Rhinitis Induced by Food Allergy

Adverse nasal symptoms are often attributed to food ingestion. Although many patients associate the ingestion of cow's milk and other dairy products with an increase in the production and thickness of nasal secretions, this generally cannot be attributed to a specific allergic reaction. Pinnock and co-workers (28) investigated the relationship

Table 14–1.
Respiratory Tract Symptoms: Differential Diagnosis

1. Infectious Illnesses
   - Viral upper respiratory tract infections
   - Recurrent otitis media
   - Rhinosinusitis
2. Allergic Diseases
   - Allergic rhinitis (seasonal, perennial)
   - Asthma
   - Food allergy
3. Non-allergic Rhinitis/Irritant Rhinitis/Gustatory Rhinitis
4. Rhinitis Medicamentosa
5. Anatomic Abnormalities
   - Nasal polyps
   - Deviated nasal septum
   - Foreign body
   - Enlarged tonsil and adenoids
   - Ciliary dyskinesia
6. Cystic Fibrosis

between milk intake and mucus production in adult volunteers challenged with rhinovirus-2. Milk and dairy product intake was not associated with an increase in upper or lower respiratory tract symptoms of congestion or nasal secretion weight. Overall, no statistically significant association was detected between milk and dairy product intake and symptoms of mucus production in healthy adults, either asymptomatic or symptomatic, with rhinovirus infection. Another investigation used a randomized, crossover, double-blind, placebo-controlled trial to examine the effects of dairy products in patients who believed that their asthma worsened following the ingestion of milk products (29). For both forced expiratory volume in 1 second (FEV_1) and peak expiratory flow rate, there were no statistically significant differences in the group mean between active challenges and placebo challenges, between sequences of administration, or between perceptions of dyspnea. The investigators concluded that it is unlikely that dairy products have a specific bronchoconstrictor effect in most patients with asthma, regardless of their perceptions.

Nasal symptoms, especially rhinitis, can certainly be observed during positive blinded oral food challenges. For example, rhinitis accounted for 70% of the overall respiratory symptoms observed in a large group of children undergoing double-blind placebo-controlled food challenges (DBPCFCs) (30). These symptoms typically occur in association with other clinical manifestations (i.e., cutaneous and/or gastrointestinal symptoms) during allergic reactions to foods, and rarely occur in isolation (12, 30).

Several other studies have identified patients who develop nasal symptoms due to IgE-mediated hypersensitivity. In a survey of 323 patients (ages 4 years and older) with chronic rhinitis attending an allergy clinic, 21 (6.5%) appeared to experience clearing of their nasal symptoms when placed on a strict milk exclusion diet (31). However, only 2 of these 21 patients (0.6%) had nasal symptoms reproduced during two consecutive DBPCFCs. In a retrospective review of 25,000 patients presenting to an allergy clinic over 5 years, 400 (1.6%) were diagnosed by history and laboratory studies (32). Of 400 patients diagnosed with food allergy, 12 (3%) were diagnosed with food-induced nasal symptoms. This would suggest that 0.05% of patients attending an allergy clinic had food-induced rhinitis.

In three epidemiological surveys of infants through their first 3 years of life, milk-induced nasal symptoms were rare. Despite the notion that milk ingestion frequently leads to nasal congestion, only 0.08%–0.2% of infants developed nasal symptoms following a milk challenge. Host and Halken (33) found 14 (36%) of 39 milk-allergic infants that developed nasal symptoms during oral milk challenges, whereas Schrander and colleagues (34) found only 1 (3.8%) of 26 experienced nasal symptoms following milk challenge (4%). In their study of 100 infants with milk allergy, Hill and co-workers (35) reported that 20 children (20%) developed rhinitis during oral milk challenges.

One form of food intolerance that provokes nasal symptoms has been termed "gustatory rhinitis" (36). In a questionnaire survey of 60 adults, more than 60% reported rhinorrhea following the ingestion of very spicy foods such as hot chili peppers, horseradish, or hot and sour soup. Unlike typical rhinitis, affected individuals do not develop sneezing, congestion, or pruritus. The spicy food elicits rhinorrhea within a few minutes of ingesting the food and resolves almost immediately after the spicy food is eaten. The reaction results from the stimulation of muscarinic receptors, which can be inhibited by atropine (36).

Serous Otitis Media Induced by Food Allergy

Serous otitis media has multiple etiologies, of which the most prominent is viral upper respiratory tract infection. Allergic inflammation in the nasal mucosa may cause eustachian tube dysfunction and subsequent otitis media with effusion. The role of food allergy in recurrent serous otitis media has been proposed; however, this association has been overestimated and poorly supported by scien-

tific studies (37). Respiratory atopy (e.g., allergic rhinitis, allergic asthma) may be a more important predisposing factor than food allergy alone (38). In contrast, another report cautiously suggested that in a subset of infants with recurrent otitis media, IgG complexes with food antigens, particularly cow's milk proteins, might contribute to the middle ear inflammation in this disorder (39). Obviously, more data are needed from well-controlled investigations before general recommendations can be made regarding this association.

Food-Induced Pulmonary Hemosiderosis (Heiner's Syndrome)

In 1960, Heiner reported a syndrome in infants consisting of recurrent episodes of pneumonia associated with chronic rhinitis, pulmonary infiltrates and hemosiderosis, gastrointestinal blood loss, iron-deficiency anemia, and failure to thrive (40). This rare syndrome is most often associated with a non-IgE-mediated hypersensitivity to cow's milk proteins, but reactivity to egg and pork have also been reported (41). Although peripheral blood eosinophilia and multiple serum precipitins to cow's milk are common, the specific immunologic mechanisms responsible for this disorder are not known. The diagnosis is suggested when elimination of the precipitating allergen leads to resolution of symptoms. Characteristic laboratory data, such as precipitating antibodies to cow's milk, are also considered necessary to the diagnosis. Avoidance of the precipitating allergen leads to resolution of symptoms, but the natural history of this disorder is not known.

Asthma Induced by Food Allergy

The majority of information on food allergy and respiratory tract symptoms focuses on asthma. In one investigation, 300 consecutive patients with asthma (age range 7 months to 80 years) were evaluated in a pulmonary clinic (7). Twenty-five (8%) patients had a history of food allergy with clinical symptoms and/or positive tests of food-specific IgE antibodies. Food-induced wheezing was documented in 6 (2%) of the cases; all were children aged 4–17 years. In another investigation, 140 children aged 2–9 years with asthma were screened by clinical history and testing for food-specific IgE antibodies (42). Of these children, 32 were able to undergo blinded food challenges, from which 13 (9.3%) had food-induced respiratory symptoms and 8 (5.7%) had specific asthmatic reactions. Only one patient had asthma as the sole symptom during a positive food challenge. Interestingly, the patients with food allergy and asthma were generally younger and had a past medical history of atopic dermatitis (AD). Oehling and co-workers reported that food-induced bronchospasm was present in 24 (8.5%) of 284 asthmatic children evaluated (43). The majority of the allergic sensitization occurred in the first year of life and was caused by a single food, especially egg. Finally, Businco and colleagues (44) evaluated 42 children (age range 10–76 months) with AD and milk allergy. Eleven (26%) of these patients developed asthmatic symptoms during a positive food challenge.

The prevalence of food-related wheezing does appear to be highest in younger patients with atopic disease. Hill and co-investigators (35) studied 100 children (mean age 16 months) in Australia who had clinical histories of adverse reactions to cow's milk. The children were categorized into three groups based on symptoms. The first group consisted of 27 infants who reacted acutely to cow's milk ingestion (eight [29%] had lower airway responses on oral challenges) and had cow's milk-specific IgE antibodies. The second group consisted of 53 infants with primarily non-IgE-mediated gastrointestinal reactions to cow's milk challenges. Only 2 (4%) in this group experienced lower airway symptoms. The third group included 20 patients with late-onset reactions to oral challenges with cow's milk. The majority of these patients had chronic asthma or AD, and 10 (50%) had wheezing after the milk challenges. In contrast, an investigation from Turkey confirmed that food allergy can elicit asthma in children less than 6 years old, but the incidence is low (4%), even with major food allergens such as egg and cow's milk (45).

Respiratory reactions induced by food challenges in 598 children with pulmonary disease were reported by Bock (46). Of the 410 (69%) children with a history of asthma, 279 (68%) had a history of food-induced asthma. Food challenges were positive in 168 (60%) of the 279 patients. This investigation documented that 67 (24%) of the 279 children with a history of food-induced asthma had a positive blinded food challenge that included wheezing. The foods responsible for these reactions included peanut (19 [28.4%]), cow's milk (18 [26.9%]), egg (13 [19.4%]), tree nuts (10 [14.9%]), and all other foods (7 [10.4%]). Interestingly, only 5 (1.8%) of these patients had wheezing as their sole objective adverse symptom.

A total of 320 children with AD undergoing blinded food challenges were also monitored for

respiratory reactions (30). The patients, ages 6 months to 30 years, were highly atopic, had multiple allergic sensitivities to foods, and over one half had a prior diagnosis of asthma. Food allergy was confirmed by blinded challenges in 205 (64%) of these patients; almost two thirds of these patients experienced respiratory reactions during their positive food challenges (i.e., nasal 224 [70%], laryngeal 154 [48%], and pulmonary 86 [27%]). Overall, 34 (17%) of 205 children with positive food challenges developed wheezing as part of their reaction. Furthermore, 88 (43%) of these patients were monitored for pulmonary function during positive and negative food challenges. Thirteen (15%) developed lower respiratory tract symptoms including wheezing, but only six (6.8%) patients had a greater than 20% decrease in FEV_1. As documented in the investigations cited earlier, wheezing as the only manifestation of the allergic reaction was rare.

Airway Hyperresponsiveness Induced by Food Allergy

Food allergens contributing to increases in airway hyperresponsiveness, and in some cases exacerbation of asthma, have been investigated. In one investigation, 26 children with asthma and food allergy were evaluated for changes in their airway hyperresponsiveness before and after blinded food challenges (47). Airway hyperresponsiveness was measured with standardized methacholine inhalation challenges both at baseline (i.e., before blinded food challenges) and 4 hours after the food challenge. Of the 22 positive blinded food challenges, 12 (54%) involved chest symptoms (e.g., repetitive cough, laryngeal reactions, and/or wheezing). Another 10 (45%) positive food challenges included laryngeal, gastrointestinal, and/or skin symptoms without any apparent chest symptoms. Significant increases in airway hyperresponsiveness occurred in seven (58%) of the 12 patients who experienced chest symptoms during positive food challenges. Decreases in FEV_1 were not generally observed in these seven patients, suggesting that significant changes in airway reactivity can occur without demonstrable changes in spirometry in a preceding food challenge. These data indicate that food-induced allergic reactions may increase airway hyperresponsiveness in a subset of patients with moderate to severe asthma despite the absence of symptoms immediately after ingestion.

In contrast, another investigation concluded that food allergy is an unlikely cause of increased airway hyperresponsiveness (48). Eleven adults with asthma, a history of food-induced wheezing, and positive skin prick tests (SPTs) to the suspected foods were evaluated. Equal numbers of patients had increased airway hyperresponsiveness, as determined by methacholine inhalation challenges following blinded food challenges to either food allergen or placebo. Unfortunately, the small number of patients investigated and the lack of environmental controls prior to the repeat methacholine challenges raise doubt about the investigators' conclusions.

These results suggest that respiratory symptoms may be provoked by food allergens in a subset of patients with asthma. Table 14–2 summarizes the major considerations for defining a role of food allergy in respiratory tract symptoms. Table 14–3 compares the prevalence of food allergy-induced asthmatic reactions in different patient populations.

Respiratory Symptoms Induced by the Inhalation of Food Allergens

Occupational exposures to airborne food allergens can also result in chronic asthma. For example, baker's asthma is caused by occupational

Table 14–2.
Respiratory Tract and Food Hypersensitivity

1. Food-induced respiratory tract symptoms are typically accompanied by either cutaneous or gastrointestinal symptoms.
2. Food-induced respiratory tract symptoms rarely occur in isolation.
3. Egg, milk, peanut, soy, fish, shellfish, and tree nuts are the most common food allergens confirmed to elicit respiratory reactions.
4. Allergic sensitization (positive tests) or clinical reactions to foods in infancy predict the later development of respiratory allergies and asthma.
5. Food-induced asthma is more common in young pediatric patients than in older children and adults.
6. Children with AD, especially those with food reactions confirmed during blinded food challenges, are at increased risk for food-induced asthma.
7. Food-induced allergic reactions may increase airway hyperresponsiveness in patients with moderate to severe asthma, and may do so without inducing obvious acute asthmatic symptoms.
8. The role of food allergy in otitis media in patients without other manifestations of atopy (e.g., AD, allergic rhinitis) is controversial and probably is extremely rare.
9. Asthma reactions to food additives can occur but are very uncommon.
10. Respiratory symptoms, especially asthmatic reactions, induced by food allergens are risk factors for fatal and near-fatal reactions.

Table 14–3.
Estimated Prevalence of Food Allergy-Induced Asthmatic Reactions

Clinical Population	*Estimated Prevalence*
Infants with cow's milk allergy	29%
Food-induced wheezing	2%–24%
Food additive-induced wheezing	<5%
Patients with AD	17%–27%

exposure to airborne cereal grain dust (25). Patients with this disorder experience cough and shortness of breath following the inhalation of wheat proteins while baking. Affected patients usually have positive skin tests to extracted wheat proteins (25). Allergic reactions associated with airborne fish particles have been reported in patients with fish allergy (49). Of the 21 children in the study who reported allergic reactions upon incidental inhalation of fish odors or fumes, nine (43%) had wheezing or rhinitis alone, and three (14%) had respiratory and cutaneous symptoms together. Methods of exposure included boiling or frying fish and simple exposure to fish. Finally, Sicherer and colleagues (24) recently reported that some peanut-allergic patients experience adverse respiratory reactions when they are exposed to peanut dust on airline flights serving peanut snacks.

Two recent investigations have highlighted cases of respiratory allergic reactions that were precipitated by inhalation of airborne food allergens. The first report focused on three patients who developed asthma and rhinitis caused by exposure to raw, but not cooked, green beans and chards in a non-occupational environment (50). The investigators observed only minor differences in IgE reactivity between nitrocellulose-blotted raw and boiled green bean extracts. The second report highlighted an observation that inhalation of lupine seed flour may be an important cause of allergic sensitization in exposed workers and may actually give rise to occupational asthma and food allergy (26). Three patients reported work-related symptoms immediately after being exposed to lupine. SPT results with an extract of lupine seed flour were positive in all three patients; lupine-specific IgE antibodies were detected in two of the three subjects. Interestingly, one patient underwent a bronchial provocation with lupine seed flour extract and experienced an immediate 25% decrease in FEV_1. In summary, lupine seed flour may be a potential sensitizing agent by inhalation in exposed workers and may give rise to occupational asthma and food allergy.

Food-Induced Anaphylactic Reactions

Respiratory symptoms induced by food allergens, especially acute bronchospasm, are very worrisome and dramatic clinical manifestations because they are major risk factors for fatal and near-fatal reactions following food ingestion (2, 3, 21). These symptoms typically include pruritus in the oropharynx, angioedema (e.g., laryngeal edema), stridor, dysphonia, cough, dyspnea, and wheezing. In a survey of six fatal and seven near-fatal anaphylactic reactions following food ingestion, all patients had severe wheezing and respiratory symptoms as part of their clinical presentation. The foods responsible for these serious reactions included peanut, tree nuts, egg, and cow's milk. Another report summarized acute allergic reactions to peanut and/or tree nuts in 122 atopic children. In the group, 63 (52%) had lower respiratory tract symptoms as part of their overall reactions (51). A recent Italian investigation summarized the clinical characteristics and treatment of 113 episodes of acute anaphylaxis triggered by different agents including medications (49%), hymenoptera venom (29%), and food allergens (8%) (52). Most of the events occurred at home (63%) and the most frequent symptoms involved respiratory (90%) and cutaneous symptoms (78%). Initial symptoms never involved the cardiovascular system. Trigger foods included mustard, mussels, shrimp, soy, peanut, and fish. In summary, the presence of asthma and/or related respiratory findings and a small number of common foods are significant risk factors for serious and even fatal cases of food-induced anaphylaxis (2, 53).

Food Additives and Respiratory Symptoms

Despite public perceptions, there is conflicting evidence that some individuals with asthma are more likely to have adverse effects from MSG compared to the general population. Woods and coworkers (54) designed a randomized, double-blind, placebo-controlled, MSG challenge protocol for identifying early and late asthmatic reactions. They were unable to demonstrate MSG-induced immediate or late asthmatic reactions in a group of 12 adult asthmatics who reported that MSG worsened their overall asthma control. In addition, these investigators observed no significant changes in bronchial hyperresponsiveness or soluble inflammatory markers (e.g., eosinophil cationic protein [ECP], tryptase) during this investigation protocol. In another study, investigators performed double-

blind, placebo-controlled oral challenges with MSG in subjects who had histories of adverse reactions to this food additive (55). Although the participants experienced no specific upper or lower respiratory complaints, 22 (36%) of the 61 enrolled subjects had confirmed adverse reactions to MSG that included headache, muscle tightness, numbness, general weakness, and flushing.

Conclusions

Previous investigations have established clearly the pathogenic role of food allergy in respiratory tract symptoms, which are usually accompanied by skin and gastrointestinal manifestations. Specific foods have typically been implicated in these reactions. Moreover, allergic sensitization to egg and cow's milk in infancy appear to constitute positive predictive factors for the development of respiratory tract allergic disease, including asthma, later in life. Food allergy and respiratory tract symptoms are usually observed in younger patients, especially those with a current or past history of AD. In addition, food allergy is a trigger in a subset of patients with asthma. This specific etiology should be considered if a patient has recalcitrant or otherwise unexplained acute, severe asthma exacerbation, asthma triggered by ingestion of particular foods, or asthma that is accompanied by other manifestations of food allergy (e.g., anaphylaxis, moderate to severe AD). Respiratory symptoms, especially asthmatic reactions, induced by food allergens should be considered a risk factor for fatal and near-fatal reactions. Practice parameters for the diagnosis and treatment of asthma have highlighted the potential role of food allergy in asthma in some patients (56).

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15

Anaphylaxis and Food Allergy

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Introduction

Although fatal allergic reactions have been recognized for more than 4500 years (1), it was not until this century that the syndrome of anaphylaxis was fully characterized. In their classic 1902 study, Portier and Richet (2) described the rapid death of several dogs that they were attempting to immunize against the toxic sting of the sea anemone. Because this reaction represented the opposite of their intended "prophylaxis," they coined the term "anaphylaxis," or "without or against protection." From these studies, they concluded that anaphylaxis required a latent period for sensitization and reexposure to the sensitizing material. Shortly thereafter Schlossman (3) reported a patient who developed acute shock after ingesting cow's milk. The first contemporary descriptions of food anaphylaxis in humans was published in 1969 by Golbert and colleagues (4). They described 10 cases of anaphylaxis following the ingestion of various foods, including different legumes, fish, and milk. The reports by Yunginger (5), and then Sampson (6) and Bock (7) further characterized the natural course of near-fatal and fatal food-induced anaphylactic reactions.

Definition

Clinical anaphylaxis is a syndrome of diverse etiology and dramatic presentation of symptoms associated with the classic features of Type I, IgE-mediated hypersensitivity (8). Typically the term "anaphylaxis" refers to an immunologically mediated event that occurs after exposure to certain foreign substances, whereas the term "anaphylactoid" indicates a clinically indistinguishable reaction that is not believed to be IgE-mediated but probably involves many of the same mediators, e.g., histamine. The syndrome results from the generation and release of a variety of potent biologically active mediators and their concerted effects on various target organs. Anaphylaxis is defined by cutaneous, respiratory, cardiovascular, and gastrointestinal (GI) signs and symptoms occurring singly or in combination. Because anaphylactic reactions can present with such a varied constellation of signs and symptoms, Table 15–1 presents a scoring system to grade the severity of food-induced anaphylaxis. This chapter focuses on allergic reactions to foods that manifest as signs and symptoms involving multiple target organs or the cardiovascular system alone.

Prevalence

The prevalence of anaphylaxis is unknown because, unlike many disorders, there is no requirement to report such reactions to a national register. In addition, it is likely that many cases are misdiagnosed (9). Also contributing to this lack of scientific data is the fact that many patients who experience a mild anaphylactic reaction recognize the causative relationship to a specific food and simply avoid that food rather than consult a physician. Sorensen and colleagues (10) reviewed all cases of anaphylactic shock occurring outside the hospital in the Thisted Hospital catchment area, in Denmark. Twenty cases of anaphylaxis were identified, or 3.2 cases per 100,000 inhabitants per

Table 15–1.
Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms

Grade	*Skin*	*GI Tract*	*Respiratory Tract*	*Cardiovascular*	*Neurological*
1	Localized pruritus, flushing, urticaria, angioedema*	Oral pruritus Oral "tingling" Mild lip swelling			
2	Generalized pruritus, flushing, urticaria, angioedema*	Any of the above* Nausea and/or one episode of emesis	Nasal congestion and/or sneezing		Change in activity level
3	Any of the above*	Any of the above*, plus repetitive vomiting	Rhinorrhea, marked congestion Sensation of throat pruritus or discomfort	Tachycardia (increase >15 beats/min)	Change in activity level plus anxiety
4	Any of the above*	Any of the above* plus diarrhea	Any of the above* Hoarseness "Barky" cough Difficulty swallowing Dyspnea Wheezing Cyanosis	Dysrhythmia and/or mild hypotension	"Light-headedness" Feeling of "impending doom"
5	Any of the above*	Any of the above* Loss of bowel control	Any of the above* Respiratory arrest	Severe bradycardia and/or hypotension or cardiac arrest	Loss of consciousness

*All symptoms are not mandatory. The severity score should be based on the organ system most severely affected, e.g., if grade 3 respiratory symptoms are present but only grade 1 GI symptoms, the anaphylaxis severity score would be "grade 3."

year, and 5% were fatal. If similar rates were found in the US (and there are reasons to believe that anaphylaxis is slightly more common in the US), about 8300 cases of anaphylaxis could be anticipated each year, with about 415 deaths attributed to this cause. As suggested above, these investigators found that eight (40%) of the 20 cases had been given an incorrect International Classification of Diseases code at the time of discharge. In this series, drugs, food, and insect stings accounted for virtually all of the anaphylactic reactions.

Unfortunately there is no International Classification of Diseases code for food-induced anaphylaxis, so it is extremely difficult to obtain reliable information about the prevalence, incidence, or mortality rates for these reactions. In a retrospective survey, Yocum and Khan (9) reviewed all cases of anaphylaxis treated in the Mayo Clinic Emergency Department (US) over a 3.5-year period. Records were reviewed on all patients experiencing respiratory obstructive symptoms and/or cardiovascular symptoms plus evidence of allergic mediator release, e.g., urticaria. Overall, 179 patients were identified; 118 (66%) were female, 88 (49%) were atopic, and 66 (37%) had experienced an immediate reaction to the responsible allergen in the past. A probable cause was identified in 142 cases (Table 15–2): food, 59 cases (33%); bee sting, 25 cases (14%); medications, 23 cases (13%); and exercise, 12 cases (7%). Allergic reactions to food were the most common cause of anaphylactic reactions outside of the hospital, more frequent than reactions to bee sting and drugs combined. Extrapolating from the Sorensen data (10) would suggest that there are about 2500 food-induced anaphylactic reactions in the US each year, with about 125 deaths. Peanuts and nuts were the most common foods causing serious anaphylactic reactions. Bock surveyed 73 emergency departments in Colorado over a 2-year period and identified 25 cases of severe anaphylactic reactions to food with one death (11) The author concluded that at least 950 cases of severe food-induced anaphylaxis occur in the US each year, but he cautioned that his survey was an underestimate of the problem because patients had also been referred to him who were not included in the survey, and the

Table 15–2.
Three-Year Retrospective Survey of 179 Cases of Anaphylaxis Occurring Outside of the Hospital Treated by the Mayo Clinic Emergency Department (9)

Presumed Etiology of Anaphylaxis	*Number*	*Percentage*
Food*	59	33%
Idiopathic	34	19%
Hymenoptera	25	14%
Medications	23	13%
Exercise	12	7%
Other	8	4%
False Diagnosis	18	10%

*Foods implicated in 18 patients who were skin-tested: peanut, 4; nuts, 9; cereals, 6; milk, 2; egg, 2.

proportion of reactions was higher in the rural emergency departments serving smaller populations than in the busier metropolitan departments. In a more recent US survey, Yocum and colleagues (12) reported an annual incidence of food-induced anaphylaxis of 7.6 cases per 100,000 person-years and a food-induced anaphylaxis occurrence rate of 10.8 per 100,000 person-years. The figures were based on a review of the medical records of Olmsted County inhabitants followed in the Rochester Epidemiology Study from 1983 to 1987. Assuming that the US population is now 280 million, and that the prevalence of food allergy did not increase since the late 1980s, about 30,000 food-induced anaphylactic episodes should occur in the US each year that result in about 2000 hospitalizations and 150 deaths. Food-induced anaphylactic reactions account for over one third of the anaphylactic reactions treated in emergency departments and are most often due to peanut, tree nuts, fish, or shellfish (13). Pumphrey (13) and Moneret-Vautrin (14) reported similar findings in the United Kingdom and France, respectively. Novembre (15) reported that food allergy was responsible for about one half of severe anaphylactic episodes in children treated in emergency departments in Italy. Similarly, a survey of South Australian preschool and school-age children revealed a parent-reported food-induced anaphylaxis rate of 0.43 per 100 school children, which accounted for over one half of all cases of anaphylaxis in this age group (16). A 5-year survey of anaphylactic reactions treated at the Children's Hospital of Philadelphia also showed that food allergy was the most common cause of anaphylaxis outside of the hospital (17).

The first of several reports on fatal food-induced anaphylaxis came in 1988 with seven cases of fatal anaphylaxis evaluated during a 16-month period (5). In all but possibly one case, the victims unknowingly ingested a food that had previously provoked an allergic reaction. Similarly, six fatal and seven near-fatal food-induced anaphylactic reactions in children (ages 2–17 years) were reported from three metropolitan areas over a 14-month period (6). Common risk factors were noted in these cases: all patients had asthma (although it was generally well controlled); all patients were unaware that they were ingesting the food allergen; all patients had experienced previous allergic reactions to the incriminated food, although in most cases symptoms had been much milder; and all patients had immediate symptoms with about half experiencing a quiescent period prior to a major respiratory collapse. In both studies, no patient who died received adrenaline immediately; however, three patients with near-fatal reactions did receive adrenaline within 15 minutes of developing symptoms but still went on to respiratory collapse and hypotension that required mechanical ventilation and vasopressor support for 12 hours to 3 weeks. Interestingly, patients with life-threatening food-induced anaphylaxis had no significant increase in serum tryptase, raising some question about the exact mechanism of food-induced anaphylaxis (6).

A more recent report (7) analyzed 32 cases of fatal food-induced anaphylaxis. As in other studies, peanuts and tree nuts accounted for more than 90% of the fatalities. In this report, all but one of the patients was known to have asthma, and most of the individuals did not have epinephrine available at the time of their fatal reaction. Of 32 fatal food anaphylaxis cases reported, two (6%) of the 32 individuals who died had received intramuscular epinephrine immediately but failed to respond. In an earlier study of 48 fatal cases reviewed by Pumphrey (13), three (6%) patients died despite receiving epinephrine from a self-administration kit at the onset of their reaction.

The incidence of food-dependent exercise-induced anaphylaxis appears to be increasing, possibly due to the increased popularity of exercising over the past decade. Two forms of food-dependent exercise-induced anaphylaxis have been described: reactions following the ingestion of specific foods such as egg, celery, shellfish, and wheat (18–24), and rarely reactions following the ingestion of any food (25). Anaphylaxis occurs when a patient exercises within 2–4 hours of ingesting a food, but otherwise the patient can ingest the food without any apparent reaction and can exercise without any apparent reaction as long as the specific food (or any food in the case of nonspecific reactors) has not been ingested within the past several hours. This disorder is twice as common in females and >60% of cases occur in individuals less than 30 years of age. In a survey of 199 individuals experiencing exercise-induced anaphylaxis, ingestion of food within 2 hours of exercise was thought to be a factor in the development of attacks in 107 (54%) of the cases (26). Symptoms generally start with a sensation of generalized pruritus which progresses to urticaria and erythema, respiratory obstruction, and cardiovascular collapse. Patients with specific food-dependent exercise-induced anaphylaxis generally have positive skin prick tests (SPTs) to the food that pro-

vokes symptoms, and occasionally they have a history of reacting to the food when they were younger. As discussed below, specific management of this disorder involves identifying the foods that cause the reaction (i.e., double-blind placebo-controlled food challenge [DBPCFC] with exercise). Several factors appear to predispose an individual to food-induced anaphylaxis, including a personal history of atopy, family history of atopy, age, and dietary exposure. Atopic patients with asthma are at increased risk of developing more severe food-allergic reactions (6, 27, 28).

In the reports of Yunginger et al (5), Sampson et al (6), and Bock et al (7), the majority of individuals were highly atopic, and all had histories of asthma. Although atopy reportedly does not predispose individuals to an increased risk of anaphylaxis (29), it does tend to predispose to more severe reactions. In general it has been thought that individuals inherit the ability to produce antigen-specific IgE to food proteins, and that hypersensitivity to a specific food is not inherited. However, a significant concordance rate of peanut allergy among monozygotic twins compared to dizygotic twins was recently reported (30), suggesting strongly that there is a major genetic influence on the inheritance of peanut allergy.

Age may play a factor in predisposing an individual to food-induced anaphylaxis. The incidence of food allergy appears greatest in the first 2 years of life and decreases with age. Consequently, foods introduced during the first year (e.g. cow's milk, egg, soy, wheat, and in the US, peanut [as peanut butter]) are more likely to induce hypersensitization. Allergic reactions to milk, egg, soybean, and wheat are generally "outgrown" with age (31, 32). Milk allergy usually develops in the first year of life, with about 85% of infants "outgrowing" their sensitivity by the third to fourth year of life (33, 34). Whereas most food hypersensitivities are outgrown during childhood, food sensitivity to peanuts, tree nuts, fish, and shellfish often persist into adulthood (27, 35). It had been thought to be quite rare to find an allergic patient that develops clinical tolerance to peanuts and tree nuts, although recent evidence (36) suggests that 15%–20% of children diagnosed with peanut allergy early in life do outgrow their peanut allergy.

Dietary exposure can influence the occurrence of food-induced anaphylaxis in several ways. Different populations and nationalities may consume more of certain foods, and the increased exposure may result in an increased prevalence of that specific food allergy. In the US, allergy to peanut is one of the most common (37, 38). The American population ingests several tons of peanuts daily (35). In contrast, in Scandinavia, where fish consumption is high, the incidence of allergic reactions to codfish is increased. Rice and buckwheat allergies are quite rare in the US but not uncommon in Japan, where these foods are eaten in large quantities.

Etiology

A large variety of foods have been reported to have precipitated anaphylactic reactions. The list of foods that may induce an anaphylactic reaction is almost unlimited, and in theory, any food protein is capable of causing an anaphylactic reaction. As indicated in Table 15–3, certain foods tend to be cited more frequently as the cause of anaphylaxis, although any food may be the cause. Foods most often responsible for anaphylactic reactions include peanuts (and to a much lesser extent other legumes such as soybeans, pinto beans, peas, green beans, garbanzo beans), fish (e.g., cod, whitefish), shellfish (shrimp, lobster, crab, scallops, oyster), tree nuts (hazelnuts, walnuts, cashew, almonds, pistachio), cow's milk, egg, fruits (banana, kiwi), seeds (cotton seed, sunflower seed), and cereals or grains (wheat, rice, rye, millet, buckwheat).

Recent reports indicate that certain foods are more likely to induce severe anaphylactic reactions. These foods include peanuts and tree nuts (5, 6, 9), fish (38), and shellfish (38). In addition, these food sensitivities are typically not outgrown, in contrast with those to milk, eggs, and soybeans. The potency of particular foods to induce an anaphylactic reaction appears to vary and depends on the sensitivity of the individual. In general, it

Table 15–3.
Foods Most Frequently Implicated in Food-Induced Anaphylaxis

Peanut	
Tree Nuts	Hazel nuts (filberts), walnuts, cashews, pistachios, Brazil nuts
Fish	Less often tuna
Shellfish	Shrimp, crab, lobster, oyster, scallop
Cow's Milk	Goat Milk
Hen's egg	
Seeds	Cotton seed, sesame seed, pine nuts, sunflower seed
Beans	Soybeans, green peas, pinto beans, garbanzo beans, green beans
Fruit	Banana, kiwi
Cereal Grains	Wheat, barley, oat, buckwheat
Potato	

appears that for some foods, such as peanuts, microgram quantities may be sufficient to induce a reaction.

When food-allergic patients are challenged on a regular basis (e.g., annually) over a period of years, patients who eventually become tolerant to a food often tolerate more of the antigen in successive years. For example, the initial challenge may be positive after 500 mg of the food, and then in the challenge 1 year later the patient may tolerate 5 g of the food. After the challenge the following year, the patient may no longer be sensitive to that food.

Prior exposure and sensitization to food allergens theoretically must precede the initial anaphylactic reaction. However, numerous reports exist of anaphylactic reactions after the first known exposure to a food substance. In one group of children allergic to peanuts and tree nuts, a significant number of these patients reacted to their first known exposure to the food (39). Several possible explanations exist for this apparent paradox: sensitization often occurs to food antigens passed in maternal breast milk during lactation; sensitization may occur following an unknown exposure to a food antigen (e.g., milk formula given during the night in the newborn nursery, food given by another caregiver, or food contained in another product that was not suspected of containing the antigen in question); and sensitization may occur because of cross-sensitization to a similar allergen (e.g., kiwi or banana allergy in a latex-sensitive individual [40]). Some data suggest that sensitization may also occur in utero (41).

Although food additives are often suspected of provoking anaphylactic reactions, the only food additives for which there is substantial evidence of precipitating anaphylactic reactions are sulfites and papain. One of the initial reports detailed an atopic, non-asthmatic patient who experienced an anaphylactic reaction after consuming a restaurant meal that contained a large amount of sodium bisulfite (42). Specific IgE to sodium bisulfite was demonstrated by SPT and transfer of passive cutaneous anaphylaxis, and an oral food challenge caused itching of the ears and eyes, nausea, warmth, cough, tightness in the throat, and erythema of the shoulders. These symptoms resolved following treatment with epinephrine. There have been other scattered case reports in the literature confirming sulfite-induced anaphylaxis (43–45).

One patient was reported with papain-induced anaphylaxis following the ingestion of a beefsteak that had been treated with papain as a meat tenderizer (46). The patient had specific IgE to papain by SPT and experienced a positive oral challenge to papain with palatal itching and throat tightness. One study suggested that monosodium glutamate (MSG) could provoke asthma and anaphylaxis in some patients, but this remains controversial (47).

Clinical Features

The hallmark of a food-induced anaphylactic reaction is onset of symptoms within seconds to minutes of the ingestion of the food allergen. The time course of the appearance and perception of symptoms and signs differ among individuals. Almost invariably, at least some symptoms begin within the first hour after the exposure. Generally, the later the onset of anaphylactic signs and symptoms, the less severe the reaction. About 25%–30% of patients experience a biphasic reaction (6) in which they develop classical symptoms initially, appear to be recovering (and may even become asymptomatic), and subsequently experience the recurrence of significant, often catastrophic, symptoms. The intervening quiescent period may last up to 1–3 hours. In the report by Sampson and colleagues (6), three (43%) of seven patients with near-fatal anaphylaxis experienced protracted anaphylaxis, with symptoms lasting from 1 day to 3 weeks. Most reports suggest that the earlier epinephrine is administered in the course of anaphylaxis, the better the chance of a favorable prognosis, but no data indicate that the timing of epinephrine affects the prevalence of biphasic or protracted symptoms. In addition, it should be noted that in about 5% of cases in which patients have received a single injection of epinephrine almost immediately, they still progressed to fatal anaphylaxis. Even with appropriate treatment in a medical facility, it rarely may be impossible to reverse an anaphylactic reaction once it has begun.

The symptoms of anaphylaxis are generally related to the GI, respiratory, cutaneous, and cardiovascular systems. Other organ systems may be affected but much less commonly. The sequence of symptom presentation and severity varies from one individual to the next. Additionally, one patient who experiences anaphylaxis to more than one type of food may experience a different sequence of symptoms with each food. Although many patients develop similar allergic symptoms on subsequent consumption of a food allergen, patients with asthma and peanut and/or nut allergy seem to be less predictable. Many peanut-allergic

children who reacted with minimal cutaneous and GI symptoms as a young child later developed asthma and then experienced a catastrophic anaphylactic event after ingesting peanut in their teenage years.

The first symptoms often involve the oropharynx. Symptoms may include edema and pruritus of the lips, oral mucosa, palate, and pharynx. Young children may be seen scratching at their tongue, palate, anterior neck, or external auditory canals (presumably from referred pruritus of the posterior pharynx). Evidence of laryngeal edema includes a "dry staccato" or croupy cough and/or dysphonia and dysphagia. GI symptoms include nausea, vomiting, crampy abdominal pain, and diarrhea. Emesis generally contains large amounts of stringy mucus. Respiratory symptoms may consist of a deep repetitive cough, stridor, dyspnea, and/or wheezing. Cutaneous symptoms of anaphylaxis may include flushing, urticaria, angioedema, and/or an erythematous macular rash. Cardiovascular symptoms, along with airway obstruction, are of greatest concern in anaphylactic reactions. Cardiovascular symptoms include syncope, a feeling of faintness, and/or chest pain. Hypotension or shock may be the result of vascular collapse, cardiac arrhythmia, or asphyxia. Anaphylaxis may be complicated by myocardial ischemia.

Other signs and symptoms commonly reported in anaphylaxis include periocular and nasal pruritus, sneezing, diaphoresis, disorientation, fecal or urinary urgency or incontinence, and uterine cramping (manifested as lower back pain similar to "labor" pains). Patients often report a sense of "impending doom." In some instances the initial manifestation of anaphylaxis may be loss of consciousness. Death may ensue in minutes but has been reported to occur days to weeks after anaphylaxis (48). Late deaths are generally manifestations of organ damage experienced early in the course of anaphylaxis.

Several factors appear to increase the risk of more severe anaphylactic reactions. Patients taking β-adrenergic antagonists or calcium channel blockers may be resistant to standard therapeutic regimens and therefore at increased risk for severe anaphylaxis (49). Patients with asthma may be at increased risk for severe symptoms, as noted in a number of reports of fatal and near-fatal food anaphylactic reactions (5–7). Similar findings have been reported in patients with insect sting allergy (50) and from patients experiencing anaphylaxis as a result of immunotherapy (51). In these patients, acute bronchospasm developed along with other symptoms of anaphylaxis.

In a review of 43 fatal cases of anaphylaxis, approximately 80% of which were due to injections of medications, Delage and Irey (52) noted that symptoms developed within 20 minutes in 37 (86%) cases, and death ensued within 30 minutes in 14 (33%) individuals, within 1 hour in 22 (51%), and within 5 hours in 8 (19%). Respiratory distress and circulatory collapse were the presenting symptoms in (16) 37% and 14 (33%) of patients, respectively, and skin symptoms were the presenting symptom in only 3 (7%) of the cases (Table 15–4). Pathologic findings consisted of pulmonary congestion in 39 (90%) of patients, pulmonary edema in 22 (50%), intra-alveolar hemorrhage in 19 (45%), tracheobronchial secretions in 19 (45%), and laryngeal edema in 16 (38%) of cases (marked laryngeal edema appeared in only 4 [10%]). In 6 cases of fatal food-induced anaphylaxis in children (6), initial symptoms developed within 3–30 minutes and severe respiratory symptoms within 20–150 minutes. Symptoms involved the lower respiratory tract in all 6 children, the GI tract in 5 of 6, and the skin in only 1 of 6. Anaphylaxis should never be considered ruled out on the basis of absent skin symptoms. In evaluation of Hymenoptera-allergic patients undergoing venom immunotherapy, intentional bee stings resulted in 14 systemic reactions (48). Three patients experienced severe bronchospasm and hypotension that was initially refractory to epinephrine and large volumes of fluid; none developed any skin symptoms.

Diagnosis

Because of its abrupt and dramatic nature, the diagnosis of systemic anaphylaxis usually is readily apparent (Table 15–5). Often when a food is implicated, the inciting food is obvious from the temporal relationship between ingestion and the onset of symptoms. The initial step in determining the cause of an episode of anaphylaxis is a very

Table 15–4.
Presenting Symptoms in 43 Fatal Cases of Anaphylaxis

Symptoms	*Number*	*Percentage*
Respiratory	16	37%
Circulatory Collapse	14	33%
Seizures	11	26%
Cyanosis	11	26%
Nausea and Vomiting	6	23%
Dizziness and Weakness	6	14%
Skin Eruption	3	7%

From Delage and Irey (52).

Table 15–5.
Clinical Signs and Symptoms of Anaphylaxis

Organ System		*Signs and Symptoms*
Respiratory (major shock organ)	Laryngeal	Pruritus and sensation of tightness in the throat, dysphagia, dysphonia and hoarseness, dry "staccato" cough, sensation of itching in the external auditory canals
	Lung	Shortness of breath, dyspnea, chest tightness, "deep" cough, wheezing
	Nose	Pruritus, congestion, rhinorrhea, sneezing
Cardiovascular		Feeling of faintness, syncope, chest pain, arrhythmia, hypotension
Skin		Flushing, pruritus, urticaria, angioedema, morbilliform rash, pilor erecti
Gastrointestinal		Nausea, abdominal pain (colic), vomiting (large amounts of stringy mucus), diarrhea
Oral		Pruritus of lips, tongue and palate; edema of lips and tongue
Other		Periorbital pruritus, erythema and edema; conjunctival erythema, and tearing; uterine contractions in women; sense of "impending doom"

careful history, especially when the cause of the episode is not straightforward. Specific questions to ask the patient include the type and quantity of food eaten, the last time the food was ingested, the time frame between ingestion and onset of symptoms, the nature of the food (cooked or uncooked), other times when similar symptoms occurred (and if the food in question was eaten on those occasions), and whether any other precipitating factors appear to be involved, e.g., exercise, alcohol, NSAIDs.

Any food may precipitate an anaphylactic reaction, but there are a few specific foods that are often implicated in the etiology of food-induced anaphylactic reactions: peanuts, tree nuts, fish, and shellfish. When the etiology of the anaphylactic reaction is not apparent, a dietary history should review all ingredients of the suspect meal, including any possible concealed ingredients or food additives. The food provoking the reaction may be merely an unintentional contaminant in the meal. For example, peanuts or peanut butter are frequently added to cookies, candies, pastries, or sauces such as chili, spaghetti, and barbecue sauces. Chinese restaurants frequently use peanut butter to hold together the overlapping ends of an egg roll, pressed or "extruded" peanut oil in their cooking, and the same wok to cook a variety of different meals resulting in contaminant carry-over. Another infrequent (but not rare) cause of food contamination occurs during the manufacturing process. This contamination may happen with scraps of candy that are "reworked" into the next batch of candy or in processing plants when there is a production change from one product to the next. As an example, a reaction to almond butter by a peanut-allergic patient started an investigation that determined that 10% of the almond butter produced in that plant was contaminated with peanut butter. This occurred after the manufacturer changed production from peanut butter to almond butter. A more recent study by the FDA found that 25% of unlabeled foods processed in plants making products that contained peanuts, milk, or egg were contaminated with these allergens. Other examples include Popsicles run on the same line as Creamsicles, fruit juices packaged in individual cartons where milk products have been packaged, and milk-free desserts packaged in dairy plants (53). Food items with "natural flavoring" designated on the label may contain an unsuspected allergen, e.g., casein in canned tuna fish, hot dogs, or bologna, and soy in a variety of baked goods. Some foods do not indicate the presence of certain proteins when they represent less than 2% of total protein, e.g., Ritz crackers contain milk protein and Fig Newtons contain egg.

Food allergy can develop at any age, although it appears more commonly in the first 3 years of life. Often, a patient who has tolerated a food (e.g., shrimp) for his/her entire life experiences a major allergic reaction after ingestion the food sometime in mid-adulthood. These patients may experience no forewarning of their impending episode, but on detailed questioning may describe some minor symptoms previously, such as oral pruritus or nausea and cramping. It is also possible that cooking or processing of some foods may remove, diminish, or even enhance their allergenicity.

Some conditions may be confused with food anaphylaxis. Among these clinical problems are scombroid poisoning, factitious allergic emergency, and vasovagal collapse. In the absence of urticaria and angioedema, one must consider arrhythmia, myocardial infarction, hereditary angioedema, aspiration of a bolus of food, pulmonary embolism, and seizure disorders.

With the presence of laryngeal edema, especially when accompanied by abdominal pain, the diagnosis of hereditary angioedema must be considered. In general, this disorder is slower in onset,

does not include urticaria, and often there is a family history of similar reactions. Systemic mastocytosis results in flushing, tachycardia, pruritus, headache, abdominal pain, diarrhea, and syncope. A factitious allergic emergency may occur when patients knowingly and secretively ingest a food substance to which they are known to be allergic (54).

In vasovagal syncope, the patient may collapse after an injection or a painful or disturbing situation. The patient typically looks pale and complains of nausea prior to the syncopal episode, but does not complain of pruritus or become cyanotic. Respiratory difficulty does not occur and symptoms are almost immediately relieved by recumbence. Profuse diaphoresis, slow pulse, and maintenance of blood pressure generally complete the syndrome. Hyperventilation may cause breathlessness and collapse. It is usually not associated with other signs and symptoms of anaphylaxis, except peripheral and perioral tingling sensations. Blood pressure and pulse are generally normal.

Laboratory Evaluation

The laboratory evaluation of patients with an anaphylactic reaction should be directed at identifying specific IgE antibodies to the food in question. IgE antibody can be recognized in vivo by SPT. Although not absolutely proven in patients with anaphylaxis, a negative SPT is an excellent predictor for a negative IgE-mediated food reaction to the suspected food. In contrast, a positive SPT does not necessarily mean that the food is the inciting agent, but in a patient with a classic history of anaphylaxis to ingestion of an isolated food and a positive SPT to that food, this laboratory test appears to be a good positive predictor of allergic reactivity.

Skin testing has some limitations that should be recognized. An SPT performed shortly after the occurrence of anaphylaxis may fail to yield a positive response owing to temporary anergy. Although not demonstrated in food allergy, this phenomenon has been demonstrated in Hymenoptera sensitivity following an insect sting (55). Possible causes of false-negative SPTs include improper skin test technique, concomitant use of antihistamines, or the use of food extracts with reduced or inadequate allergenic potential. With some foods, the processing of the food for commercial extracts may diminish antigenicity (56). This is especially true for some fruits and vegetables. However, if suspicion is high that a food may have precipitated an anaphylactic reaction even though the SPT is negative, the patient should be tested with the natural food utilizing the "prick-plus-prick" method, in which a needle is used to prick the food and then the material is used to prick the skin, to ensure an absence of detectable IgE antibody (57). Caution should be exercised in doing this procedure, because the amount of antigen on the prick device is not controlled. Appropriate negative controls also should be performed.

Appropriate skin testing is indicated in each patient, although in vitro measurement of food-specific IgE may be evaluated initially. In many patients with anaphylaxis, limited SPT is necessary to confirm the etiology of the anaphylactic reaction. In cases of idiopathic anaphylaxis, more extensive SPT may be helpful for diagnosis (43). The clinician must decide how many SPTs are practical and justified, taking into account the anticipated low yield of positive results in idiopathic anaphylaxis and the value of discovering an etiology in this serious disorder.

Intradermal skin tests are performed by some physicians following negative SPTs in other allergic diseases, but the diagnostic significance of a positive intradermal test following a negative SPT is dubious and of no clinical benefit (48). Fatal anaphylactic reactions have been documented following intradermal skin tests to foods (51), so extra caution should be exercised if intradermal tests are performed (if done at all). Under no circumstances should an intradermal skin test be performed before an SPT. When extreme hypersensitivity is suspected, alternative approaches may be warranted, including further dilution of the food extract used in SPT, or use of a food-specific in vitro test such as radioallergosorbent test (RAST). Overall, the RAST is considered less sensitive and specific than the SPT, but the new CAP-System FEIA appears to be slightly more sensitive than the standard RAST. In high quality laboratories a 3+ to 4+ RAST (on a 1+ to 4+ Phadebas RAST scale) probably has a similar positive predictive value as an SPT 3 mm greater than the negative control (58). Recent studies suggest that the CAP-System FEIA can give better predictive values for predicting a positive food challenge for at least milk, egg, and peanuts (59, 60).

Massive activation of mast cells during anaphylaxis results in a dramatic rise in plasma histamine and somewhat later a rise in plasma or serum tryptase (61–63). Plasma histamine rises over the first several minutes of a reaction and generally remains elevated for brief period of time; it requires

special collection techniques and will degrade unless the plasma sample is frozen immediately. Consequently, measurement of plasma histamine to document anaphylaxis is impractical except in research situations. Whether measurement of urinary methylhistamine is useful in the documentation of anaphylaxis remains to be demonstrated. Serum tryptase rises over the first hour and may remain elevated for many hours. It is fairly stable at room temperature and can even be obtained from postmortem specimens. Tryptase is markedly elevated in most cases of bee sting or drug-induced anaphylaxis (62, 63). Unfortunately, tryptase is not usually elevated in food-induced anaphylaxis (6). A recent study found that tryptase was not elevated in many patients seen in the emergency room for treatment of anaphylaxis (64). The reason for this lack of elevation is not clear, but it suggests that other cells, such as basophils or monocytes/macrophages may be more important in food-induced anaphylaxis.

DBPCFCs are contraindicated in patients with an unequivocal history of anaphylaxis following the isolated ingestion of a food to which they have evidence of specific IgE antibodies. However if several foods were ingested and the patient has positive SPTs to several foods, it is essential that the responsible food be identified. Patients have experienced repeated anaphylactic reactions because physicians incorrectly assumed that they had identified the responsible food (6). Young children who experience anaphylactic reactions to foods other than peanuts, tree nuts, fish, and shellfish may eventually outgrow their clinical reactivity, so an oral challenge may be warranted after an extended period of food elimination with no history of reactions to accidental ingestions.

Treatment

Treatment of food-induced anaphylaxis may be subdivided into acute and long-term management. Although management of an acute attack is something physicians spend hours preparing for, it is the long-term measures that provide the best quality of life for the food-allergic patient.

Acute Management (Table 15–6)

Fatalities may occur if treatment of a food-induced anaphylactic reaction is not immediate (5, 6). Data from a review of fatal bee sting-induced anaphylactic reactions indicate that the longer the initial therapy is delayed, the greater the incidence of complications and fatalities (48). Initial treatment must be preceded by a rapid assessment to determine the extent and severity of the reaction; the adequacy of oxygenation, cardiac output, and tissue perfusion; any potential confounding medications (e.g., β-blockers); and the suspected cause of the reaction (8). Initial therapy should be directed at maintaining an effective airway and circulatory system. Epinephrine (adrenaline) is the drug of choice in the treatment of anaphylaxis. The first step in the management of anaphylaxis is the intramuscular (IM) injection of 0.01 mL/kg of aqueous epinephrine 1:1000 (maximal dose 0.3–0.5 mL, or 0.3–0.5 mg). Intravenous (IV) administration of epinephrine may cause fatal arrhythmias or myocardial infarction, particularly in adults, and should be reserved for refractory hypotension requiring cardiopulmonary resuscitation (CPR). In patients with pulmonary symptoms, supplemental oxygen should be administered.

The importance of epinephrine in the treatment of anaphylaxis is best seen in fatal and near-fatal food-induced anaphylaxis. In general, patients who die from the anaphylactic reaction have received no epinephrine or an inadequate dose

Table 15–6.
Acute Management of Anaphylaxis

Rapid Assessment of:	
	• Extent and severity of symptoms • Adequacy of oxygenation, cardiac output, and tissue perfusion • Potential confounding medications • Suspected cause of the reaction
Initial therapy:	
	• Epinephrine, 0.01 mg/kg/dose up to 0.3–0.5 mg SC or IM up to three times every 20 minutes • Oxygen, 40%–100% by mask • Intravenous fluids, 30 mL/kg of crystalloid up to 2 L (or more, depending on blood pressure and response to meds)
Secondary Medications:	
	• Nebulized albuterol; may be continuous • Antihistamines: H_1 antagonist (diphenhydramine, 1 mg/kg up to 75 mg) H_2 antagonist (cimetidine, 4mg/kg up to 300 mg) • Corticosteroids: solumedrol, 1–2 mg/kg/dose IV, prednisone, 1–2 mg/kg/dose orally • Dopamine, 2–20 μg/kg/min, for hypotension refractory to epinephrine • Norepinephrine, for hypotension refractory to epinephrine • Glucagon, for hypotension refractory to epinephrine and norepinephrine, especially patients on β-blockers

during their acute reaction (5, 6, 48). In contrast, patients who have survived a near-fatal anaphylactic reaction generally received epinephrine early in the course of their reaction, and many have received repeated doses of epinephrine.

To ensure that patients receive epinephrine as early as possible, it is important that they, their family members, and other care providers are instructed in self-administration of epinephrine. Preloaded syringes with epinephrine are available and should be given to any patients at risk for food-induced anaphylaxis, i.e., patients with a history of a previous anaphylactic reaction and patients with asthma and food allergy, especially if they are allergic to peanuts, nuts, fish, or shellfish. In the US, premeasured doses of epinephrine can be obtained in two forms: Epi-Pen and Epi-Pen Junior (distributed by Dey Laboratories). The Epi-Pen is a disposable drug delivery system with a spring-activated concealed needle intended for a single IM injection. Epi-Pen contains 0.3 mg (adult dose), and Epi-Pen Junior contains 0.15 mg, the dose for children less than 17–18 kg. Young children are advanced to the regular Epi-Pen when they reach 20–25 kg, depending on the severity of previous reactions. Children with a history of severe symptoms should be advanced earlier than those with milder reactions. The device is pressed firmly into the thigh muscle (or trigger-activated, with the newer version of the Epi-Pen) and held in place for several seconds to allow the medication to be injected. Although some epinephrine remains in the device after it is used, no more epinephrine is accessible once the needle has been exposed. Because the Epi-Pen can deliver only a single dose, two Epi-Pens may be prescribed for patients who have experienced a previous anaphylactic reaction or who are at high risk and do not have ready access to a medical center (the kit now comes from the manufacturer with two pens). It is imperative that the patient and/or family members practice with appropriate training devices to ensure their ability to use the device proficiently in case of an emergency. Also, it should be made clear to the patients that these preloaded devices carry a 1-year shelf life and therefore should be renewed each year.

Sustained-release preparations of epinephrine are not appropriate treatment for acute anaphylaxis. Inhaled epinephrine (either nebulized or via metered-dose inhaler as Primatene Mist in the US) has been recommended by the European Academy of Allergy and Clinical Immunology (65). A minimum of 20 puffs inhaled correctly can produce blood levels similar to an injection of 0.3–0.5 mg of epinephrine in adults, and 10–15 puffs in a child can deliver the equivalent of 0.15 mg injected subcutaneously (SC) (66, 67). A study by Simons and coworkers (68) demonstrated that most children are not capable of inhaling 10–15 puffs of adrenaline, so this method should not be considered in children. Lesser doses may help reverse laryngeal edema or persistent bronchospasm.

Once epinephrine has been administered, other therapeutic modalities may be beneficial. A combination of an H_1 antihistamine (i.e., diphenhydramine, 1 mg/kg up to 75 mg) either IM or IV, and an H_2 antihistamine (i.e., cimetidine, 4 mg/kg up to 300 mg) IV may be more effective than either administered alone (69). Both histamine antagonists should be infused slowly if given IV, because rapid infusion of diphenhydramine is associated with arrhythmias, and cimetidine with falls in blood pressure. The role of corticosteroids in treating anaphylaxis remains unclear. However, most authorities recommend prednisone (1 mg/kg orally) for mild to moderate episodes of anaphylaxis, and Solu-Medrol (1–2 mg/kg IV) for severe anaphylaxis in an attempt to modulate the late-phase response. Patients who have been receiving glucocorticosteroid therapy for other reasons should be assumed to have hypothalamic-pituitary-adrenal-axis suppression and should be administered stress doses of hydrocortisone intravenously during resuscitation. If wheezing is prominent, an aerosolized β-adrenergic agent (i.e., albuterol) is recommended intermittently or continuously, depending on the patient's symptoms and the availability of cardiac monitoring. IV aminophylline may also be useful for recalcitrant respiratory symptoms. Aerosolized epinephrine may be useful for preventing life-threatening upper airway edema; however, in about 10% of patients a tracheotomy is required to prevent fatal laryngeal obstruction (52). Hypotension due to a shift in fluid from the intravascular to extravascular space may be severe and refractory to epinephrine and antihistamines. Depending on blood pressure, large volumes of crystalloid (e.g., lactated Ringer's solution or normal saline) infused rapidly are frequently required to reverse the hypotensive state. An alternative to crystalloid solution is the colloid hydroxyethyl starch. Children may need up to 30 mL/kg of crystalloid over the first hour (70), and adults up to 2 L total (56), over the first hour to control hypotension. Patients taking β-blockers may require much larger volumes (e.g., 5–7 L) of fluid before pressure is stabilized (71). Although epinephrine and fluids are the mainstay of treatment

for hypotension, the use of other vasopressor drugs may be necessary. Dopamine administered intravenously at a rate of 2–20 μg/kg/min while carefully monitoring the blood pressure may be lifesaving. In addition, 1–5 mg of glucagon given as an intravenous bolus followed by an infusion of 5–15 μg/min titrated against clinical response may be helpful in refractory cases or in patients taking β-blockers. The best treatment of patients experiencing anaphylaxis while taking β-adrenergic blocking drugs remains a matter of some concern. If combined β_1 and β_2 receptor blockers (e.g., propranolol) are used, it may be possible to administer epinephrine for its α-adrenergic activity, and isoproterenol to attempt to overcome the β blockade. Because patients may experience a biphasic response, all patients should be monitored for a minimum of 4 hours, longer in cases of more severe anaphylaxis.

Although still somewhat controversial, some authorities have suggested the use of activated charcoal in an attempt to prevent further absorption of food allergens from the gut. Others have suggested that some attempt should be made to evacuate the stomach if vomiting has not already occurred. Some have advocated the use of gastric lavage when large amounts of the allergen have been ingested. Whether or not these measures are beneficial in ameliorating food-induced anaphylaxis remains to be demonstrated.

Patients who are at risk for food-induced anaphylaxis should have medical information about their condition available with them at all times, e.g., a Medic Alert bracelet. An emergency treatment plan, such as that posted on the Food Allergy and Anaphylaxis Network's web site (foodallergy.org), should be available to anyone caring for the food-allergic patient. This information may be lifesaving, because it can expedite the diagnosis and appropriate treatment of a patient experiencing an anaphylactic reaction.

Long-Term Management (Table 15–7)

The life-threatening nature of anaphylaxis makes prevention the cornerstone of therapy. If the causative food allergen is not clearly delineated, an evaluation to determine the etiology should be promptly initiated so that a lethal reoccurrence can be prevented, as discussed above. The central focus of prevention of food-induced anaphylaxis is appropriate identification and complete dietary avoidance of the specific food allergen. Certain factors, such as history of food allergy or asthma, place some individuals at increased risk for severe anaphylactic reactions (Table 15–8). Education is imperative to ensure that the patient and family understands how to avoid all forms of the food allergen and the potential severity of a reaction if the food is inadvertently ingested. The Food Allergy and Anaphylaxis Network is a nonprofit organization in Fairfax, VA (phone 703-691-3179 or 800-929-4040; fax 703-691-2713; website http://www.foodallergy.org) that can assist patients by providing information about food allergen avoidance, and that has programs for schools and parents of children with food allergies and anaphylaxis.

Patients who have already had a food allergic reaction often subsequently demonstrate instinctive avoidance of that food. This may be typified by extreme dislike for the taste or even smell of the offending food. However, the sensitized person must become proactive to completely avoid a food that has caused an anaphylactic reaction. For many this may even require total removal of the food from the household. Educational measures must be directed at the patient, his/her family, and school personnel and other caretakers or fellow workers so that they understand the potential severity and scope of the problem. If a patient consumes a food prepared outside the home, he/she must always be very cautious and not hesitate to ask very specific and detailed questions about in-

Table 15–7.
Long-Term Management of Food-Induced Anaphylaxis

1) Identify the food that provoked the anaphylactic reaction.
2) Educate patient, family, and/or care providers how to avoid all exposure to the food allergen.
3) Provide patient with self-injectable epinephrine and thoroughly teach him/her when and how to use it (i.e., practice with Epi-Pen trainer).
4) Provide patient with liquid antihistamine (diphenhydramine or hydroxyzine) and teach them when and how to use it.
5) Establish a formal emergency plan in case of a reaction: proper use of emergency medications, transportation to nearest emergency facility capable of resuscitation and endotracheal tube placement.

Table 15–8.
Factors That Suggest Increased Risk of Severe Anaphylaxis

- History of a previous severe anaphylactic reaction
- Patient with asthma, especially if poorly controlled
- Allergy to peanuts, nuts, fish, and/or shellfish
- Patients on β-blockers or ACE inhibitors
- Female (?)
- Adolescents and young adults (?)

gredients of foods they are planning to eat. Unfortunately, patients dining in restaurants often ingest an allergy-provoking food that they were assured did not exist in the meal they were eating.

Although changes in food labeling laws in the US have improved the reading of labels for food-allergic individuals, several problems still remain. These problems fall into five categories: 1) misleading labels, e.g., "non-dairy" creamers usually contain some milk proteins; 2) ingredient switches, e.g., a name brand food may alter the ingredients with no significant change on the label; 3) "natural flavoring" designation often allows a product to contain but not identify a small amount of other food proteins for flavoring purposes, e.g., casein in canned tuna fish; 4) legal labeling loopholes allow proteins at less than 2% of total protein to be included in a product without indicating its presence; and 5) inadvertent contamination that may occur when more than one product is run on a line and residual protein from the previous run contaminates the subsequent run, e.g., nondairy ice cream desserts. It is still imperative that patients and their families be taught the many "words" indicating a particular food protein as one of the ingredients of a food product, and that they try to be aware of unexpected product contents.

A recent phase I/II trial indicated that the use of humanized, recombinant anti-IgE antibody in peanut-allergic individuals can significantly increase the quantity of peanut necessary to induce an allergic response (72). Future studies should demonstrate whether the prophylactic use of anti-IgE will prevent severe IgE-mediated food-allergic reactions.

Prognosis

Many young children diagnosed with anaphylaxis to foods such as milk, egg, wheat, and soybeans, may well outgrow their clinical sensitivity after several years. Children who develop their food sensitivity after 3 years of age are less likely to lose their food reactions over a several-year period. About 20% of children who develop peanut allergy early in life will outgrow this sensitivity (36). Allergies to foods such as tree nuts, fish, and seafood are generally not outgrown, no matter at what age they develop. These individuals are likely to retain their allergic sensitivity for a lifetime. With better characterization of allergens and understanding of the immunologic mechanisms involved in this reaction, investigators have developed several therapeutic modalities potentially helpful in the treatment and prevention of food allergy. Among the therapeutic options currently under investigation are peptide immunotherapy, mutated allergen protein immunotherapy, DNA immunization, immunization with immunostimulatory sequences, and anti-IgE therapy. These novel forms of treatment for allergic disease hold promise for the safe and effective treatment and prevention of food allergies (73).

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16

Infantile Colic and Food Hypersensitivity

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Introduction

Persistent crying is a common pediatric problem that affects a large number of young infants (1). In the majority, the distressed behavior commences at about 6 weeks and gradually improves by 3–4 months of age (2, 3). Although the etiology of infantile colic is not understood, increasing evidence links persistent crying and distress in the young infant to food hypersensitivity (4–6). Interactive factors and behavior patterning may also influence the clinical course of infantile colic (7).

Crying and fussing, especially in the evening, are normal developmental phenomena in the first months of life (2). Unexplained paroxysms of irritability, fussing, or crying that persist for more than 3 hours per day, for more than 3 days per week, and for at least 3 weeks, are considered a separate clinical condition termed colic (3, 8). During such episodes the legs may be drawn up to the abdomen and the infant may become flushed. Abdominal distension and increased passage of flatus are often noted. Parents may attribute these episodes to pain. However, the infant appears generally well, and in less than 5% of infants is an underlying medical etiology identified (7).

Epidemiology of Colic

Prevalence figures for infantile colic vary greatly depending on the definition of colic and the recruitment method used in epidemiological studies (9). No population-based prevalence study using generally accepted diagnostic criteria for colic has yet been performed. In general, the methodological quality of prevalence studies has been poor, with likely recruitment bias toward severe colic and families presenting in crisis (1). Several factors may explain the difficulties in obtaining a reliable prevalence estimate. Mothers with depressive symptoms may be more likely to seek help for their crying infant (10, 11). Furthermore, parents may perceive persistent crying as more worrisome if it is associated with symptoms such as regurgitation (12) or feeding difficulties (13). However, because of spontaneous improvement of the condition, some parents of infants with true colic may not seek or need medical help (1). Recent studies have estimated a prevalence for infantile colic of 5%–19% (1, 14, 15). Table 16–1 shows the varying prevalence of colic reported from different Western countries.

Etiology of Infantile Colic and Distressed Behavior

The etiology of infantile colic is multifactorial. Our understanding of the mechanisms leading to distressed behavior in early infancy is still incomplete and often based on observation rather than evidence. The term "colic" implies that the infants' distress is related to pain or spasm, although such a mechanism has never been conclusively demonstrated. For this reason, alternative

Table 16–1.
Prevalence of Infantile Colic

Author	*Country*	*Year*	*Prevalence*
Wessel et al (8)	U.S.A.	1954	48%
Illingworth (3)	England	1954	20%
Boulton and Rowley (101)	Australia	1979	41%
Thomas (102)	Australia	1981	35%
Hide and Guyer (29)	England	1982	16%
Rubin and Prendergast (103)	England	1984	26%
Carey (104)	U.S.A.	1984	10%
Lothe (105)	Sweden	1989	17%
Michelsson et al (106)	Finland	1990	14%
Hogdall et al (107)	Denmark	1991	19%
Rautava et al (11)	Finland	1993	28%
Lehtonen and Korvenranta (108)	Finland	1995	13%
Canivet et al (14)	Sweden	1996	11%
Canivet et al (15)	Sweden	2002	9.4%

terms such as "persistent crying" or "distressed behavior" have been used. In the following discussion, the term "colic" will be used interchangeably with each of these terms without implying a particular pathological mechanism.

Three major theories have emerged about the etiology of infantile colic (16):

- Colic is part of normal emotional development, in which an infant has diminished capacity to regulate crying duration. This may lead to a disturbance in mother-infant interaction (7).
- Colic is distinct from normal crying behavior and results from an adverse reaction to foods (4).
- Colic is due to pain or discomfort associated with gastrointestinal (GI) abnormalities, including gastroesophageal reflux (GER) and esophagitis (17–19), lactose malabsorption (20–22), or GI motility disturbance (23–25).

Infantile and Parental Factors Associated with Infantile Colic

Infantile Factors

Brazelton (2) used parental recording on cry charts to document the natural history of distressed behavior in infancy. Figure 16–1 summarizes the pattern of crying and fussing in a group of 80 infants studied in the first 12 weeks of life. Distressed behavior frequently worsened until the children were about 6 weeks of age and then gradually improved. In the majority of infants, fussing behavior occurred during the late afternoon and evening (2, 8). Brazelton (2) found that in six of

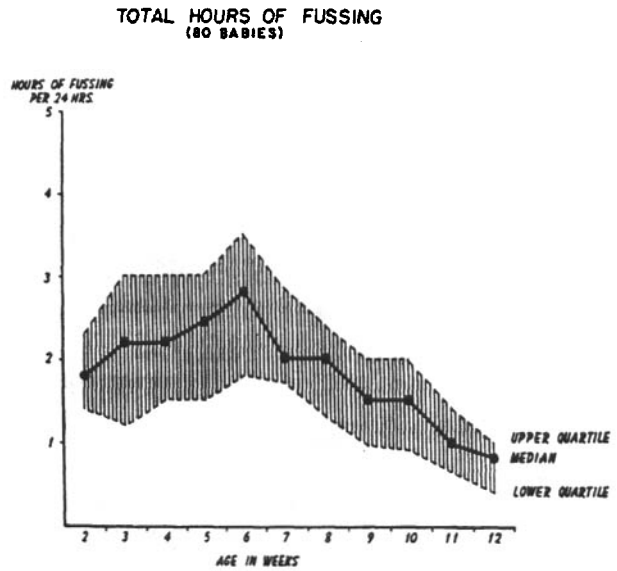

Figure 16–1. Total crying time of 80 infants in the first 12 weeks of life. From Brazelton (2).

these infants, distress was more marked, peaked beyond 6 weeks of age, and persisted to the 12-week follow-up.

Barr et al (26) developed a 24-hour crying chart that has been validated against objective measurements of infant distress. Information charted by parents was compared with voice-activated audiotape recordings (VAR). Their study of 10 infants showed a good correlation between crying diaries and VAR. Using these validated cry charts, Hunziker and Barr (27) confirmed Brazelton's (2) previous findings on the natural history of distressed behavior in young infants.

Studies by our group compared the pattern and duration of distressed behavior in 30 colicky and non-colicky infants (28). Figure 16–2 shows the higher levels of distressed behavior in the colicky infants than in the non-colicky infants. These findings were confirmed in a separate group of 90 colicky infants. The evaluation of distressed behavior on an hour-by-hour basis confirmed the predominance of nocturnal symptoms, but like Hide and Guyer (29), we found that in colicky infants distressed behavior frequently occurred during other time periods (Fig. 16–3).

Children with a past history of colic are at increased risk of experiencing negative emotions and negative moods during meals, and are more likely to report abdominal pain in early childhood, suggesting that infant temperament may be a factor contributing to infantile colic (30). However, the majority of colicky infants develop normal parent-

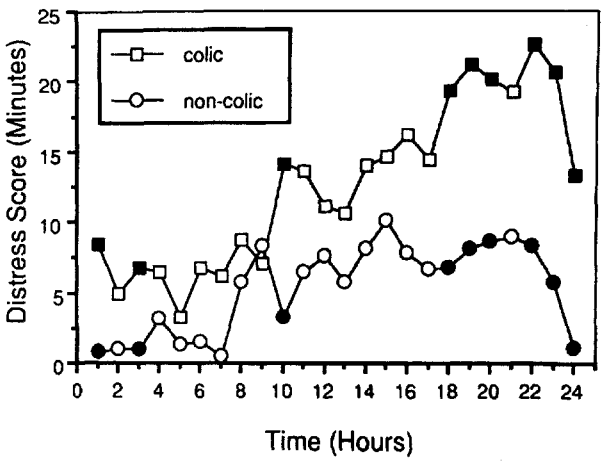

Figure 16–2. Duration of total distress (the sum of cry and fuss time) recorded over 24 hours in 30 colicky and 30 non-colicky infants (Group 1). The colic group showed significantly more total distress than the non-colic group; horizontal bars indicate median ($P < .001$). The median total distress score of a second group of 90 colic infants (Group 2) was 280 minutes (28).

child interaction and relationships, and only a small number will progress to a more generalized "persistent mother-infant distress syndrome" (31).

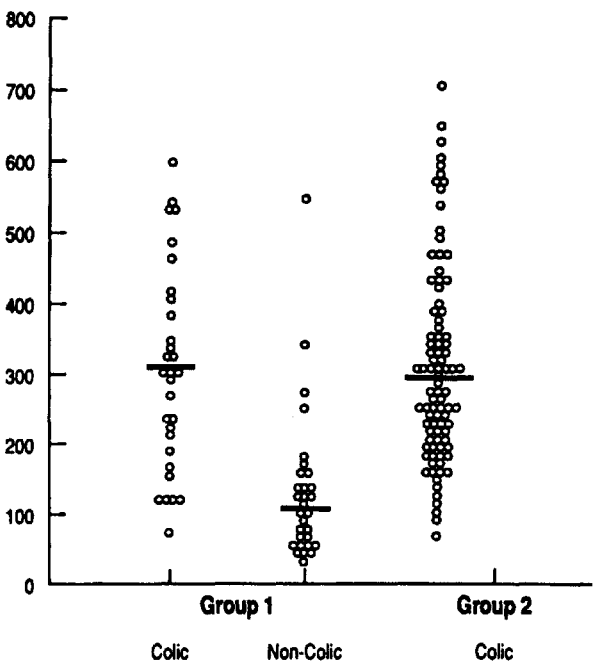

Figure 16–3. One-hour distress scores (minutes). Hourly mean duration of crying and fussing time recorded over 24 hours. Colicky infants showed significantly more distress than non-colic infants. Filled symbols mark periods with a significant difference in crying and fussing between groups ($P < 0.05$) (28).

Maternal Factors

The unpredictable, prolonged, and unexplained nature of crying in colicky infants is a source of great concern and anxiety for parents (32, 33). Studies by Rautava et al (34) in Finnish infants suggested an association between colic and maternal distress during pregnancy and childbirth or unsatisfactory sexual relationships, but not between colic and sociodemographic factors. Mothers who report excessive infant crying are also more likely to perceive a lack of positive reinforcement from their infant (35).

Infants with colic sleep significantly less than non-colicky infants, although sleep polygraphic findings did not indicate a sleep disorder in these infants (36). Maternal reports of infants' sleep problems were significantly associated with depressive symptoms (10), suggesting that maternal depressive symptoms during the early infant period may be caused or compounded by sleep deprivation that is caused by an infant with persistent crying.

Behavior Interventions and Parental Support

Several studies have assessed the importance of behavioral and interactive factors in infantile colic. The results of these studies are summarized in Table 16–2. Taubman (37) compared parental counseling and dietary interventions in a study of 21 colicky infants. He found that increasing parental responsiveness had a similar effect on persistent crying as the introduction of a cow's milk-free diet. Interestingly, the distressed behavior of diet-responsive colicky infants decreased further with parental counseling. Taubman concluded that infant distress may result from parental misinterpretation of infant behaviors. However, in view of the small number of patients and the difficulty of blinding counseling procedures, these results need to be interpreted cautiously.

Hunziker and Barr (27) also suggested that distressed behavior in infancy might reflect parental misinterpretation of normal crying behavior. Following their observation that normal infants cried less when regularly nurtured by supplemental carrying, Barr et al (38) studied the effect of supplemental carrying on 66 colicky infants. In 6-week-old colicky infants, however, a significant treatment benefit of supplemental carrying could not be demonstrated (38). Hunziker's group con-

Table 16–2.
Studies Investigating Disturbed Family Interaction as a Cause of Colic

Author	*Study Details*	*Outcome: Change in Distress (hours per 24-hour period)*
Hunziker and Barr (27)*	Carrying (N = 49)	1.2
	Control (N = 50)	2.2 ($P < .001$)
Barr et al (38)	Carrying (N = 31)	3.3
	Control (N = 35)	3.4 ($P > .05$)
Taubman (37)	Counseling (N = 10)	3.2 vs 1.06 ($P = .001$)
	Diet (N = 10)	3.2 vs 2.03 ($P = .01$)
Wolke et al (39)	Empathy (N = 27)	6.3 vs 3.7 ($P < .001$)
	Behavior modification (N = 21)	5.8 vs 2.8 ($P < .001$)†
	Control (N = 44)	5.7 vs 3.7 ($P < .001$)

*Study of non-colic infants; †behavior modification superior to empathy and control ($P < .02$).

cluded that this difference in response to carrying may be due to an underlying pathological process such as protein hypersensitivity or irritable bowel. Wolke et al (39) examined the effect of different behavior strategies in 92 pairs of mothers and colicky infants. After 3 months, infants' distress had improved in all patients. Infants of mothers who received advice on behavior modification improved their distress by 51%, compared with 37% in mothers who received empathic support and 35% in the control group.

Colic as a Manifestation of Food Protein Allergy

Several trials have demonstrated a treatment benefit for soy and extensively hydrolyzed formulas in infants with colic, even when no other symptoms of food protein allergies were evident (Table 16–3). Irritability is common in infants with hypersensitivity to cow's milk and other food proteins (4). Infants hypersensitive to cow's milk frequently demonstrated similar reactions to other foods, including eggs, peanuts, nuts, wheat, soy formula, and extensively hydrolyzed casein and whey hydrolysate preparations (40, 41).

Cow's Milk Allergy and Colic

In a sequential cohort of 100 patients with challenge-proven cow's milk allergy (CMA), 44% of infants displayed irritable and colicky behavior during the cow's milk challenge procedure (42). Cow's milk challenge in young children suspected of having CMA elicited a range of manifestations

Table 16–3.
Studies Supporting the Role of Diet as a Cause of Infantile Colic

Author	*Study Details*	*Outcome*
Jakobsson and Lindberg (88)	Breast (N = 10)	Conditional probability of 95% that intact whey protein is implicated in colic
Evans et al (89)	Breast (N = 20)	Range of maternal diet significant ($P < .05$)
Lothe and Lindberg (91)	Formula (N = 24)	Casein hydrolysate 1.0 hour vs intact whey protein 3.2 hours ($P < .001$)
Forsyth (92)	Formula (N = 17)	Cow's milk distress > casein hydrolysate distress ($P < .01$)
Lucassen et al (84)	Formula (N = 43)	Difference in decrease of crying by 63 minutesper day for extensively hydrolyzed whey formula compared to standard formula ($P < .05$)

(42–45). Three clinical groups of CMA were identified on the basis of timing of reactions (immediate, intermediate, and late onset) (42). Children who developed immediate reactions responded to small volumes of cow's milk within 1 hour of commencing the cow's milk challenge. In contrast, children with intermediate reactions tolerated 60–200 mL of cow's milk before vomiting and diarrhea developed over several hours. The third group (late-onset reactions) usually tolerated near-normal volumes of cow's milk for 24–72 hours before symptoms of CMA developed. The prevalence of distressed behavior after cow's milk challenge was similar in the three groups, suggesting that distressed behavior in infants with CMA may be due to several immunological mechanisms.

Sleep disturbance is a major feature of infants with colic. Kahn et al (46) identified 15 infants in whom sleep disturbance resolved within 5 weeks of commencing a cow's milk-free diet. More than half of the infants had eczema, vomiting, and diarrhea before receiving the cow's milk-free diet, consistent with a clinical diagnosis of CMA. In subsequent double-blind, placebo-controlled challenges (DBPCFCs), the sleep disturbance recurred within 4 days of the reintroduction of cow's milk. The effect of milk exclusion and reintroduction was monitored by polysomnography to document arousal and sleep disturbance patterns and was evaluated by measurement of skin water evaporation during non-rapid eye movement sleep. GER and cardiorespiratory dysrhythmias had

been excluded as sources of the distressed sleep pattern.

Food Allergy in Breast-Fed Infants

Evidence is increasing that maternal ingested food antigens are secreted into human milk that may sensitize the breast-fed infant (47–50). Sensitization to multiple food antigens has been described in breast-fed infants (51). Several intact dietary antigens have been demonstrated in breast milk, including β-lactoglobulin (49), ovalbumin (50), peanut (52), and gliadin (53).

Allergic IgE sensitization can occur during the fetal period (54, 55). Immunologic host factors appear to mediate sensitization to food allergens in breast milk. Transforming growth factor-beta (TGF-β) is an important immunoregulator in promoting development of oral tolerance. During early infancy, breast milk is the main source of TGF-β. Kalliomäki et al (56) found that TGF-β promotes specific IgA production in human colostrums. IgA antibodies in human milk have a protective effect on sensitization to food allergens as they may prevent antigen entry at the intestinal surface of infants (57). Other factors that may mediate the immune response to ingested food antigens include regulatory lymphokines, such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). TNF-α is produced by activated macrophages and T lymphocytes in human milk, and it upregulates HLA class II expression. Defective TNF-α production may be a factor impeding the development of oral tolerance to ingested food antigens in breast-fed infants (58).

Differences Between Breast- and Formula-Fed Infants

In contrast to breast-fed infants, formula-fed infants often develop infantile colic before 6 weeks of age (2, 59). Diurnal variation of distressed behavior differs significantly between breast- and formula-fed infants. In our studies on colicky infants, we found that although the total distress levels were similar over a 24-hour period, formula-fed infants showed significantly more distress in the morning hours than breast-fed infants, whereas breast-fed infants were more distressed in the afternoon (4, 28) (Fig. 16–4). Axelsson et al (60) noted that about 4 hours after a mother ingested cow's milk, β-lactoglobulin appeared in her breast milk, and the highest concentrations were found 8

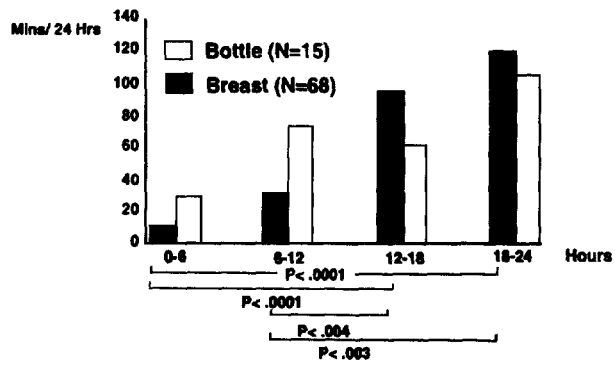

Figure 16–4. Bottle-fed infants show more distress before midday, whereas breast-fed infants have significantly more distress in the afternoon and evening (4).

to 12 hours after ingestion. Paganelli et al (61) demonstrated that cow's milk antigen appeared in serum within 1 hour of ingestion. Thus, formula feeding with a large dose of ingested antigens may elicit a more rapid distress response than prolonged low-dose antigen exposure through breast milk. These observations may explain the differences in age of onset and diurnal variation of distressed behavior between breast- and formula-fed infants with colic.

Development of Food Allergy in Children Presenting with Colic

A recent study from Finland suggested that infants with infantile colic are significantly more likely to develop atopy compared to non-colicky infants (62). In that study, 44 (38%) of 116 high-risk infants had developed atopy by 2 years of age. At 12 weeks of age, these atopic infants had presented with significantly more colic-type distress than the non-atopic infants. However, another prospective study of 983 infants found no evidence of an increased risk for asthma and other atopic manifestations in colicky infants (63). The absence of atopic manifestations in the majority of colicky infants suggests that non-IgE-mediated mechanisms are important in the pathogenesis of infantile colic.

Infants with colic may respond to cow's milk protein exclusion but often do not display other clinical manifestations of CMA. In a study of 70 infants with severe colic, Iacono et al (64) used soy formula in 70 cow's milk formula-fed infants with severe colic. Fifty (71%) of the infants had improvement of their colic after a change to soy formula, and their distress relapsed within 24 hours

of reintroduction of cow's milk into their diets (64). Within 3 weeks, 8 (16%) of 50 infants developed soy allergy, and at the age of 9 months, 18 developed other symptoms of CMA at challenge. Lothe et al (65) noted a similar phenomenon. Of 43 infants with colic who responded to exclusion of cow's milk, 8 (18%) showed other features of CMA by the age of 6 months, and 6 (13%) retained these features to at least 12 months of age (65). Although the severity of colic at presentation in these infants may have overshadowed other minor features of CMA, it is more likely that colic represented one early manifestation of true food protein hypersensitivity.

Colic and Gastrointestinal Disorders

Gastroesophageal Reflux, Esophagitis and Infantile Colic

Persistent distress and feeding refusal in the early infant period are frequently attributed to GER (17, 18, 66). This is based on the assumption that acid reflux, even in the absence of esophagitis, may be associated with pain and feeding resistance. Crying itself does not appear to increase GER (67). Distressed infants are often empirically treated with antireflux medications (68). However, a causal relationship between GER and distress has never been demonstrated (17).

GER is considered pathological if it is associated with acid-peptic complications (esophagitis, esophageal strictures, etc.), failure to thrive, or respiratory complications (aspiration, persistent wheeze, stridor, apneic episodes). In three retrospective series of infants with severe persistent distress, abnormally frequent acid reflux was demonstrated in 15%–25% of infants studied by 24-hour esophageal pH monitoring (68–70). This exceeds the expected prevalence of 5%–10% in young infants (71) and may partly be explained by selection bias in infants referred for gastroenterological investigation. Abnormally frequent or prolonged GER on pH monitoring usually presented with overt regurgitation, and non-regurgitant "silent" GER was uncommon (69, 70). The duration of daily crying and fussing did not correlate with the severity of GER, thus a direct causal relationship between acid reflux and crying appears unlikely (69).

Esophageal 24-hour pH monitoring is the definitive diagnostic test for GER. In a study of 125 distressed infants with symptoms of GER, one quarter had an abnormal pH study, and one quarter had histologic esophagitis. However, we found poor diagnostic agreement between abnormal pH monitoring and histological evidence of esophagitis (70). This may indicate a non-acid-peptic etiology of the esophagitis in these infants. Esophagitis was frequently associated with gastritis or duodenitis, suggesting the presence of a more generalized upper GI inflammatory process in infants with persistent distress (70, 72).

Evidence supports the hypothesis that GER and esophagitis in infancy are caused by food hypersensitivity. Clinical evidence of gastric dysrhythmias was found in infants with CMA presenting with reflex vomiting and GER (73). Iacono et al (74) demonstrated that, in more than 42% of infants with histologic esophagitis, reflux symptoms improved on hydrolyzed formula and relapsed on subsequent blinded formula challenges. We described a group of infants that were intolerant to soy and extensively hydrolyzed formula and had persistent distress attributed to reflux esophagitis; these infants responded to a hypoallergenic amino acid formula-based diet (72). Older children with idiopathic eosinophilic esophagitis have also successfully been treated with amino acid-based formula (AAF) (75). These findings support the hypothesis of an immunologically mediated esophagitis. The infiltrate in idiopathic eosinophilic esophagitis in adult patients was characterized as a Th2-type allergic inflammatory response (76). Assessing the cause of esophagitis in irritable infants is complicated by the possible clinical overlap between acid-peptic and allergic esophagitis. Further studies are needed to assess the role of GER in persistent distress, and to establish whether treatment with H_2-receptor antagonists or proton pump inhibitors, in addition to an hypoallergenic diet, offers a therapeutic benefit in infants with esophagitis.

Colic and Intestinal Spasm

In a systematic review of treatments for infantile colic, the anticholinergic agent dicyclomine was effective in the treatment of colic (5). However, it is no longer used for colic because of its potentially serious side effects in infancy (77). The therapeutic effect in colicky infants is poorly understood but may be due to antispasmodic properties on intestinal smooth muscle. Recently, another anticholinergic agent, cimetropium bromide, has been shown to significantly shorten the duration of crying episodes in infants with colic (78). This drug, a synthetic scopolamine derivative, appears

to have fewer serious side effects than dicyclomine. About three quarters of infants responded to treatment with cimetropium bromide. The mean duration of crying episodes was 17.3 min for active medication and 47.5 min for placebo ($P < .005$). Although not conclusive, these findings may add further weight to the hypothesis that infantile colic is associated with spasmodic visceral pain, which is relieved by these medications.

Animal models of food hypersensitivity have provided direct evidence of GI spasm and motility disturbance in response to dietary antigen challenge (23). In sensitized rats, mucosal exposure with food protein antigens resulted in gastric (79) or intestinal smooth muscle contraction (24, 80). The potential importance of disturbed gut motility in colic is further supported by the finding of increased levels of the hormone motilin, a prokinetic GI hormone, both postnatally and at the age of onset of colicky behavior (25, 81). These findings provide some clues to the etiology of distress in young infants and should stimulate further research into the role of gut motility in the etiology of "colic" in young infants.

Lactose Intolerance

The role of lactose intolerance in infants with colic has remained inconclusive (5, 6). Lactose intolerance may occur as a result of small intestinal mucosal damage and disaccharidase depression. Moore et al (20) examined the effect of lactose-containing formula on breath hydrogen production in colicky and non-colicky infants. That study found that, after intake of human milk or lactose-containing formula, breath hydrogen concentrations were higher in colicky than in non-colicky infants. However, two subsequent randomized controlled trials found no significant benefit for lactase treatment of human milk or cow's milk formula (21, 22). A recent double-blind placebo-controlled study in 53 colicky infants has found a modest benefit of colic from preincubation of foods with lactase (82). The response to lactase treatment was variable, however, and the trial remained inconclusive. Low-lactose formula or pretreatment of feeds with lactase are therefore not recommended as treatments for infantile colic (5, 6).

Dietary Treatment of Colic

The self-limiting course of infantile colic makes the assessment of therapeutic interventions difficult, and no firm conclusions can be drawn unless proper double-blind placebo-controlled randomized trials are performed. However, only few well-designed randomized trials on the treatment of colic have so far been conducted, and many previous studies had shortfalls in methodology or study design. This review will focus predominantly on the role of hypoallergenic diets in the treatment of infantile colic.

Hypoallergenic Formulas

Several studies have assessed the effect of dietary interventions on persistent crying, including treatment with soy- (37, 83), extensively hydrolyzed whey- (84), extensively hydrolyzed casein- (85), and amino acid-based formulas (86, 87).

Jakobsson et al (88) noted that one third of breast-fed infants with colic developed remission and then relapse of colic when mothers excluded and reintroduced cow's milk into their diet. Evans et al (89), however, were unable to confirm these findings but linked symptoms of colic to a range of foods in the maternal diet, including cow's milk, egg, chocolate, fruit, and nuts. Lothe et al (65) reported that 11 (18%) of 60 infants with colic on cow's milk formula responded to soy formula; another 32 (53%) improved after administration of a casein hydrolysate formula. These preliminary studies have been criticized because of some methodological limitations (46, 89, 90).

Other investigations have addressed some of these shortcomings. Lothe et al (91) implemented a 5-day cow's milk-free diet using casein hydrolysate. A marked reduction of distressed symptoms occurred in 24 (89%) of 27 colicky infants. In these infants, the total crying time decreased from 5.6 hours to 0.7 hours ($P < .001$). The 24 responding infants then entered into a randomized double-blind, crossover trial of intact whey protein formula. Of the 24 infants challenged, 18 (two thirds of the original study population) demonstrated increased distress on whey protein challenge.

In a blinded cross-over study of 17 colicky infants, casein hydrolysate alone or casein hydrolysate plus cow's milk formula were fed in sequence for four 4-day periods (92). Significant decreases in distressed behavior were noted after the first two formula change periods only. Over the four formula challenge periods, only 2 (11.8%) of the infants showed a reproducible effect of formula change on colic behavior. Forsyth concluded that diet was likely to be only one factor in

the causation of colic (92). He drew particular attention to the feelings of helplessness, frustration, and decreased confidence in parenting ability that parents of colic patients may experience.

The incomplete response to treatment with hypoallergenic formulas may be due to hypersensitivity to extensively hydrolyzed whey or casein formula (93, 94). An estimated 10%–15% of infants with CMA are also intolerant of extensively hydrolyzed formula (95). In these infants, treatment with AAF has proved effective and safe (96, 97). Several groups have assessed the effect of AAF on persistent crying (41, 72, 86, 87, 98). These preliminary studies provided evidence that AAF is effective in reducing persistent crying. However, further prospective randomized trials are required to assess the efficacy and cost-effectiveness of this approach in the community.

Elimination Diets—The Melbourne Colic Study

Allergen avoidance is one of the key principles in the treatment of food allergies (99). The Melbourne Colic Study examined the role of a hypoallergenic diet on crying and fussing in a cohort of 115 colicky infants (100). Infants were referred from community-based pediatric facilities and were studied over a 1-week period. All mothers of breast-fed infants were placed on an artificial color-, preservative-, and additive-free diet. In addition, those assigned the active low-allergen diet excluded cow's milk and other common food allergens, including egg, wheat, peanut, nuts, fish, and shellfish, from the maternal diet. Formula-fed infants were randomly assigned to a casein hydrolysate preparation (low-allergen diet) or cow's milk-based formula. The response to diet was assessed by comparing the level of distress at the outset and at the end of 1-week diet treatment. Parents recorded distress levels on the previously validated infant distress charts (28).

Clinical improvement was defined as a reduction in distress of 25% or more, and on the basis of this definition, infants on the active diet had a significantly higher response rate than those on the control diet (odds ratio [OR] 2.32; 95% confidence interval [CI] 1.07–5.0; $P = .03$). In addition, the results were assessed by comparing the distress ratio of day 8 to day 1 for infants assigned the active diet compared with those assigned the control diet. Distress was reduced by 39% (95% CI 26–50) in infants on the active diet compared with 16% (95% CI, 0–30) for those on the control diet. After adjusting for age and feed mode, these differences remained statistically significant ($P = .012$).

These findings have general applicability because community-based pediatricians, family practitioners, and child health practitioners referred these infants. One important finding that emerged from that study was the difference in the effect of the two diet programs on breast-fed infants less than 6 weeks of age. The infants on the active (low allergen) diet *decreased* distress by 24% (73 minutes over 24 hours), whereas those on the control (allergen containing) diet *increased* their distress by 34% (67 minutes over 24 hours). These results suggest that breast-fed infants under 6 weeks of age may benefit most from maternal elimination diets.

Conclusion

Infantile colic is a common pediatric problem in the first months of life. No consensus has emerged about its etiology, except that is it likely multifactorial. Infants with colic appear generally well, and in less than 5% of distressed infants can a medical explanation for the distress be found (7). On the basis of their responses to hypoallergenic formula or maternal elimination diets, a large proportion of infants with colic may have underlying food protein hypersensitivity. Other conditions with a close relationship to food allergies, such as GER and esophagitis, may also be found in these infants. Whether these associated conditions are the cause of the distress remains unclear.

In formula-fed infants with moderate to severe unremitting colic, a trial of hypoallergenic formula should be attempted. There is also preliminary evidence that breast-fed infants with colic respond to a strict maternal elimination diet by reducing the antigen load in breast milk. The therapeutic effect of elimination diets appears to be greatest in young infants under 6 weeks of age. Elimination diets should be closely supervised to prevent insufficient macro- or micronutrient intakes for both mother and infant.

We hypothesize that infantile colic in the first weeks of life is due to a transient hypersensitivity to one or several food proteins, which is often associated with GI inflammation and motility disturbance. Infantile colic usually resolves by 3 months of age; however, a significant number of infants present with distress persisting beyond this period. In infants with unremitting colic due to

persistent intolerances, secondary behavior patterning may develop. Infant temperament and interactive factors are likely to affect the subsequent clinical course of infantile colic. Successful management of these infants therefore needs to treat not only the underlying food protein intolerances but should also address the adverse psychological effects of prolonged parental stress and sleep deprivation on family dynamics and the mother-infant relationship.

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17

Eosinophilic Esophagitis, Gastroenteritis, Gastroenterocolitis, and Colitis

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Introduction

The eosinophil was first described by Paul Ehrlich only 125 years ago as a normal cellular component of the blood and certain tissues, including the spleen, lymph nodes, thymus, and submucosa of the gastrointestinal (GI), respiratory, and genitourinary tracts (1). It was initially believed that the eosinophil was a precursor of red blood cells (2), but during the latter half of the twentieth century the biological properties of this cell and its life cycle were more accurately elucidated.

The eosinophil is formed in the bone marrow, where it spends about 8 days maturing under the regulation of interleukin (IL)-5 (3–5). It subsequently relocates into the peripheral circulation for 8–12 hours, and finally moves to specific tissues where it remains for at least 1 week (6, 7). The eosinophil is a multifunctional proinflammatory leukocyte implicated in the pathogenesis of numerous inflammatory processes, especially allergic disorders (8, 9); in addition, it was recently recognized that it may have a physiological role in organ morphogenesis (e.g., postgestational mammary gland development) (10). Known eosinophil functions include chemotaxis and chemokinesis (3); phagocytosis and endocytosis (7, 11); cytotoxicity (7, 11), especially against parasites (7, 8, 12, 13); bactericidal activity (7, 11); and effector of hypersensitivity and modulator of inflammatory responses (3, 7, 11, 13). The eosinophil has a bilobed nucleus (6), and its cytoplasm is filled with approximately 200 large granules (8) containing hydrolases, cationic proteins, and cytokines (6, 8). Specific granule proteins, such as major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN, also sometimes called eosinophil protein X [EDX]) are capable of inducing tissue damage and dysfunction. MBP, EPO, and ECP are toxic to a variety of tissues, including the heart, brain, lung, and intestines (14–17). ECP and EDN are members of the ribonuclease (RNAse) family and in this capacity they are linked with eosinophil-mediated antiviral activity. Interestingly, these two enzymes have the most divergent coding sequence in the human genome, indicating strong evolutionary pressure directed at eosinophils (18). Thus, eosinophils are likely to have a critical biological role, perhaps related to the antiviral activity of eosinophil RNAses. The degree of tissue injury is related to the duration of eosinophilia and the level of eosinophil activation, as well as the type of stimulus attracting the eosinophil. On one end of the spectrum, for example, in drug reactions eosinophils are likely to be innocent bystander cells; the other end of the spectrum is represented by conditions such as the idiopathic hypereosino-

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philic syndrome (IHES) where the eosinophil is linked with severe tissue pathology (12, 19).

Eosinophils reside predominantly in the gastric and intestinal lamina propria under healthy conditions (20, 21). Numerous inflammatory mediators have been implicated in regulating eosinophil accumulation, including IL-1, IL-3, IL-4, IL-5, IL-13, and granulocyte-macrophage colony stimulating factor (GM-CSF); and the chemokines RANTES, monocyte chemoattractant protein (MCP)-3, MCP-4, macrophage inflammatory protein (MIP)-1α, and eotaxin-1, eotaxin-2, and eotaxin-3 (3, 22, 23). IL-3 and GM-CSF, in association with IL-5, enhance eosinophil development, migration, and effector function, whereas IL-1, IL-4, IL-13, and tumor necrosis factor (TNF)-α regulate eosinophil trafficking by promoting adhesive interactions with the endothelium (24, 25). In collaboration with IL-5, chemokines and lipid mediators (platelet-activating factor [PAF] and leukotriene C_4) induce eosinophil trafficking by promoting chemoattraction. Of the mediators implicated in modulating eosinophil accumulation, only IL-5 and the recently described chemokine subfamily termed eotaxins are specific for eosinophils (22) (Fig. 17–1). Recent studies suggest that eotaxin-1 has a key role in the modulation of eosinophil accumulation in the GI tract and that its effect is primarily tissue-specific (20, 21, 26). For example, eotaxin-1-deficient mice have a defect in eosin-

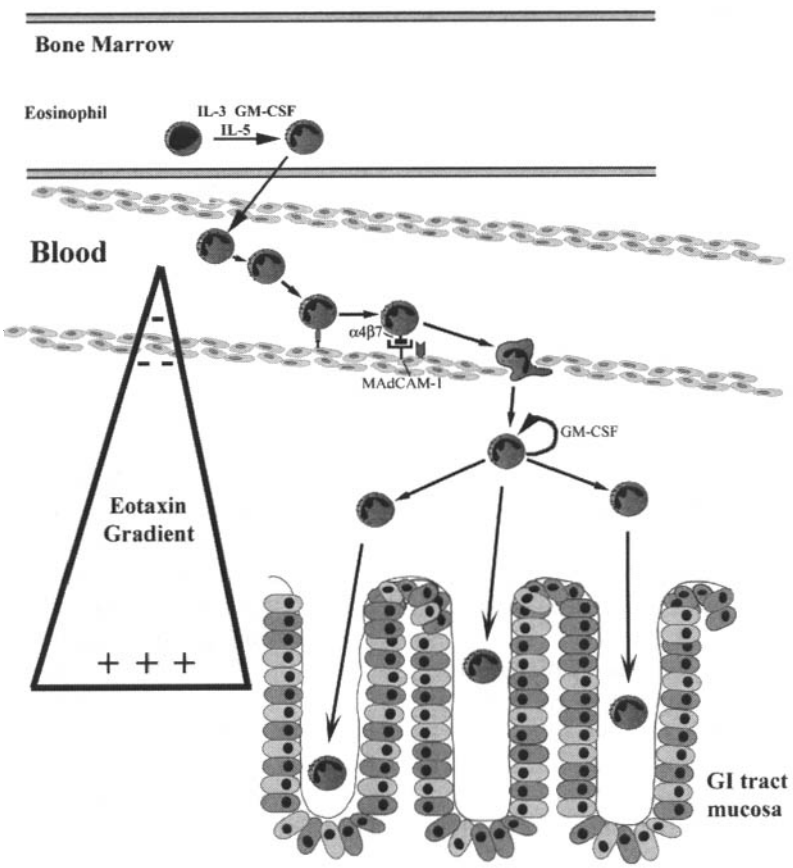

Figure 17–1. Eosinophil development and trafficking. Eosinophils develop in the bone marrow under a process regulated in part by IL-3, IL-5, and GM-CSF. Mature eosinophils exit the bone marrow in response to IL-5 and circulate in the blood stream (at low levels) before trafficking into the GI tract. Eosinophil homing to the GI tract is mediated by the interaction of the eosinophil adhesion molecule α4β7 and the endothelial receptor MAdCAM-1. Eosinophil trafficking to the GI tract is regulated by local generation of the eosinophil chemoattractant eotaxin. Under baseline conditions, most eosinophils reside in the lamina propria at the base of the crypts. During inflammatory responses, eosinophils migrate into the villi in response to the eotaxin concentration gradient.

ophil trafficking to the GI tract and are protected from experimental oral antigen-induced GI pathology. Recent studies have shown that eotaxin-induced GI eosinophilia depends on the interaction of the eosinophil adhesion molecule $\alpha4\beta7$ and the endothelial receptor mucosal addressin cell adhesion molecule (MadCAM)-1 (27, 28) (Fig. 17–1). Likewise, IL-5-deficient mice fail to expand eosinophils in the bone marrow and blood, and have impaired eosinophil accumulation in the allergen-challenged lung (29).

Overview of Eosinophilia in the Gastrointestinal Tract

Pathologic eosinophil accumulation in the GI tract occurs in a variety of processes including diseases limited to specific anatomical areas such as eosinophilic esophagitis (EE), eosinophilic colitis (EC) (30–33), and gastroesophageal reflux disease (GERD) (34–36), as well as diseases that affect multiple segments of the gut such as eosinophilic gastroenteritis (EGE) (37–39), and systemic diseases such as IHES (40–42), inflammatory bowel disease (IBD) (43–45), parasitic infections (46), and iatrogenic processes such as drug reactions (3). The exact incidence and prevalence of the primary eosinophil disorders of the GI tract (EE, EGE, and EC) are not known, but these diseases are occurring or being diagnosed with increasing frequency, and are especially prominent in pediatric populations. The underlying causes of these disorders are not yet understood; however, several investigations have demonstrated an association with atopy. For example, nearly half of the patients with eosinophilic GI disorders are atopic as defined by elevated levels of total IgE or food-specific IgE (47–52), and IgE-mediated mast cell degranulation has been demonstrated in patients with EGE (53); however, anaphylactic food-induced IgE-mediated reactions occurs in only a minority of patients (54, 55). Eosinophils are the predominant cellular infiltrate in EE, EGE, and EC, and are believed to be critical effector cells in the pathological manifestations of these diseases. It is currently thought that eosinophils may augment and sustain GI inflammatory responses through the release of proinflammatory mediators and/or granule cationic proteins that are toxic to the mucosa (1, 48, 54, 56). In vitro studies have shown that eosinophil granule constituents are toxic to many tissues including the intestinal epithelium (15). Electron microscopy studies have revealed ultrastructural changes in the secondary granules (indicating eosinophil degranulation and mediator release) in duodenal samples from patients with EGE (37), and clinical investigations have demonstrated extracellular deposition of eosinophil-derived MBP and ECP in the small bowel of these patients (37, 39, 45, 55, 57). Furthermore, Charcot-Leyden crystals (CLCs), which are remnants of eosinophil degranulation, are commonly found on microscopic examination of stools obtained from patients with EGE (49, 58). The level of eosinophils is correlated with disease severity (55, 59). In addition, in an experimental model of oral antigen-induced EGE, eosinophils have a critical effector role in the pathogenesis of these disorders (60). In particular, eosinophils have been directly implicated in allergen-induced cachexia and gastromegaly, and may mediate the pathology by causing axonal necrosis. However, these conclusions are primarily based on the recent exploitation of murine models of disease and on evaluation of clinical tissue from patients with a variety of eosinophil-associated GI disorders.

Eosinophilic Esophagitis

Introduction

The esophagus is normally devoid of eosinophils (1, 48), and the finding of eosinophils in this segment of the GI tract denotes pathology. The first description of eosinophilic infiltration of the esophagus is attributed to Dobbins et al (61) when they reported a patient with extensive EGE in 1977.

Definition and Classification

Many disorders are accompanied by eosinophil infiltration in the esophagus, such as EE, EGE, GERD, recurrent vomiting, parasitic and fungal infections, allergic gastroenteropathy, IBD, IHES, esophageal leiomyomatosis, Hodgkin's disease, myeloproliferative disorders, carcinomatosis, periarteritis, allergic vasculitis, scleroderma, and drug injury (62). Eosinophil-associated esophageal disorders are classified as primary and secondary. The primary subtype includes the atopic, nonatopic, and familial variants, and the secondary subtype is divided into two groups, one composed of systemic eosinophilic disorders (EGE and IHES) and the other of non-eosinophilic disorders (Table 17–1). Primary EE has also been

Table 17–1.
Classification Scheme for Eosinophil-Associated Gastrointestinal Disorders

Eosinophilic Esophagitis
Primary
Atopic
Non-atopic
Familial
Secondary
Eosinophilic disorders
Eosinophilic gastroenteritis
Hypereosinophilic syndrome
Non-eosinophilic disorders
Iatrogenic esophagitis
Infection-associated esophagitis
Primary gastroesophageal reflux
Esophageal leiomyomatosis
Connective tissue disease (scleroderma)
Eosinophilic Gastroenteritis
Idiopathic
Mucosal form
Muscularis form
Serosal form
Protein-induced syndromes (allergic eosinophilic gastroenteritis)
Protein-induced enterocolitis syndrome
Protein-induced enteropathy
Secondary
Eosinophilic disorders
Hypereosinophilic syndrome
Non-eosinophilic disorders
Infection-associated gastroenteritis
Inflammatory bowel disease
Celiac disease
Connective tissue disease (scleroderma)
Eosinophilic Colitis
Idiopathic
Protein-induced syndromes
Protein-induced proctocolitis syndrome (allergic colitis)
Secondary
Eosinophilic disorders
Eosinophilic gastroenteritis
Hypereosinophilic syndrome
Non-eosinophilic disorders
Iatrogenic colitis
Infection-associated colitis
Inflammatory bowel disease
Connective tissue disease (scleroderma)

called idiopathic EE or allergic esophagitis. Eosinophilic infiltration of the esophagus may occur with or without generalized GI involvement, but if multiple segments of the GI tract are concurrently involved, the entity is called EGE (see below), not EE (currently there is controversy over whether EE is an entity by itself or a subcategory of EGE [63]).

Etiology

EE is a poorly characterized and incompletely understood disease, but food allergy has been implicated. Esophageal eosinophilic inflammation may also be mechanistically linked with pulmonary inflammation. The latter theory is based on the finding that repeated intranasal *Aspergillus fumigatus* antigen challenges (under conditions that promote allergic airway inflammation) induce EE in mice (64, 65). It is unclear whether the trigger that initiates eosinophil accumulation in GERD is secondary to the reflux of acidic gastric contents into the esophagus, or if eosinophils themselves are pathogenically involved.

Clinical and Diagnostic Studies

Patients with primary EE commonly report symptoms that include vomiting, epigastric or chest pain, dysphagia, diarrhea, and respiratory obstructive problems (66, 67). These patients are predominantly young males (66, 68, 69) (Table 17–2) that have relatively high levels of esophageal eosinophils (>20–24 eosinophils/high power field [hpf] [400×] [70, 71]) in the esophageal mucosa, extensive epithelial hyperplasia, and a high rate of atopic disease when compared to patients with GERD (72). As stated above, the number and location of eosinophils is helpful when trying to differentiate EE from GERD. Up to 7 eosinophils/hpf is most indicative of GERD; 7–24 eosinophils/hpf likely represents a combination of GERD and food allergy; and more than 20–24 eosinophils/hpf is

Table 17–2.
Comparison of Eosinophilic Esophagitis and Gastroesophageal Reflux

Characteristic Features	*Eosinophilic Esophagitis*	*Gastroesophageal Reflux*
Clinical		
Presence of atopy	Very high	Normal (possibly elevated)
Food sensitization	Very high	Above normal
Marked male gender preference	Yes	No
Abdominal pain and vomiting	Common	Common
Investigative Findings		
pH probe	Normal	Abnormal
Endoscopic furrowing	Very common	Occasional
Histopathology		
Involvement of proximal esophagus	Yes	No
Involvement of distal esophagus	Yes	Yes
Epithelial hyperplasia	Severely increased	Increased
Eosinophil levels in mucosa	>20/hpf	0–7/hpf
Treatment		
H_2-blockers	Not helpful	Helpful
Proton pump inhibitors	Not helpful	Helpful
Glucocorticoids	Helpful	Not helpful
Elemental diet	Helpful	Not helpful*

*Unless co-occuring food allergy exists.

characteristic of EE (70). The anatomical location of eosinophils to both the proximal and distal esophagus denotes EE, whereas accumulations of eosinophils mainly in the distal esophagus is characteristic of GERD. Remarkably, EE has been associated with esophageal dysmotility, and the etiology of the motor disturbance is unclear, but eosinophil activation and degranulation has been postulated as a possible etiology (71, 73). Radiographic and endoscopic studies have shown many findings including strictures, mucosal rings, ulcerations, whitish papules, and polyps (35, 74–78). The assessment of EE includes an extended allergy evaluation for food sensitization by either skin testing or radioallergosorbent testing (RAST), and the exclusion of GERD as well as other causes of eosinophils in the esophagus. If GERD is diagnosed (by pH probe or upper GI radiographic imaging), a trial of antireflux therapy might be indicated with further assessment. The presence of GERD does not exclude the diagnosis of food allergy (79), emphasizing the importance of a food allergy evaluation in these patients.

Treatment

A trial of food avoidance is often indicated for patients with atopic EE, and if unsatisfactory or practically difficult (when patients are sensitized to many allergens), an elemental formula diet can be used. Interestingly, it has been shown that an elemental diet frequently improves symptoms and reduces the number of eosinophils in the esophageal biopsies in patients with primary EE (80). Patients on elemental diets often require a gastrostomy tube to achieve adequate caloric support. Steroids (systemic [35] or topical [81]) have also been used with satisfactory results. Systemic steroids are used for acute exacerbations, whereas topicals are used for long-term control. For topical steroids we recommend the use of a metered-dose inhaler without a spacer. The patient should swallow the dose to promote deposition on the esophageal mucosa. In the authors' experience, fluticasone dipropionate is the drug most used, with a wide range of dosages based on severity and level of response.

Prognosis

EE is a chronic disease with a genetic predisposition that is exacerbated by environmental conditions and requires prolonged treatment, similar to allergic asthma.

Eosinophilic Gastroenteritis

Introduction

The stomach and intestine have many histological differences when compared to the esophagus. One of those differences is the presence of eosinophils in the intestinal wall during healthy conditions. It is the increased number of eosinophils that denote pathology as first described by Kaijser in 1937 (82).

Definition and Classification

EGE is a histologically supported diagnosis denoting selective infiltration of eosinophils into the stomach and/or small intestine with variable involvement of the esophagus and/or large intestine (37–39, 83). EGE encompasses multiple disease entities and is subcategorized into three types based on the level of histological involvement: mucosal, muscularis, and serosal forms (58).

Etiology

EGE is an idiopathic disorder. Its pathogenesis is not well characterized, but in a subset of patients an allergic mechanism has been suggested (84, 85). It is in this subgroup that IgE levels are elevated and food-specific IgE has been detected. In contrast, syndromes with focal erosive gastritis, enteritis, and occasionally esophagitis with prominent eosinophilia, such as dietary (food) protein-induced enterocolitis and dietary protein enteropathy, are characterized by negative skin tests and absent specific IgE RAST (86–91). Interestingly, EGE might be an example of a delayed-type of food hypersensitivity syndrome (92). It is important to bear in mind that EGE and the dietary protein-induced syndromes (enterocolitis, enteropathy, and colitis) are a continuum of eosinophil-associated GI disorders that might be very closely related with similar underlying immunopathogenic mechanisms.

Clinical Picture and Diagnostic Studies

The constellation of symptoms of patients with EGE is related to the degree and area of the GI tract affected. The mucosal form (the most common variant) is characterized by vomiting, diarrhea, blood loss in stools, iron-deficiency anemia, malabsorption, protein-losing enteropathy, and growth retar-

dation (83). The muscularis form is characterized by infiltration of eosinophils predominantly in the muscularis layer, which causes thickening of the bowel wall that may result in GI obstructive symptoms mimicking pyloric stenosis or other causes of gastric outlet obstruction (93). The serosal form occurs in a minority of patients with EGE. It is characterized by exudative ascites with higher peripheral eosinophil count when compared to the other forms (55). No standards for the diagnosis of EGE exist (33), but a few findings support the diagnosis. For example, elevated eosinophils in biopsy specimens from the GI tract wall, lack of involvement of other organs, and the exclusion of other causes of eosinophilia (infections, IBD, etc.) are supportive of EGE. Food allergy and peripheral eosinophilia are not required for diagnosis. In one study, 9 of 40 (23%) patients with EGE lacked peripheral eosinophilia, but up to 50% of patients with the mucosal form had a history of food allergy or intolerance (55, 58). In a recent survey, 35 of 57 (61%) patients with EGE reported food allergy (94). Histological analysis of the small bowel from patients with EGE reveals extracellular deposition of MBP and ECP (37, 39, 45, 55, 95). Patients with allergic EGE have increased secretion of IL-4 and IL-5 by peripheral blood T cells (85).

Treatment

Elimination diets have transient and unsatisfactory results (96), but complete resolution has been reported with elemental diets (47, 97, 98). There are reports of improvement with drugs such as cromoglycate (99–101), montelukast (102), ketotifen (103), suplatast tosilate (104), and an "alternative Chinese medicine" (105), but controlled trials are lacking. In our institution, a therapeutic approach includes a trial of food elimination if sensitization is found by food skin testing and/or RAST. If no sensitization is found, or if specific food avoidance is not feasible, elemental formulas are instituted. In severe cases, intravenous alimentation has been used (authors' unpublished results). Anti-inflammatory drugs (systemic or topical steroids) are the mainstay of therapy when diet restriction is not feasible or has failed to improve the disease.

Prognosis

No standard criteria exist for the diagnosis of EGE, and many symptomatic patients with eosinophilic infiltration of the GI tract may actually have EGE. Therefore, the prognosis of EGE depends on several factors. If EGE is associated with food protein intolerance, specific protein avoidance should produce clinical improvement. A general consensus is that nearly 80% of children with eosinophil infiltration of the GI tract and food intolerance during infancy are able to tolerate the offending protein (cow's milk protein) by age 3 years (91). In contrast, idiopathic EGE is likely to be chronic and require prolonged treatment.

Eosinophilic Colitis

Introduction

Eosinophils accumulate in the colon of patients with a variety of disorders, including EGE, allergic colitis of infancy (dietary protein-induced proctitis or proctocolitis of infancy syndrome), parasitic infections, drug reactions, and IBD (43–46, 68, 106, 107).

Definition and Classification

EC is a biopsy-based diagnosis and is the most frequent cause of eosinophil infiltration in the colon (79, 108).

Etiology

Allergic colitis is usually a non-IgE-mediated disease (although often associated with IgE production), and some studies point to a T lymphocyte-mediated process, but the exact immunologic mechanism responsible for this condition has not been identified (91, 109). In a murine model of oral antigen-induced diarrhea, colonic T cells transferred the disease to naïve mice (110). Allergic colitis of infancy might be a early expression of protein-induced enteropathy or protein-induced enterocolitis syndrome (79). Cow's milk and soy proteins are the foods most frequently implicated in allergic colitis, but other food proteins can also provoke the disease (wheat, corn, fish, seafood, and nuts) (91, 111). Interestingly, this condition can also occur in infants exclusively breast-fed and in those fed with protein hydrolysate formulas (which may contain small amounts of protein) (112–115).

Clinical Picture and Diagnostic Studies

The mean age at diagnosis for allergic colitis is approximately 60 days (116), but older children have been reported with this condition (117).

Bloody diarrhea precedes the diagnosis for several weeks (116), and anemia due to blood loss is occasionally found (30–32, 87, 118). The majority of infants affected do not have constitutional symptoms and they otherwise look healthy. Endoscopic examination reveals patchy erythema, loss of vascularity, and lymphonodular hyperplasia mostly localized to the rectum, but that might extend to the entire colon (87, 108, 112). Histological examination shows that the overall architecture of the mucosa is well preserved, but there is focal eosinophilic infiltration in the lamina propria, crypt epithelium, and muscularis mucosa, and occasionally multinucleated giant cells in the submucosa (108, 116, 119, 120). No single test is the gold standard for diagnosis, but peripheral eosinophilia or eosinophils in stool suggest allergic colitis.

Treatment

The eosinophil infiltration and symptomatology often improves upon withdrawal of the protein triggers in the diet; gross blood in the stools disappears within 72 hours, but occult blood loss may persist longer (32, 113). Withdrawal of the presumed allergen with resolution of symptoms is usually sufficient to make the diagnosis, although some authors suggest a food challenge to aid in the diagnosis (121, 122). The management of breast-fed infants requires an elimination diet in the mother, aiming to restrict cow's milk, egg, and/or soy (113).

Prognosis

The majority of patients with allergic colitis are able to tolerate the suspected culprit foods by 1–3 years of age (123, 124). Several studies found an association between allergic colitis and later development of IBD, but this association was not proven in a 5- to 10-year follow-up (124–126). The prognosis of other diseases associated with EC is related to their proposed etiologic agents.

Summary

The eosinophil-associated GI disorders are being recognized more frequently, as highlighted by a recent mini-epidemic of EE in the pediatric population. At the authors' institution, a World Wide Web survey (*www.cincinnatichildrens.org/eosinophils/*) was developed to further characterize these disorders (94). Electronically submitted information is being collected and its analysis will provide insight in areas that need further development. It is important that the medical community be aware of the eosinophil-associated GI disorders, and be updated with the latest approaches and management. The therapeutic armamentarium against these disorders is currently limited (food elimination, elemental diets, or steroids), but as research advances, new approaches will be developed on the basis of the immunopathologic mechanisms. Clinical trials using monoclonal antibodies against IL-5 and eotaxin inhibitors are underway. Hopefully therapy that targets a proposed dysregulated immunological pathway will soon be available, making the management of these disorders more successful.

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18

Food Protein-Induced Enterocolitis, Enteropathy, and Proctocolitis

Anna Nowak-Wegrzyn

Introduction

Food protein-induced enterocolitis syndrome (FPIES), enteropathy, and proctocolitis are non-IgE-mediated gastrointestinal (GI) food hypersensitivities. Food-allergic reactions that affect the GI tract have been described since ancient times. Hippocrates noted that cow's milk could cause GI symptoms as well as urticaria, and that some infants fed cow's milk developed prolonged diarrhea, vomiting, and failure to thrive that resolved only after removal of cow's milk from their diet (1). Exact prevalence of FPIES, enteropathy, and proctocolitis is not known, but they affect predominantly young infants, and together with other GI food hypersensitivity syndromes, they account for up to 30%–40% of cow's milk protein hypersensitivity (2–8). Although well established as distinct clinical entities, their pathophysiology requires further characterization (9). Current evidence indicates that T cell-mediated responses play an important role, whereas IgE antibodies to the offending foods are of minimal or no significance in the pathogenesis of these disorders. In the absence of definitive laboratory tests, diagnosis relies predominantly on clinical responses to elimination diets with resolution of symptoms, oral food challenges (OFCs) with reappearance of symptoms following ingestion of the offending food, endoscopy and biopsy findings, and exclusion of causes such as infections, inflammatory bowel disease (IBD), ischemia, and others.

Table 18–1 summarizes the most important features of the four clinical conditions reviewed in this chapter that are induced in children by dietary proteins, including food protein-induced enterocolitis, enteropathy, proctocolitis, and iron-deficiency anemia caused by cow's milk. They were defined using the consensus criteria developed by a workshop jointly sponsored by the European Society of Pediatric Gastroenterology, Hepatology and Nutrition, and the North American Society for Pediatric Gastroenterology and Nutrition, in November 1998 (9).

Food Protein-Induced Enterocolitis Syndrome

FPIES manifests as profuse vomiting and diarrhea in young infants and is most commonly caused by cow's milk and soy proteins. Other foods such as grains, meats, and fish have been reported, and onset of symptoms at older ages occurs with some foods, e.g., shellfish (9).

Historical Perspective

Gryboski (10, 11) and Powell (12, 13) described infants presenting at less than 6 weeks of life with recurrent vomiting, bloody diarrhea, and abdominal distension while being fed with cow's milk-based formula. Many of the infants appeared dehydrated and severely ill. Sepsis evaluations were negative but improvement was achieved by elimination of cow's milk, and use of intravenous (IV) fluids or casein hydrolysate formula. The

Table 18–1.
Food Protein-Induced Gastrointestinal Syndromes*

	FPIES	*Proctocolitis*	*Enteropathy*	*Iron-Deficiency Anemia*
Age at onset	1 day to 1 year	1 day to 6 months	Depends on age of exposure to antigen; cow's milk and soy up to 2 years	2–20 months
Food proteins implicated				
Most common	Cow's milk, soy	Cow's milk, soy	Cow's milk, soy	Cow's milk
Rare	Rice, chicken, turkey, fish, pea	Egg, corn, chocolate	Wheat, egg	
Multiple food hypersensitivities	>50% both cow's milk and soy	40% both cow's milk and soy	Rare	No
Feeding at the time of onset	Formula	>50% exclusive breast-feeding	Formula	Cow's milk, non-humanized cow's milk-based formula
Atopic background				
Family history of atopy	40%–70%	20%		
Personal history of atopy	30%	22%		
Symptoms				
Emesis	Prominent	No	Intermittent	No
Diarrhea	Severe	No	Moderate	Minimal
Bloody stools	Severe	Moderate	Rare	No
Edema	Acute, severe	No	Moderate	Mild
Shock	15%	No	No	No
Failure to thrive	Moderate	No	Moderate	Minimal
Laboratory findings				
Anemia	Moderate	Mild	Moderate	Moderate
Hypoalbuminemia	Acute	Rare	Moderate	Mild
Methemoglobinemia	May be present	No	No	No
Acidemia	May be present	No	No	No

Allergy evaluation				
Food skin prick test	Negative*	Negative	Negative	Negative
Serum food-allergen IgE	Negative*	Negative	Negative	Negative
Total IgE	Normal	Negative	Normal	Normal
Peripheral blood eosinophilia	No	Occasional	No	No
Biopsy findings				
Villous injury	Patchy, variable	No	Variable, increased crypt length	Mild
Colitis	Prominent	Focal	No	No
Mucosal erosions	Occasional	Occasional, linear	No	No
Lymphoid nodular hyperplasia	No	Common	No	No
Eosinophils	Prominent	Prominent	Few	No
Food challenge	Vomiting in 3–4 hours; diarrhea in 5–8 hours	Rectal bleeding in 6–72 hours	Vomiting and/or diarrhea in 40–72 hours	
Treatment	Protein elimination, 80% respond to casein hydrolysate and symptoms clear in 3–10 days; rechallenge in 1.5–2 years	Protein elimination, symptoms clear in 3 days with casein hydrolysate, resume/continue breast-feeding on maternal antigen-restricted diet	Protein elimination, symptoms clear in 1–3 weeks, rechallenge and biopsy in 1–2 years	
Natural History	Cow's milk: 60% resolved by 2 years; soy: 25% resolved by 2 years	Resolved by 9–12 months	Most cases resolve in 2–3 years	Most cases resolve by 3 years

*If positive, may be a risk factor for persistent disease.

reintroduction of the cow's milk formula resulted in recurrence of symptoms and elevation of the peripheral polymorphonuclear leukocyte count. Subsequently, Powell (14) established criteria for FPIES diagnosis and a standard challenge protocol. Reports of two series of infants with FPIES by Sicherer et al (16 patients) (15) and Burks et al (43 patients) (16) further characterized clinical features and refined the food challenge protocol.

Clinical Characteristics

FPIES is typically caused by cow's milk or soy protein in formula-fed infants, with over one half reacting to both foods. FPIES had been sporadically reported with rice, chicken, turkey, pea, and fish (15, 17–20). In the author's experience, FPIES can also be caused by other grains (oat, wheat) and vegetables such as sweet potato, and green beans (21). In adults, shellfish (shrimp, crab, and lobster) hypersensitivity may provoke a similar syndrome with severe nausea, abdominal cramps, and protracted vomiting (22).

Symptoms commonly start before 2 months of age, but they may first occur later (up to 9 months) when introduction of milk or soy protein is delayed, such as in breast-fed infants, or when solid foods are introduced into an infant's diet. FPIES has not been reported in infants while being exclusively breast-fed, even if the mother is ingesting the offending foods in her diet, suggesting an important protective role of breast-feeding in FPIES.

Infants may present with protracted vomiting and diarrhea, dehydration, and occasionally shock (12–15). Vomiting generally occur 1–3 hours, and diarrhea 5–8 hours, after feeding. Continued exposure to the food allergen may result in bloody diarrhea, anemia, hypoproteinemia, abdominal distension, and failure to thrive. Overall, 75% of infants with FPIES appear acutely ill, including 15% who are hypotensive and require hospitalization and extensive evaluation before diagnosis of FPIES is established. Methemoglobinemia is a unique feature of FPIES, and it has been reported in association with acidemia (mean pH 7.03) in 6 (35%) of 17 infants hospitalized with FPIES (23). The authors postulated that methemoglobinemia was caused by an elevation of nitrites in the intestine resulting from severe intestinal inflammation and reduced catalase activity. Other reports confirmed association of methemoglobinemia with FPIES (15).

Genetics

The genetics of FPIES and the role of heredity are unknown. Family history of atopy is positive in 40%–70% of patients reported, but only rarely is family history positive for food allergy. In the author's practice, a pair of male twins with soy-induced FPIES were evaluated, but otherwise no reports of FPIES in siblings or relatives have been published. Approximately 30% of infants develop atopic diseases such as atopic dermatitis (AD), rhinitis, or drug hypersensitivity later in life (15).

Diagnosis and Management

Diagnosis of FPIES relies on history, clinical features, exclusion of other etiologies, and OFC. The vast majority (over 90%) of patients in a large series have negative skin prick tests (SPTs) and undetectable allergen-specific IgE antibodies to the offending foods (15, 16). Although OFC is the "gold standard" for diagnosis of FPIES, most infants do not undergo confirmatory challenges, especially if they have a history of severe reactions and become asymptomatic following elimination of the suspected food. In infants with chronic diarrhea, stool samples test positive for occult blood and show the presence of intact polymorphonuclear neutrophils, eosinophils, and Charcot-Leyden crystals (CLCs) with Hansel's stain. Some patients develop carbohydrate malabsorption and show reducing substances in the stool.

OFCs involve the administration of food protein at a rate of 0.06–0.6 g/kg of body weight, with lower doses used in children with prior severe reactions (14, 15). OFCs involve significant risk to the infant and should be performed under physician supervision with fluid resuscitation immediately available (Table 18–2). About 50% of positive challenges require physician-supervised treatment (15). IV hydration is the first-line therapy, although corticosteroids are often used for severe reactions, based on the presumed pathophysiology.

Strict avoidance of the offending food protein(s) should be recommended, and appropriate guidelines for avoidance provided. Since there is a high risk of concomitant milk- and soy-induced FPIES (over 50% of cases in infants <6–8 months of age), casein hydrolysate formulas should be used (13, 15, 24). Eighty percent of patients respond to casein hydrolysate and their symptoms resolve within 3–10 days. Up to 20% of patients

Table 18–2.
Oral Food Challenge in FPIES

Challenge protocol
High-risk procedure, requires immediate availability of fluid resuscitation
Gradual (over 1 hour) administration of food protein at 0.06*–0.6 g/kg body weight
If no reaction, discharge after 6 hours
50% of positive challenges require treatment
Criteria for a positive challenge
Symptoms
Emesis (typically in 2–4 hours)
Diarrhea (typically in 5–8 hours)
Laboratory findings
Fecal leukocytes
Fecal eosinophils
Increase in peripheral polymorphonuclear leukocyte count >3500 cells/mm^3, peaking at 6 hours
Interpretation of the challenge outcome
Positive challenge: three of five criteria positive
Equivocal: two of five criteria positive

*Lower dose recommended in children with history of previous severe reaction.
Based on data from (14, 15, 24).

require amino acid-based formula or temporary IV therapy (25).

Depending on the clinical severity, follow-up challenges should be performed every 18–24 months to determine tolerance (24).

Natural History

In one series, sensitivity to milk was lost in 60% and to soy in 25% of patients 2 years after the initial occurrence (15).

Sicherer et al (15) described three patients who presented initially with positive SPTs and two who developed positive SPTs and detectable serum milk-specific IgE (1 and 3 years after the diagnosis). All five patients remained sensitive to the offending food and showed only symptoms consistent with FPIES. They had no IgE-mediated symptoms of urticaria, wheezing, or anaphylaxis when challenged. Therefore, the initial presence or development of IgE to food protein may indicate a poor prognosis. Initial and follow-up evaluations should include SPT or measurement of serum food-specific IgE levels, to identify patients at risk for persistent FPIES.

Pathology

Because the diagnosis of FPIES is based on clinical criteria, endoscopy and biopsy are not routinely performed. Observations from case reports of protracted FPIES with available biopsy data highlight inflammatory responses in the colon. Endoscopic evaluation reveals diffuse colitis with variable ileal involvement. Colonic mucosa may demonstrate mild friability to severe spontaneous hemorrhage and minute ulcers similar to those seen in ulcerative colitis (11, 26, 27). Crypt abscesses have been identified in some patients (28). Jejunal biopsies reveal flattened villi, edema, and increased numbers of lymphocytes, IgM- and IgA-containing plasma cells, eosinophils, and mast cells (MCs). Villous atrophy ranges from mild to severe (28, 29).

Pathophysiology

Cytokines secreted by food allergen-stimulated T cells may affect intestinal permeability. Interleukin (IL)-4, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α synergistically increase intestinal permeability, whereas transforming growth factor (TGF)-β1 protects the epithelial barrier of the gut from penetration of foreign antigens by antagonizing the action of IFN-γ (30–33). Several studies investigated peripheral blood lymphocyte responses and cytokine release in the patients with FPIES. Van Sickle et al (34) showed that after in vitro stimulation with milk or soy proteins, peripheral blood mononuclear cells from children with soy- or milk-induced FPIES had significantly ($P < .01$) higher geometric mean proliferation indices compared to children who had negative oral challenges to milk and soy. An increase in serum antigen-specific IgA and IgG levels was also noted in these patients (35).

Chung and colleagues (36) found generally depressed TGF-β expression in duodenal biopsies from all 28 infants with challenge-proven milk-induced FPIES. Expression of type 1 TGF-β receptors was significantly lower in patients with villous atrophy compared with patients who did not have villous atrophy ($P < .001$). This was negatively correlated with the severity of villous atrophy ($r = -0.59$, $P < .001$). TNF-α expression on epithelial and lamina propria cells was significantly greater in the patients with villous atrophy ($P < .01$). TNF-α is a potent pro-inflammatory cytokine that induces neutrophil activation and increases intestinal permeability in vitro by altering the tight junctions between epithelial cells (37). In view of these effects of TNF-α, its role in GI cow's milk hypersensitivity has been investigated in studies that included subsets of infants with milk-induced FPIES. Heyman et al (38) demonstrated that TNF-α secreted by circulating

milk protein-specific T cells increased intestinal permeability, thus contributing to the influx of antigen into the submucosa with further activation of antigen-specific lymphocytes. Patients with active intestinal cow's milk allergy require lower doses of intact cow's milk protein to stimulate TNF-α secretion, and secretion of TNF-α by peripheral blood mononuclear cells is prolonged compared to patients with cutaneous symptoms and those who outgrew cow's milk allergy (39, 40). Cow's milk-stimulated fecal TNF-α was also found in increased concentrations following positive milk challenges in children with cow's milk allergy and GI symptoms (41).

Food Protein-Induced Enteropathy

Food protein-induced enteropathy is a syndrome of small bowel injury with resulting malabsorption, similar to celiac disease (CD) although less severe (9).

Historical Perspective

The first report of malabsorption syndrome with diarrhea, emesis, and impaired growth induced by cow's milk feedings in infants was published in 1905 (42). Subsequent reports, including large series of cow's milk protein-sensitive Finnish infants defined clinical features of this disorder (43–49). The reported incidence of food protein-induced enteropathy peaked in the 1960s in Finland, with virtual disappearance of severe jejunal damage caused by cow's milk protein in the past 20 years (50). Infant feeding practices have been implicated as a cause of the changing prevalence of food protein-induced enteropathy, with the highest incidence of classic severe enteropathy attributed to feedings with non-humanized infant formulas containing a high protein content, and a low incidence of breast-feeding (51–53).

Clinical Features

Food protein-induced enteropathy presents with protracted diarrhea in the first 9 months of life, typically the first 1–2 months, within weeks of the introduction of cow's milk formula. Food proteins such as soybean, wheat, and egg have been confirmed as causes of enteropathy, frequently in children with coexistent cow's milk protein-induced enteropathy (54–57). In one report, "enteropathy" related to fish, rice, and chicken was described in three patients (19). However, careful analysis of the cases leads to conclusion that the reported condition was consistent with FPIES rather than enteropathy. In view of our evolving understanding of the GI food hypersensitivity syndromes, the older literature has to be reviewed critically and disorders reclassified on the basis of the consensus guidelines.

More than 50% of the affected infants have vomiting and failure to thrive, and some present with abdominal distension, early satiety, and malabsorption. In many infants the onset of symptoms is gradual, in others it mimics acute gastroenteritis with transient emesis and anorexia complicated by protracted diarrhea. It may be difficult to distinguish it from post-enteritis lactose intolerance, especially since these two conditions may overlap (58). Acute small bowel injury caused by viral enteritis may predispose children to subsequent food protein-induced enteropathy, or alternatively may unmask underlying food protein-induced hypersensitivity (48, 56, 58, 59). Diarrhea generally resolves within 1 week of cow's milk protein elimination, although some infants require prolonged IV nutrition (49).

Moderate anemia was present in 20%–69% of infants with cow's milk protein-induced enteropathy (49, 50). Iron deficiency was more common than anemia, and malabsorption of iron or folate is likely a major contributing factor (49). Bloody stools were absent but occult blood was found in 5% of patients (60). Malabsorption with hypoproteinemia and deficiency of vitamin K-dependent factors was reported in 35%–50%. Moderate steatorrhea was found in over 80%. The absorption of the sugar D-xylose was abnormal in up to 80% (61). Lactose was found in urine in 55% and in stool in 52% of cases, typically in the youngest infants. Lactose absorption promptly became normal after elimination of cow's milk protein (48).

Genetics

The infants with enteropathy typically do not have a predisposing family history of cow's milk allergy. AD and chronic respiratory symptoms were present in 22% of children in one study (49).

Diagnosis and Management

Food protein-induced enteropathy is diagnosed by endoscopy to confirm the presence of

villous injury, crypt hyperplasia, and inflammation in small bowel biopsies. Biopsies must be obtained in a symptomatic infant on a diet containing the offending food allergen (28, 62–64). Elimination of the food allergen should lead to resolution of clinical symptoms within 1–3 weeks (49). Villous atrophy should improve within 4 weeks but complete resolution may take up to 1.5 years. Infants with severe initial manifestations may require prolonged bowel rest and parenteral nutrition for days or weeks. Diagnostic confirmatory challenges and measurement of specific serologies for CD (see Chapter 19) may be necessary to distinguish between food protein-induced enteropathy and CD or to identify other food allergens. In clear-cut cases, oral challenges are not absolutely required for confirmation of the diagnosis. The OFCs should be performed periodically to assess the development of oral tolerance. Immunologic studies have shown increased levels of milk IgA in 74% and milk IgG precipitins in 65% of infants. Milk IgA levels decreased following cow's milk dietary elimination (49). However, the diagnostic utility of these tests is unclear, particularly in view of high prevalence of the positive results in many other GI inflammatory disorders in childhood. Classically, food-specific IgE antibodies are undetectable and SPTs are negative (65). Several studies suggested that patch skin tests may be a useful screen for GI food hypersensitivity (cow's milk, wheat) (65, 66). Biopsies were not obtained in these studies, so the association between positive patch tests and GI changes remains to be determined.

Natural History

Food protein-induced enteropathy resolves clinically in the majority of children by age 1–2 years, although the proximal jejunal mucosa may be persistently abnormal at that time (49). Mucosal healing continues during feeding with the implicated food once clinical tolerance is achieved (62). In children with less severe disease who were diagnosed at an older age, tolerance developed at an older age and most became tolerant by 3 years (52). Of note, 5 (9.3%) of 54 infants with challenge-confirmed cow's milk enteropathy were ultimately diagnosed with CD (49). In contrast, transient wheat enteropathy with or without associated cow's milk protein-induced enteropathy has been reported in a number of studies; transient wheat enteropathy has also been reported following enteritis (57, 67, 68). Two strict criteria for the diagnosis of transient wheat enteropathy have been proposed: 1) evidence of small bowel villous injury that resolves with gluten avoidance, and 2) demonstration that the small bowel mucosa remains normal for 2 or more years after the return of gluten to the diet (69). Recently, school-age children who developed delayed GI symptoms to cow's milk challenge but had no villous atrophy or malabsorption syndrome were reported (70). Twenty-seven children with suspicion of milk-related symptoms, such as history of milk allergy in infancy, abdominal pain, or diarrhea after consumption of dairy products, were placed on strict elimination of cow's milk protein for 2 weeks, followed by oral challenge over a 1-week period. Although all children responded favorably to the elimination of cow's milk, only 15 children (mean age 10 years, range 6–14) had relapse of symptoms during the 1-week challenge. When compared with control children (11 with CD, 12 without GI disease), they had significantly more frequent history of food allergy at <2 years of age, gastritis and esophagitis on biopsy, and lymphonodular hyperplasia of the duodenal bulb. An increase in γ/δ T lymphocytes occurred, although of lesser magnitude than in CD. It remains to be established whether these older children represent a milder phenotype of enteropathy or whether they have a different disease caused by cow's milk hypersensitivity. The prevalence of this problem among school-age children is unknown.

Pathologic Features

The degree of villous injury in food protein-induced enteropathy can range from mild to severe, with most biopsy specimens revealing patchy, subtotal villous atrophy (28, 49, 62–64). Intestinal mucosa is thin and crypts can be elongated (49, 52, 71). Intraepithelial lymphocytes are prominent, and infiltration with eosinophils is inconsistent (71–73) (Fig. 18–1). Lymphocytes can also be found in the lamina propria. Mucosal lipid content may be increased (74). Columnar cells of the normal jejunum are replaced by crypt cells of a more cuboidal, immature type (45). The epithelial cells bear short microvilli that contain large aggregates of lysosomes and abnormal nuclei (63). The basement membrane is unevenly thickened. The epithelial cell renewal rate is markedly increased as a result of the increased mitotic rate (75, 76). Immunohistochemical studies of the mucosal biopsies in untreated and challenge-positive in-

Image Not Available

Figure 18–1. A. Biopsy of rectal mucosa showing increased numbers of eosinophils in the lamina propria. The mucosa is otherwise unchanged (hematoxylin and eosin stain; original magnification, 200×). B. At higher power abundant eosinophils are seen that focally invade the glandular epithelium (original magnification, 400×). Slides and description generously provided by Dr. Margaret Magid, Division of Pediatric Pathology, Mount Sinai School of Medicine, New York, NY.

fants demonstrate nonspecific increases in mucosal IgA, IgG, and IgM, with inconsistent increases in IgE (77, 78). In most cases the number of both intraepithelial and lamina propria lymphocytes is increased. The histologic features of soybean-induced enteropathy are similar to those noted for cow's milk (54, 56, 79, 80).

Pathophysiology

T lymphocytes appear to play a central role in the pathophysiology of food protein-induced enteropathy. Activated T cells in the lamina propria of the fetal human small intestine have been shown to produce crypt hypertrophy and villous atrophy (81, 82). Increased intraepithelial lymphocytes are predominantly $CD3^+$ α/β suppressor/cytotoxic $CD8^+$ T cells (83). However in a small series, between 50% and 100% of patients have increased densities of γ/δ T cells in the epithelium, similar to CD and autoimmune enteropathy (84, 85). In the lamina propria, the numbers of lymphocytes (predominantly $CD4^+$ T cells), plasma cells, and eosinophils are increased (71, 73, 83). Many T cells express HLA-DR, suggesting an activated state. These cells diminish with a cow's milk elimination diet (83).

In addition to lymphocytic infiltration, electron microscopy shows edema of the lamina propria and of the endothelium of small blood vessels, as well as degranulation of MCs, eosinophils, and macrophages (64). Mucosal histamine content is high, suggesting MC activation (63, 86). In 21 patients with cow's milk enteropathy, eosinophil degranulation increased significantly, as evidenced by localization of extracellular major basic protein (MBP), compared with the non-cow's milk enteropathy control groups. The severity of villous atrophy was positively correlated with the deposition of MBP ($r = 0.79$; $P < .001$). In addition, mononuclear cells from biopsies of children with cow's milk enteropathy had significantly higher expression of vascular adhesion molecule (VCAM)-1 than those of control children (29).

The numbers of IgA- and IgM-bearing cells in the lamina propria increase significantly (average 2.4 times) following positive cow's milk challenge. In contrast, in specimens from asymptomatic patients, the IgA cells increase 1.5-fold, whereas the IgM cells do not change during 4–6 weeks of milk consumption. An elimination diet following a positive challenge results in decreased densities of IgA- and IgM-containing cells (87). Similar changes in IgA and IgM cells were observed in soy-induced enteropathy following an oral challenge with soy and reinstitution of an elimination diet (79). IgE was demonstrated in the mucosal biopsies by two groups but was not confirmed in other large studies of infants (64, 88).

Following stimulation with cow's milk protein in vitro, a higher proportion of cells isolated from jejunal specimens of patients with enteropathy secreted IFN-γ and IL-4 than did cells of control subjects; IFN-γ secreting cells were 10 times more numerous than IL-4 secreting cells (89). IL-10 secreting cells were reduced, further implicating a predominance of the Th1-type responses. The

numbers of cells secreting IL-5 were not different between the two groups.

Food Protein-Induced Proctocolitis

Food protein-induced proctocolitis typically starts in the first few months of life, with blood-streaked stools in otherwise healthy-looking infants. It is considered a major cause of colitis under 1 year of age (9, 90) (Table 18–3). Unlike other forms of GI food hypersensitivity, proctocolitis is highly prevalent in breast-fed infants, with more than 50% of infants in published reports being exclusively breast-fed (90–94).

Historical Perspective

Food protein-induced proctocolitis was originally described by Lake et al in 1982 (91), in six exclusively breast-fed, healthy-appearing infants with rectal bleeding that developed during the first month of life. Stool cultures were negative for pathogens, and radiological studies were normal. The rectal biopsies showed focal acute inflammation with prominent eosinophilic infiltrates. All infants experienced resolution of rectal bleeding within 36 hours of discontinuation of breast-feeding, and all redeveloped bleeding following the resumption of breast-feeding on unrestricted maternal diet. Two infants became asymptomatic when their mothers' diets were restricted for cow's milk, but the remaining patients required casein hydrolysate- or soy-based formulas to control rectal bleeding. All infants became clinically tolerant to milk after 1 year of age. Subsequently studies of infants with rectal bleeding caused by food protein hypersensitivity defined the clinical and histologic features of this disorder (92–97).

Table 18–3.
Differential Diagnosis of Rectal Bleeding in Infancy

Severe	*Mild-Moderate*
Necrotizing enterocolitis	Anal fissure
Sepsis	Perianal dermatitis and/or excoriations
Hirschprung's disease	Gastrointestinal infection (*Salmonella, Shigella, Campylobacter, Yersinia sp,* parasites)
Intussusception	Coagulation disorders
Volvulus	Vitamin K deficiency
FPIES	Food protein-induced proctocolitis

Clinical Features

Food protein-induced proctocolitis is typically caused by cow's milk and soybean protein in formula-fed infants, whereas cow's milk, egg, corn, and soybean proteins are most commonly implicated in breast-fed infants, with small number (5%) reacting to more than one food (93, 97, 98). Infants typically present in the first 4 months of life, usually at 1–4 weeks of age, with intermittent blood-streaked normal to moderately loose stools (94, 98) (Table 18–4). Rarely, symptoms may begin as early as the first day of life or as late as 10 months of age. Breast-fed infants are often older at the time of initial presentation and have less severe histologic findings (99) The onset may be acute (<12 hours following the first feeding of the offending food) but is more often insidious with a prolonged latent interval between the introduction of the food protein and the onset of symptoms (92). The affected infants typically appear well, although they may have increased gas (up to 30% of patients), vomiting (up to 27%), pain on defecation (22%), abdominal pain (up to 20%), or poor weight gain (up to 10%) (92, 94). No anatomic abnormalities are found, and stool cultures are negative for pathogens. Smears of the fecal mucus usually reveal increased polymorphonuclear neutrophils. Mild anemia may be present initially, although more commonly it develops in infants with continued bleeding (91, 100, 101). Occasionally, peripheral blood eosinophilia and mild hypoalbuminemia may be seen (94, 97).

Table 18–4.
Clinical Features of Food Protein-Induced Proctocolitis in 95 Exclusively Breast-Fed Infants

	Frequency (%)
Initial presentation	
Blood-tinged stools	100
Pain during defecation	22
Diarrhea or loose stools	4
Failure to thrive	0
Endoscopic findings	
Focal rectal erythema or erosions	100
Lymphoid nodular hyperplasia	48
Positive response to dietary protein elimination	
Cow's milk	65
Egg	19
Corn	6
Soy	3
Two of the above	5
Not identified	12
Response to L-amino acid formula only	4

Based on data from (98).

Genetics

The genetics of food protein-induced proctocolitis remain unknown. Up to 25% of infants may have positive family history of atopy, comparable to the general population (92, 94). Significance of the atopic background is difficult to ascertain on the basis of limited data. In a study of 95 exclusively breast-fed infants, 21 infants (22%) had moderate eczema at the time of initial presentation, but information on the development of personal atopic disease later in life was not available (98).

Diagnosis and Management

Tests for IgE-mediated food hypersensitivity are negative or inconsistent and not useful for the diagnosis of food protein-induced proctocolitis. Hence, the diagnosis relies primarily on the clinical history, findings on the biopsy, and a favorable response to the dietary elimination of the offending food protein. Avoidance of the offending protein in the diet typically leads to a clinical resolution of gross bleeding within 72–96 hours (98, 102). In breast-fed infants, Lake (98) proposed discontinuation of breast milk and feeding with a casein hydrolysate formula until resolution of bleeding is achieved, typically within 72 hours. Soy formula must be used with caution in infants reacting to cow's milk because up to 40% of infants react to both food proteins (98). Most infants respond well to casein hydrolysate and only a few require amino acid-based formulas. If breast-feeding is continued, strict avoidance of the offending food protein in the maternal diet is necessary. Rechallenge within the first 6 months usually provokes recurrence of bleeding within 72 hours. In contrast to FPIES, no peripheral blood leukocytosis is seen following the challenge. After 9–12 months of age, the infants typically tolerate an unrestricted diet. Introduction of the offending food protein is typically done at home gradually, advancing from 1 oz/day to full feedings over 2 weeks (60).

Natural History

Food protein-induced proctocolitis is a benign, transient disorder of infancy. Affected infants invariably become tolerant to the offending food by 2 years of age, and the vast majority achieves clinical tolerance by age 1 year. Formula-fed infants experience resolution of symptoms on casein hydrolysate formula, with few requiring an amino acid-based formula. Up to 20% of breast-fed infants have spontaneous gradual resolution of bleeding without changes in the maternal diet (97, 103, 104). Among the 95 exclusively breast-fed infants recently reported by Lake, 62 (65%) responded positively to milk protein elimination trial in the maternal diet, 18 (19%) to egg, 6 (6.3%) to corn, and 3 (3.2%) to soy. Only 5 (5.3%) were sensitive to more than one food. Eleven (12%) infants had persistent symptoms despite trials of maternal dietary restrictions of various proteins; 7 (64%) of these infants responded favorably to casein hydrolysate, whereas 4 (36%) required amino acid-based formula (Table 18–4). Twenty-one (22%) infants had persistent symptoms while breast-feeding because the mothers could not maintain their elimination diet or the offending food was not identified. These 21 infants were observed for more than 6 months and had persistent symptomatic proctitis, and six became mildly anemic despite oral iron supplementation. None developed increased symptoms, but serial biopsies were not performed. All were weaned to a normal diet after 1 year of age. Of the 35 infants with food protein-induced proctocolitis followed for more than 10 years, none reportedly developed IBD. This is in agreement with a report by Hill et al (105), who noted that, of 13 infants with eosinophilic colitis (presumably of allergic etiology), none developed symptoms of IBD during a 5- to 10-year follow-up (105).

Pathologic Features

Lesions of food protein-induced proctocolitis may involve any segment of the colon, but the rectosigmoid is most commonly affected (102) (see also Chapter 17). Endoscopy reveals intermittent focal erythema with frequent small mucosal nodules consistent with lymphoid nodular hyperplasia (94, 96, 97, 102, 103). In patients with more chronic manifestations at the time of diagnosis, ulcerations in the rectum have been described. Microscopically, the normal architecture of the mucosa is preserved (94). Biopsy findings reveal focal, moderate acute inflammation with epithelial erosions and eosinophilic infiltration of the lamina propria, the epithelium, and the lamina muscularis (94, 96, 97). The number of eosinophils varies from six to more than 20 per 40× high-power field; eosinophils are frequently degranulated and localized next to the lymphoid nodules (94, 97). Occasionally, crypt abscesses are noted with both neu-

trophils and eosinophils. There is no correlation between the degree of eosinophilia and the level of the biopsy within the rectosigmoid (94). Aggregates of nuclear dust consisting of apoptotic epithelial cells have been reported (106).

Pathophysiology

Lake (98) hypothesized that food protein-induced proctocolitis may be a milder form of FPIES, based on the fact that in FPIES, the maximal inflammatory response occurs usually in the rectum. Hence, proctocolitis in formula-fed infants would represent the mildest phenotype, whereas the protective effects of breast milk, such as the presence of IgA antibodies and partially processed food proteins, would prevent the expression of the full, more severe clinical phenotype in the breast-fed infants. This concept is particularly interesting in view of the fact that there are no published reports of classic FPIES in breast-fed infants. IgA or other immunologically active components of breast milk may bind with the food allergens and release them in the rectum following cleavage by microbial IgA proteases or via other mechanisms (98).

The prominence of eosinophilic infiltrates in the rectal mucosa is a hallmark of this disorder and suggests an important pathogenic role of the eosinophils. Eosinophils express a variety of cell surface receptors (i.e., for complement components, LTB4, PAF, IgG, low-affinity receptor for IgE [FcεRII], and IgA), and the presence of IgA receptor on eosinophils may be of particular relevance to GI disorders because the gut is a major site of IgA antibody production (107, 108). Eosinophil mediators induce diverse proinflammatory effects, including toxic reactions to multiple tissues (including host cells), MC degranulation, dysfunction of vagal muscarinic M2 receptors, smooth muscle constriction, and stimulation of chloride secretion from colonic epithelium (109, 110). Eosinophils often degranulate in proximity to nerves, suggesting that they may be involved in promoting changes in neurons that contribute to gastric dysmotility (111, 112). Additionally, experimental eosinophil accumulation in the GI tract is associated with weight loss (113).

Iron-Deficiency Anemia

In 1962, Wilson, Heiner, and Lahey (114) reported infants with cow's milk protein-induced occult rectal bleeding, anemia, hypoproteinemia, and respiratory signs. Symptoms presented between 2 and 20 months of age, commonly upon transition from breast-feeding or formula to cow's milk. Hypoproteinemia was secondary to increased intestinal protein leakage, but malabsorption and growth retardation were absent (115).

Subsequently, cow's milk-induced anemia and hypoproteinemia were reported in 1 (0.01%) of 7000 infants in a large prospective study from Scandinavia (116). Whole cow's milk was associated with iron depletion in a large proportion (27%) of infants ages 4–12 months, mostly attributable to reduced iron absorption (117). Heat treatment of pasteurized cow's milk reduced the incidence of occult fecal blood loss from 40% to less than 10% in 6-month-old infants, whereas feeding with humanized cow's milk-based formula completely prevented fecal blood loss (118). The pathophysiology of this disorder is unknown; limited biopsy data reveals minimal lymphocytic infiltrates and cytotoxicity, and no significant increase in local antibody synthesis (119).

Pulmonary hemosiderosis has been reported in children with cow's milk-induced anemia and respiratory symptoms of chronic cough, hemoptysis, recurrent lung infiltrates, wheezing, and persistent rhinitis (120–122). A single case of buckwheat-induced hemosiderosis and melena was described (123). Symptoms resolved upon elimination of the offending food and relapsed following oral challenge. Iron-laden macrophages were recovered from bronchial or gastric washings or at lung biopsy. SPTs and serum food-specific IgE levels were negative, but high titers of serum milk and buckwheat precipitins were reported. Biopsy specimens of the lung revealed deposits of IgG, IgA, and complement components, without evidence of IgE (122). Pulmonary symptoms were persistent, with relapses described in 6- and 8-year olds. However, the natural history of food protein-induced pulmonary hemosiderosis is unknown. Considering the seriousness of pulmonary hemorrhage, diagnostic OFCs should be performed rarely, only under close physician supervision in a hospital setting, and only when potential benefits out-weight the risks, such as identifying an offending food in a patient with ongoing symptoms, or determining tolerance after a long period of food avoidance.

Conclusion

There are four distinct food protein-induced GI syndromes in infants and children. Invariably, they respond favorably to strict dietary elimina-

tion of the offending food protein. Most are outgrown within 3 years of life. Currently no evidence exists for an association of these disorders with the development of IBD at an older age. Considering the increasing prevalence of allergic disease in general, and food hypersensitivities specifically, one may anticipate increasing frequency of these disorders (124, 125). Awareness and increased attention should lead to early, accurate diagnosis and treatment.

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19

Gluten-Sensitive Enteropathy

Joseph A. Murray

Introduction

Celiac disease (CD), also known as gluten-sensitive enteropathy, is the end result of a collision between the human immune system and the widespread cultivation of wheat (the major food source that fueled western civilization). The point of contact is the lining of the small intestine. The collision results in inflammation and architectural changes of the absorptive mucosa in those susceptible to CD. The inflammation leads to destruction and eventual loss of the absorptive surface (villi), increased net secretion, and potentially a multitude of consequences of malabsorption (Fig. 19–1).

Classically, CD causes increased loss of ingested fat and fatty acids in the stool, malnutrition, and deficiency of micronutrients (iron, folate, and the fat-soluble vitamins) that may result in a syndrome of severe malabsorption (1). However, the disorder frequently presents with only the vaguest of symptoms or, indeed, may remain entirely silent for many years despite much damage to the intestine, explaining its apparent rarity in some countries (2). The disease affects whites predominantly. Its relative absence from eastern cultures may be due to genetic differences in the population and/or the absence of wheat in the diet until recently. The disorder usually completely resolves with exclusion of gluten from the diet, but reoccurs when gluten is reintroduced (3). Although it was once thought to be a rare disease, it is now recognized as a common chronic disorder affecting as much as 1% of some western populations. CD is defined as a permanent intolerance to ingested gluten that damages the small intestine and that resolves with the removal of gluten from the diet.

Other Forms of Intolerance to Wheat/Gluten

Although CD is the best-recognized pathologic consequence of gluten ingestion, wheat and gluten may be implicated in other syndromes that partly resemble CD (4). Clinical syndromes of chronic diarrhea that respond to gluten exclusion have been described that lack the architectural changes of CD (5, 6). A recent report suggests that some patients may have signs of subtle mucosal responsiveness to gliadin, or may have microscopic changes in the colon and share the same genetic background as those with true CD; these patients' symptoms may represent incompletely expressed forms of gluten-sensitive enteropathy or colonopathy (7, 8).

In addition, wheat may be subject to incomplete absorption in adults (9). Gluten has been implicated in autism and schizophrenia; however, these have remained in the realm of uncontrolled observations. Despite the lack of scientific rigor supporting these conclusions, the prescription of a gluten-free diet (GFD) has found favor among many practitioners of alternative medicine. CD, with its many complications and implications, should be diagnosed before commencing the GFD, because without this step subsequent confirmation may be difficult.

Wheat may, of course, induce a more classic allergic response that is characterized by IgE or eosinophil-mediated responses; its diagnosis is made by eliciting a history of an immediate reaction to wheat including urticaria, wheezing, and angioedema. While skin prick testing would support suspect food items, ultimately double-blind food challenge may be needed as proof.

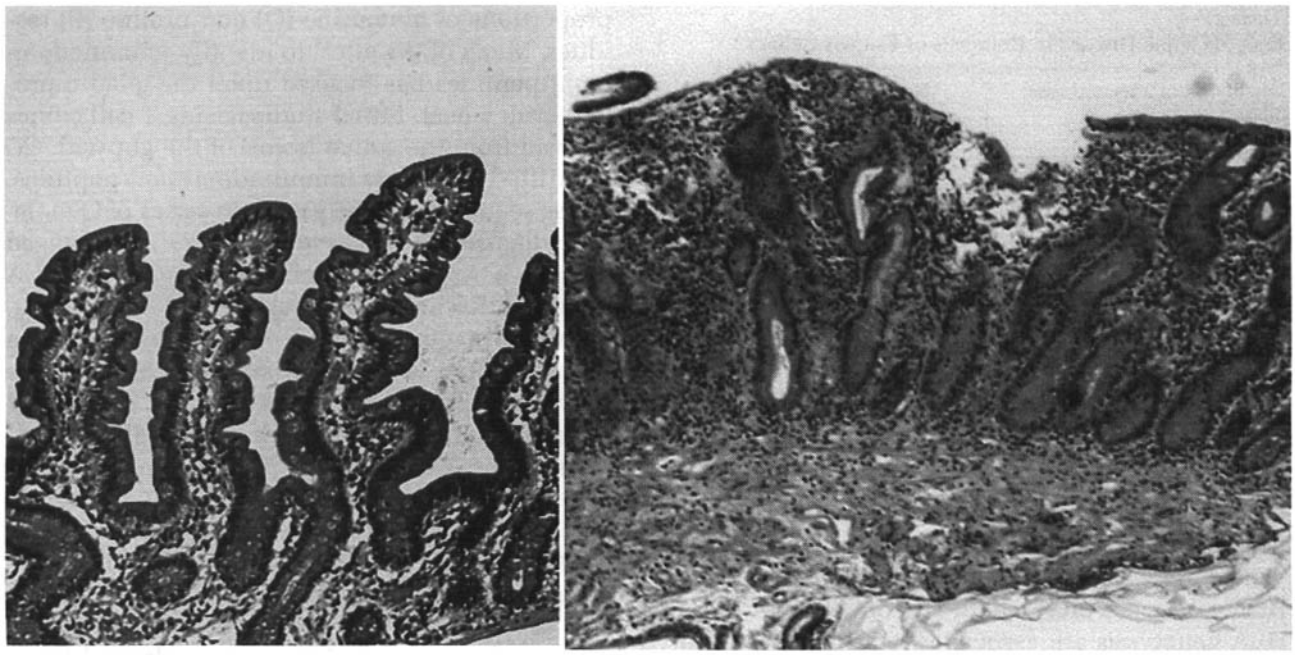

Figure 19–1. A normal mucosa on the left in contrast with the typical changes of the mucosal lesion of CD on the right. There is loss of the villous structures and hyperplasia of the crypts. Lymphocytes and plasma cells predominate in the inflamed lamina propria. Intraepithelial lymphocytes increase in density (magnification 200×).

Etiology

The intestinal lesion in CD is characterized by architectural and inflammatory changes in the mucosa of the proximal small intestine. The inflammatory response consists of increased numbers of lymphocytes, plasma cells, and macrophages in the lamina propria, and increased lymphocytes in the surface layer of the epithelium (intraepithelial lymphocytes). The surface enterocytes are shorter and wider, and have poorly ordered nuclei. The normally tall thin villi are shortened and flattened (Fig. 19–1), and the crypt layer is increased in depth. These changes may be patchy and may affect a variable length of the small intestine.

All of these changes result in substantial loss of the absorptive function of the small intestine. The inflammation also can lead to pain, scarring, and edema of the bowel wall.

The combination of genetic predisposition, environmental insults, and the intestinal immune system culminates in the intestinal mucosal damage of CD (Fig. 19–2) (4, 10, 11).

Genetics

CD has been reported predominantly in whites from different ethnic groups and geographic locations (12–18). Although it has been reported that the prevalence of CD is substantially greater in certain ethnic groups or geographic regions in Europe, there appears to be little difference among Caucasian groups when population-based screening is done. CD occurs commonly in families (Table 19–1) (19–24). The inheritance pattern is complex and determined by the effects of several genes and the environment. CD is strongly associated with the HLA class II genes that encode the molecule DQw2, and less often DQ8 (25–27). Such is the strength of the association that these HLA haplo-

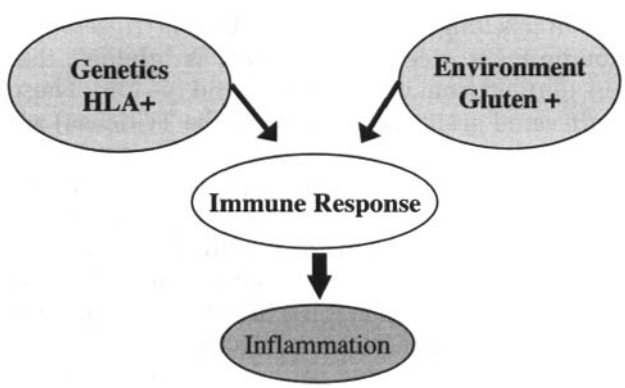

Figure 19–2. Celiac disease is a result of a unique interaction between the environment (gluten) and a genetic susceptibility that results in an aberrant immune response in the gut.

Table 19–1.
Risk of Celiac Disease in Relatives of Known Celiacs

Likelihood of a second case	50%
Sibling	10%–20%
Parent	5%–10%
Sibling sharing at-risk HLA	40%
Child	5%–10%
Niece/nephew	5% or less
Grandchild	5%

References (19, 164)

types are essential for the disease to occur. There may be a gene dosage effect in which homozygosity increases the risk of early onset and occurrence of the disease (27). There are almost certainly other genes involved in the susceptibility for CD; however, other genes have yet to be convincingly established as contributory (28–30).

There are several possible reasons that these HLA genotypes increase the risk of CD. First, these HLA genotypes are associated with an increased risk of autoimmune diseases in general. This increased risk is due to the escape of autoreactive T cells from thymic selection (31, 32). People with these HLA genotypes develop a larger repertoire of T cells that are potentially self-reactive, although they may also confer an advantage in resisting certain infections such as malaria. Second, a unique binding affinity exists between DQ2 or DQ8 and certain peptide fragments of wheat (especially if they have undergone modification) that may occur in the gut. Finally, the lack of an HLA genotype associated with CD may have some negative predictive value in people considered otherwise at risk (33, 34).

Environmental Factors

It has long been known that CD is triggered by the proteins, collectively known as "gluten", that are derived from wheat, barley, and rye (35). These cultivated grain plants (plant tribe Triticeae) are closely related grasses from the family (Poaceae). The storage proteins of these grains are needed for seed germination. The proteins most harmful to those susceptible to CD are gliadins and, to a lesser extent, glutenins in wheat, hordeins in barley, and secalins in rye. The avidins in oats, although long suspected as harmful, are probably not. The toxicity of these proteins has been verified mainly in in-vivo challenge studies (3, 36, 37). These proteins are large and complex, and they contain many separate sequences that can elicit vigorous responses in CD. These proteins consist of remarkably large proportions of glutamine (Q) and proline (P) residues. Much of the effort to identify immunodominant peptides has focused upon the gliadin protein from wheat. Initial studies using T cell clones derived from the active lesion of the gut with CD identified just a few immunodominant peptides. These sequences usually contain series of Q molecules flanked by P. These sequences are arranged in such a way to maximize binding to the HLA molecule. Recent studies suggest that, in children, a much broader selection of peptides induces a response than was previously thought (38). It is likely that gluten stimulates both the innate and adaptive immune systems. Of course, dietary proteins are broken down by digestive enzymes, but gliadin has some unique properties that enable it to resist digestion, resulting in the preservation of peptide motifs that appear to be particularly immunogenic (39). As mentioned above, the motifs that are especially likely to produce responses consist of sequences of Q residues flanked by P, and often separated by one or more other amino acids (Fig. 19–3). The toxicity of wheat is further complicated by the likelihood of further processing of the peptides by host enzymes, especially tissue transglutaminase (tTG) (40). This enzyme is released in the setting of inflammation and changes specific Q residues found within gluten peptides to glutamic acid (E) residues. This process (deamidation) causes these peptides to be more immunogenic, and it primes T cells derived from the CD-prone intestine (41). It is not clear whether this processing is required for the initiation of the disease, but it may well be required in the progression of the immune response once inflammation has occurred. The amino acid sequences in the gliadin peptides are uniquely configured to allow deamidation by tTG. This deamidation occurs at the crucial residues that theoretically increase binding to the HLA binding domain (42).

Although gluten is central to the pathogenesis of CD, other factors may trigger the onset of symptoms, including gastrointestinal (GI) surgery, pregnancy, high-dose gluten challenge, and viral infection (43). GI surgery, intercurrent gastroenteritis, and high-dose gluten challenge may merely impede the small intestine's ability to compensate for damage that has already occurred. A few patients receiving interferon (IFN)-α for chronic hepatitis developed CD (44). Similarly, viral infection that results in an IFN-α response may trigger CD in susceptible individuals (45). These viral infections may heighten the immunologic response to gluten and thus lead to a loss of tolerance. Alternatively,

Figure 19–3. The steps that lead to CD are shown in this illustration. 1) Gliadin, the alcohol-soluble fraction of wheat, and similar proteins from rye and barley, undergo partial digestion in the gut. 2) The resulting peptides cross the gut epithelial barrier. 3) Native gliadin molecules are taken up by APCs as is, or 4) they undergo deamidation (glutamine [Q] is changed to glutamic acid [E]), after which they are presented to activated T cells. These activated T cells in turn activate the 5) cellular and 6) humoral pathways. 7) The T cells cause production of more cytokines and recruitment of other inflammatory markers that lead to epithelial damage. 8) The plasma cells produce antibodies directed against both gliadin and autoantibodies. It is not clear how these antibodies cause disease in the gut, but cross-reactive antibodies may cause dermatitis herpetiformis. Both environmental (predominantly gluten) and genetic factors give rise to the inflammation that leads to the destruction of the absorptive surface of the intestine. (Adapted from *Encyclopedia of Gastroenterology 2002,* copyright The Mayo Foundation)

cross-reaction between viral proteins and gliadin could trigger CD, as has been suggested with adenovirus 12 (46). Protective factors, such as breastfeeding and delayed introduction of large quantities of gluten into the infant diet, seem to reduce the likelihood of developing CD at an early age (47).

Immunologic Factors

The intestinal mucosa responds constantly to myriad foreign antigens in the gut lumen including food, bacteria, viruses, and toxins. It must do so in a way that protects the host from pathogens and toxins but allows the controlled entry of nutrients. The gut immune system is a delicately balanced milieu in which both the innate and adaptive arms of the immune system are in a controlled state of chronic inflammation. In CD, the consumption of gluten disturbs this homeostasis, resulting in unchecked inflammation in the proximal intestinal mucosa. The intestinal lesion is an immunologically mediated inflammation of the intestine (48). Much of what is known about this response is based on the study of established intestinal lesions and may not reflect early pathogenic processes that initiate intestinal damage.

Little is known about the process that leads to CD. Peyer's patches (PPs) are immunologic sampling sites in the small intestine. Under normal conditions, luminal antigens are taken up through pinocytosis by M cells (microfold cells, which are specialized thin enterocytes) over the PP and are delivered to the subepithelial layer of the PP, where antigen-presenting cells (APCs) process the antigens. This mechanism allows controlled sampling of the antigenic milieu by the intestinal immune system. Primed B cells travel from the PPs to the mesenteric lymph nodes and return to the jejunal mucosa, where they are terminally differentiated to produce IgA directed against the antigen (49).

Gluten may disturb this delicate immunologic balance in several ways. First, it must gain inappropriate access to the lamina propria to cause sensitization. Second, gluten proteins may be more resistant to complete digestion by gut enzymes, leaving large, potentially immunogenic peptides relatively intact (39).

Innate responses to gluten can elicit effects within minutes to hours of exposure. In vitro studies demonstrate that the expression of HLA antigen on the cells in the surface layers of the intestinal mucosa increases within 2–4 hours of exposure to gluten (50). Another rapid innate response to gluten is the permeabilization of the intestine. This process also seems to be independent of the other immunological effects of gluten, because it occurs rapidly (within minutes) and anywhere along the intestine. Gluten alters the permeability by inducing the release of zonulin, a molecule that interacts with the junctional complex (51). This causes the intercellular tight junctions to loosen, allowing for the paracellular passage of antigens including gluten. It could be speculated that this is a nonspecific toxic effect of the protein, whereby the plant induces rapid transit of intact seeds through the intestine, enhancing propagation of the plant's offspring.

This induction of the innate immune response by gluten may have other important consequences. Because the gluten peptides enter into the epithelial compartment and paracellular regions, the PP pathway is not the exclusive route taken by the gluten peptides to the immune system. Bypass of the PPs may lead to a loss of tolerance and even an induction of sensitization, resulting in an uncontrolled immune response in the intestinal mucosa (45). Thus, both arms of the immune system—innate and adaptive—play a role in the development of CD, even though most attention has so far been focused on the adaptive arm (Fig. 19–4).

Adaptive Immune Response

Cellular Immunity: Cellular immunity seems to play the major role in the intestinal damage of CD. The pathogenic sequence of events has been elucidated primarily through in vivo challenge studies in treated patients with CD and in vitro challenge studies on biopsies from treated and untreated patients (50, 52, 53). T cells play a crucial role in the ongoing response to gluten. Activated T cells increase in the small intestine, and many of them respond specifically to gluten. This mechanism is dominated by a Th1 type cytokine response, with cytotoxic and helper T cells predominating.

T cell clones reacting specifically to gluten in an HLA-restricted fashion have been isolated from the lamina propria of affected intestinal tissue and the peripheral blood (54, 55). These cells release IFN-γ, tumor necrosis factor (TNF), and other proinflammatory cytokines (56). These cytokines induce a migration of lymphocytes into the surface epithelium, subsequent recruitment of activated lymphocytes, macrophages, and plasma cells into the lamina propria, and deposition of complement in the subepithelial layer (57).

The surface epithelial layer is infiltrated with an increased number of T lymphocytes. These cells predominantly express the αβ receptor, but an increased proportion of cells express the γδ receptor. The intraepithelial cells in the surface layer also express the natural killer (NK) cell surface marker CD94 (58). These cells migrate into the intraepithelial compartment in response to gliadin, an effect that may be mediated by interleukin (IL)-15, which is released by enterocytes (59). These cells may be affected by the innate response to gluten or other noxious stimuli to provide the costimulatory signals needed to expand the initial adaptive response. Enterocytes, monocytes, and dendritic cells (DCs) may also play a crucial role in amplifying the initial adaptive response. The inflammatory sequence depends on a competent cellular immune response in the small intestine and shares many features with those of experimental graft vs host disease (60). The inflammatory response likely also damages the structural support and the microcirculation of the villus, causing the villus to collapse (61). The complex interrelationship between the surface enterocytes and the supporting fibroblasts is disrupted, leading to loss of the orderly migration and differentiation of the villous surface. The thickening of the crypt is not so much a response to loss of surface entero-

Figure 19–4. The panel on the left represents the role of the thymus in the development of its T cell repertoire during development. The MHC molecules (DQ2 or DQ8) have a crucial role in presenting self-antigens to the thymic cells. Individuals with the genotypes DQ2 and DQ8 may be at greater risk of autoimmunity as autoreactive cells escape from the thymus. The panel on the right illustrates that innate responses are rapidly elicited, permeabilizing the gut, allowing gluten to gain access to the lamina propria. Innate responses to the gluten and/or intercurrent infections results in the initial release of inflammatory cytokines. The local damage that ensues leads to the release of tTg from the fibroblasts. This then alters the peptide residues of the gliadin, presenting a neoepitope (deamidated gliadin) that stimulates the adaptive response both toward this new epitope(s) and toward itself. If this response overcomes the toleragenic effects of regulatory T cells, inflammation ensues with increased release of cytokines and recruitment of other inflammatory cells. It takes the congruence of genes, exposure to gluten, and probably another trigger to generate the complex that is established CD. (Copyright, The Mayo Foundation, 2002.)

cytes as it is the result of inflammation and remodeling of the mucosa (62). This damage is the most intense in the proximal small intestine, and it decreases caudally. The extent of damage to the intestine determines the malabsorptive consequences of the disease. Surface lymphocytic infiltration of the stomach and colon may also be seen (63). The rectal mucosa of untreated CD responds to rectal exposure to gluten (64).

Humoral Response: A potent humoral response occurs in untreated CD (65). The intestinal mucosa in CD contains increased numbers of plasma cells secreting IgA, IgG, and IgM directed against gluten peptides, and antibodies against connective tissue autoantigens (66). Those antibodies are found in the intestinal juice and the serum (67, 68). The dynamics of the humoral response seem to parallel those of cellular injury, although antibodies may arise before mucosal relapse and disappear before healing. Secreted IgA against gliadin may be a vain attempt to exclude a harmful antigen, while anti-connective tissue antibodies may target host antigen(s) in the connective tissue of the jejunum, umbilical cord, and endomysium (68, 69). The main autoantigen target is the enzyme tTG (70).

The role of antibodies in the pathogenic process has been discounted because of the predominant role of the cellular immune response in the small intestinal lesion, and case reports that CD can occur in the setting of hypogammaglobulinemia. However, the coincidence of tTG as the autoantigen, its release in CD, and a unique interaction with gliadin have renewed interest in the humoral response in CD pathogenesis (71, 72). Gliadin antibodies can be seen in other intestinal conditions, but the connective tissue antibodies are highly specific to CD (73). The humoral response may have a role in some of the extrain-

testinal processes seen in CD, including dermatitis herpetiformis, hyposplenism, IgA nephropathy, and hypoparathyroidism (74, 75).

Host Modification of Gluten by Tissue Transglutaminase

An intriguing feature of tTG is that its substrate is glutamine, which constitutes 35% of gliadin's amino acids. Tissue transglutaminase itself complexes with gliadin and may allow gliadin-responsive T cells to help tTG-responsive but inactive B cells to generate a potent self-directed antibody response (72). One report suggests that tTG modifies the gliadin peptides, increasing the binding affinity for the antigen-presenting site of the two HLA molecules that seem to be necessary for CD (DQw2 and DQw8) (41). Interestingly, the deamidated peptides are not recognized in the context of DQ types that are not involved in CD (76). This host modification of the external antigen may be a crucial step in expanding the immune response to the exogenous gliadin molecule once tTG has been released (42, 72).

Presentation

The classic constellation of symptoms and signs characterizing the malabsorptive syndrome of CD includes steatorrhea, weight loss or failure to thrive, bloating, and flatulence with multiple deficiency states. More common and difficult to recognize are the other ways in which CD presents. It can mimic many common clinical entities, including irritable bowel syndrome (IBS), diabetic diarrhea, and dyspepsia. Other atypical modes of presentation include deficiencies of single micronutrients, especially iron, folate, B12, and the fat-soluble vitamins D, E, A, and K. Nonspecific gastrointestinal (GI) complaints such as bloating, abdominal pain, diarrhea, constipation, flatulence, secondary lactose intolerance, dyspepsia, and non-GI complaints such as fatigue, depression, arthralgias, milk intolerance, osteomalacia or osteoporosis, and iron-deficiency anemia are common consequences of CD (Table 19–2) (22, 77–79). The latter can occur with or without heme-positive stool (80). The presentation of CD in children similarly can result in stunting of growth and intellectual development, epilepsy, and dental abnormalities as single symptoms without the more classic malabsorptive symptoms of the malnourished pot-bellied infant with steatorrhea (Table 19–3) (81–83).

Table 19–2.
Prevalence of Celiac Disease in Associated Diseases

Population	*Risk %*	*Reference*
Diarrhea-predominant IBS	5%	85
Lactose intolerance		142
Osteoporosis	5%	143
Type I diabetes mellitus	3%–7%	20, 21
Sjögren's disease	3%	22
Thyroid disease	4%	23
Myocarditis	4%	165
Selective IgA deficiency	7%	24

Several studies have shown that there is a significant delay (often of many years) between the initial onset of symptoms in a patient and the diagnosis of CD (84). These patients' symptoms are often ascribed to a functional cause such as IBS, and a high fiber diet or other non-specific dietary measure is prescribed to little avail (85). Unfortunately, referral for psychiatric evaluation is all too common prior to diagnosis (86). It is unusual for the patient to identify gluten as the source of the symptoms, but when they do, the physician should not ignore it. CD is occasionally found incidentally during endoscopy or by abnormal liver blood tests on routine chemistries (87, 88). Sometimes CD presents with severe liver failure (89). Although CD is associated with many possible symptoms, several misconceptions still occur. Obesity, normal or tall stature, absence of diarrhea, advanced age, or even the absence of a deficiency state, do not discount the possibility of CD.

Table 19–3.
Presentations of Celiac Disease

Gastrointestinal	*Non-Gastrointestinal*
Steatorrhea	Dermatitis herpetiformis
Duodenal obstruction	Infertility, recurrent fetal loss
Chronic diarrhea	Anemia
Elevated transaminases	Dementia
Weight loss	Folate and/or iron deficiency
Recurrent pancreatitis	Ataxia
Constipation	Neuropathy
Heme-positive stools	Tetany
Bloating, abdominal pain	Osteoporosis
Enteropathy-associated T cell lymphoma	Arthralgia
	Developmentally synchronous dental enamel defects
Failure to thrive	Fatigue
Vomiting	Osteomalacia
Dyspepsia	Seizures
	Brittleness of diabetic control
	Depression

Epidemiology

CD is common in whites from many different ethnic groups and is not limited geographically to Europe (14–16). It is rarely seen in the Far East and in sub-Saharan Africa. Cases predominate in females and adults (90). The age at diagnosis is rising in most populations, with some patients being diagnosed over the age of 60 years (12). The prevalence of diagnosed CD varies widely, from 1:300 to 1:5000 persons. This greatly underestimates that estimated by serological screening, which suggests as many as 1 in 100–300 persons may be affected (Fig. 19–5) (2, 13, 17, 18, 70, 91–93). A heightened suspicion or awareness of CD results in a substantially increased rate of diagnosis (94–96). But even with such active case findings, many cases remain undiagnosed because most present with vague symptoms and few nutritional abnormalities (91, 97).

Several other diseases are associated with a high prevalence of usually undiagnosed CD. It is particularly common in patients with type 1 diabetes mellitus, thyroid disease, Addison's disease, osteopenic bone disease, Down's syndrome, and rheumatologic complaints (Table 19–2) (20, 21, 98). The associations with autoimmune diseases are probably due to shared genetic risk factors for both, though it has been suggested that untreated CD may predispose children to diabetes or other autoimmune disease (42, 99).

Diagnosis

The pre-modern diagnosis of CD was based on the constellation of features, especially steatorrhea and weight loss or failure to thrive, that are hallmarks of frank malabsorption, because the pathogenesis of the disease was not well understood (100). Almost simultaneously in the 1950s, advances in understanding of the specific pathologic lesion in the small intestinal mucosa and its gluten-induced etiology enhanced the precision with which the disease could be diagnosed; diagnosis included the response to therapy (GFD) (101). CD is most precisely defined by work groups of ex-

Image Not Available

Figure 19–5. The icebergs of known and hidden CD. Numbers represent the prevalence in numbers per thousand ($^0/_{00}$). The tips represent the prevalence of diagnosed CD, and the submerged portions represent undiagnosed patients.

perts, and it requires histologic evidence of enteropathy and a positive response of symptoms or signs to a GFD (102, 103). The earlier requirement for three biopsies was both cumbersome and, in most cases, unnecessary to establish and confirm a diagnosis of CD. Three biopsies may be needed in individuals diagnosed under 3 years of age, when the population from which the individual comes is subject to common alternative diagnoses, and when the original diagnosis is uncertain or is challenged later.

Serological testing has greatly facilitated the identification of CD in people with clinical presentations too mild to justify the invasiveness of a biopsy as the first test. Its ready availability has made diagnosis accessible to doctors and their patients in primary care. Not only are more people being diagnosed, but many other issues have arisen about the accuracy of the diagnosis and even the definition of the disease. Such terms as latent, silent, atypical, or subclinical CD are used, leaving many clinicians and their patients mystified as to what, exactly, they have or do not have.

If the patient's symptoms are characteristic of malabsorption, then the adjective "'classical" is used, and if they are not, then "non-classical" is used. (Some authors use the term "atypical" CD; however, recent work suggests that "atypical" really may be the most common form.) The total absence of any measurable clinical consequence would imply "silent" CD, which may not be at all rare and is of unclear significance. "Latent" CD can be defined as a state in which there is evidence of an immune response to the autoantigen without architectural changes in the small intestine. In vitro challenge of the mucosa with gluten or some other triggering event may result in the fully expressed disease, which may manifest as endomysial antibodies in the setting of normal small bowel histology. A variant of latent CD is the closely related phenomenon of non-CD gluten sensitivity, in which a symptomatic patient who has the same HLA type that is associated with CD, and has an increased secreted antibody to gliadin or tTG, can have symptomatic relief from gluten avoidance (5, 6, 8). The precise extent of this population is not known, but it may be common; the clinical consequences of continued gluten in the diet are unknown beyond the symptoms. It is clear from these and other observations that CD is a spectrum of clinical and pathologic entities that are closely related and overlap considerably (4).

Dermatitis Herpetiformis

Dermatitis herpetiformis a pruritic rash that causes bullae on extensor surfaces of the limbs, trunk, and scalp (Fig. 19–6). It is a skin manifestation of the intestinal immune response to ingested gluten that is characterized by the deposition of IgA granules at the dermo-epidermal junction (104). The source of these IgA deposits in the skin is unknown, but they may be produced in the intestinal mucosa and are likely cross-reactive with the closely related skin-based autoantigen epidermal transglutaminase, which is similar to tTG (the primary target of the humoral system in the gut) (75). The enteropathic damage in the intestine may be asymptomatic at the time of presentation of the skin rash, but it is indistinguishable from that seen in CD. Some patients with essentially normal intestinal biopsies develop frank enteropathic damage on a regular gluten-containing diet. The presence of endomysial or tTG antibodies correlates with the degree of enteropathy in individuals with dermatitis herpetiformis (105, 106). A positive serological test strengthens the certainty of the skin diagnosis, and would also mandate examination of the patient for consequences of malabsorption. However, it is not necessary to perform these antibody tests or even an intestinal biopsy to establish the etiologic role of intestinal gluten exposure in dermatitis herpetiformis. That can be reliably inferred by the demonstration of the granular IgA deposits in the skin (Fig. 19–4). The serology test may be useful in cases in which there remains some doubt, for example in distinguishing it from bullous linear IgA disease, which is not a gluten-sensitive disorder. Gliadin antibodies may be seen in other bullous skin disorders and are not particularly helpful in this setting (107, 108).

Many patients in the US with dermatitis herpetiformis have not been treated with a GFD, but rather with dapsone, which suppresses only the skin rash. Dapsone does not prevent intestinal damage, but its benefit on the rash does delay or prevent appropriate dietary measures. Many of these patients may present years later with GI symptoms or anemia. CD should be high on the list of possible explanations for the patient's problem. Often patients do not volunteer a history of skin rash. Intestinal biopsies at this point may help to confirm enteropathic damage as a cause for the GI symptoms and convince the patient to commence a GFD.

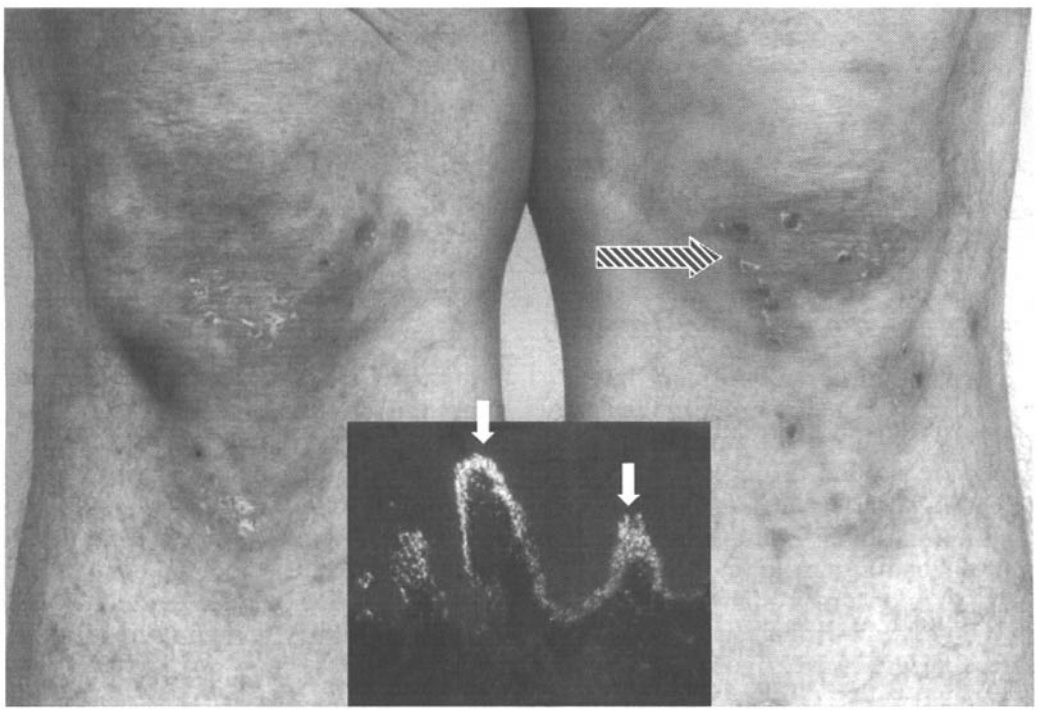

Figure 19–6. This photograph demonstrates the typical distribution of the rash of dermatitis herpetiformis. The skin biopsy demonstrates the granular pattern of IgA deposits on the dermoepidermal junction typical of dermatitis herpetiformis (magnification 100×).

Diagnostic Tests for Celiac Disease

An important consideration is whether the patient has been on a GFD prior to testing. All of the tests, including the intestinal biopsies, may have returned to normal, making confirmation difficult without reintroducing gluten into the diet (see Gluten Challenge, below).

Serology

Two types of serological tests exist for CD. The first are tests for IgA and IgG antibodies to gliadin. Although these have been used widely in screening, both have low specificity for CD, because false positives may occur in many other small intestine conditions, such as Crohn's disease, cow's milk protein intolerance, or bacterial overgrowth (109, 110). The second class of antibodies are those directed at connective tissue autoantigens, endomysium, or tTG; these tests are supplanting the gliadin-based tests. The so-called endomysial (EMA-IgA) or tTG (tTg-IgA) antibodies correlate much better with actual damage in the intestine, because they are both more sensitive and more specific for CD (Table 19–4) (111). The EMA-IgA test is an indirect immunofluorescent assay (IFA) that depends on the identification of a specific pattern of staining on a substrate (usually primate esophagus or human umbilical cord) (112–114). It is usually negative in the normal population. Any positive samples are titered in serial dilutions until the signal is lost, thereby quantifying the level of antibody. The EMA-IgA does have drawbacks, such as a limited supply of substrate and inter-observer variability. Because the tTg-IgA test is an enzyme-linked immunosorbent assay (ELISA), it is much easier to perform and standardize between labs than the EMA. The result is expressed in assay units with a threshold that is considered above normal. A number of different substrates for the tTg-IgA ELISA have been used, but of these human tTg seems to be the best (115). Occasionally, false positive tTg-IgA will be detected in conditions such as autoimmune liver disease and other autoimmune diseases (116). One of the major limitations of current serology is the inability to accurately detect CD in patients with unrecognized IgA deficiency, a common association (117). In those circumstances, a tTg-IgG-based test or an intestinal biopsy is needed (112). IgG-based tTg ELISAs seem to have some promise.

Table 19–4.
Serological Tests for Celiac Disease

Substrate/Antigen	*Antibody isotype*	*Test type*	*Sensitivity*	*Specificity*
Gliadin	IgA	ELISA	31%–100%	85%–100%
Gliadin	IgG	ELISA	46%–100%	67%–100%
Endomysium	IgA	IFA	57%–100%	95%–100%
Tissue transglutaminase	IgA	ELISA	92%–98%	98%*

References (70, 111, 113, 114, 144, 166–175)
*Lower in autoimmune disease; IFA, immunflorescence assay.

Family members of CD patients may have positive EMA-IgA or tTg-IgA antibodies in the absence of histological changes in the gut. These patients have a high likelihood of developing CD, whereas those patients who have gliadin antibodies alone with a normal biopsy have a relatively lower risk of subsequently developing CD (118). The converse can also occur, in that histological damage and even symptoms can occur in affected families who have negative serology (119).

Clinical Use of Serologic Tests: Serologic tests for CD are used for screening projects, in initial diagnoses, as a triage for subsequent biopsy, and in follow-up of patients. The predictive value of the tests depends on the pretest likelihood of the disease in the individual patient's population (120). If the issue is diagnosis in a patient in whom CD is highly likely, a positive anti-endomysial antibody by IFA may be specific enough that biopsy is unnecessary in CD (121, 122).

The gliadin antibody test is the cheapest test, but it does not have the specificity required to confirm the diagnosis. The tTG ELISA test is very close to the EMA-IgA test but occasional false positives are seen, and a negative test does not rule out the possibility of CD.

For patients with a lower pretest probability of disease (~5% risk), the serologic tests are accurate enough to triage patients for subsequent intestinal biopsy, but produce too many false positives to allow avoidance of biopsy. In patients with frank symptoms or signs of malabsorption, intestinal biopsy should be done regardless of the antibody results, to detect other mucosal diseases that can cause malabsorption (123). The EMA-IgA may be especially useful in populations who have a high prevalence of other causes of flat mucosa. In these cases, a flat biopsy with positive EMA would differentiate it from conditions such as cow's milk and other enteropathies. However, in patients with incomplete enteropathy, negative serological tests do not exclude CD, but should prompt consideration of other diseases.

Screening at-risk populations for CD is based on the known prevalence of these diseases, and the potential for future malignant complications and prevention or correction of subtle but important problems such as osteopenic bone disease (Table 19–2) (124). However, formal outcome studies of cases diagnosed by screening are lacking.

Other Non-Invasive Tests

Tests for deficiency in hemoglobin and red cell indices, carotene, vitamin D, prothrombin time, and iron and folate levels, although important in themselves, are neither specific nor sensitive for CD. Similarly, tests of absorption, including the D-xylose test and qualitative or quantitative fecal fat tests, have the same drawbacks. For example, fecal fat may be normal in 30% of celiacs. Many patients with CD have normal hemoglobin and vitamin levels.

Identification of CD by contrast radiography depends on very subtle signs of edema of the mucosa folds or jejunalization of the ileum. Patterns of flocculation described in the past rarely occur, because modern barium emulsions resist flocculation. It is therefore a very insensitive test for CD. The major use of the contrast study is to identify a complication of CD such as ulcerative jejunitis, strictures, and neoplasms of the small intestine.

Measurement of intestinal permeability has become fashionable and may be a sensitive test for intestinal damage. The simplest method is the measurement of the ratio of urinary lactulose and mannitol following an oral challenge. Lactulose, the larger molecule, is absorbed in greater proportion to the smaller mannitol, indicating increased permeability to macromolecules. Although the test may be sensitive, it is not specific for CD (125, 126).

Salivary and stool antibodies to gliadin may indicate some degree of humoral response to gluten, but they have not been validated as tests for CD. IgE-based tests to wheat are not useful in the diagnosis of CD. IgG-based tests for "food allergies" may or may not help identify CD, and they have very low specificity and sensitivity for CD.

Skin testing to intradermal gliadin is relatively insensitive and can be rather painful. Rectal challenge to gliadin does produce a measurable change in the number of intraepithelial T lymphocytes in untreated CD and could serve as a surrogate test but, as currently performed, does not help in diagnosing the patient already on a GFD. The in vitro response to gluten of biopsies cultured from the small intestine holds some promise for the patient already on a GFD (67).

HLA typing to identify and exclude the possibility of CD in those lacking the required HLA genotypes DQ2 or DQ8 may be useful in some circumstances (33, 127).

Intestinal Biopsies

Biopsies may be obtained either by endoscopy or by the various devices designed to obtain pieces blindly. Endoscopy is more widely available, more comfortable for the sedated patient, and allows for multiple directed biopsies to be taken from the distal duodenum. Assuming that an adequate number of well-oriented samples are taken, a definite diagnosis should be obtainable in the vast majority of cases. Biopsies obtained too close to the stomach may be distorted by underlying Brunner's glands or coincidental peptic inflammation (128). Pathologists need to be aware of the spectrum of change that occurs in CD. The histologic changes in CD vary from severe villous atrophy to more subtle changes well-characterized (53, 61) in acute challenge studies carried out in treated CD patients. The earliest stages include increased density of intraepithelial lymphocytes, crypt hyperplasia, and villous atrophy (129).

The pathologist should consider the villous-to-crypt ratio, the intraepithelial lymphocyte-to-enterocyte ratio, and chronic inflammation of the lamina propria. The term "chronic non-specific duodenitis" should be used and interpreted with caution, as this has often been understood by the pathologist or gastroenterologist to imply a peptic process, thereby missing the significance of the finding in a patient with CD. Review of prior histological specimens may reveal changes suggestive of CD. Occasionally, an incorrect diagnosis of CD may have been made on badly oriented or fragmented biopsies.

Villous atrophy is not specific for CD, but there are only a few other conditions that occur in developed countries associated with villous atrophy, especially in adults. Graft-vs-host disease, radiation, and ischemia are readily differentiated by history or other tests. Tropical sprue is a consideration in those that have traveled or lived in tropical areas. These patients should be treated for tropical sprue and discouraged from avoiding gluten. A failure to respond to the appropriate therapy for tropical sprue should suggest the possibility of CD. Serologic tests may help distinguish the two but few cases of tropical sprue have been included in the validation studies for serological testing. Intestinal lymphangiectasia, Whipple's disease, and amyloidosis are readily differentiated by the histologic appearances of the intestine. Immune deficiency states that may coexist with CD can usually be identified by electrophoresis or HIV testing. Giardiasis rarely produces the severe damage seen in CD except in the setting of immunodeficiency states. In very young children, cow's milk, soy, and rarely other foods may cause a similar picture, although the damage is usually not as severe as it is in CD. Crohn's disease of the duodenum is rare but may mimic CD, and occasionally the two entities coexist (130).

Gluten Challenge

It is no longer necessary to rechallenge most patients who have a well-established diagnosis of CD. However, in patients first diagnosed under the age of 3 years or those who have already embarked on a GFD and are seeking a confirmation of the diagnosis, a formal gluten challenge may be desirable. This is not usually needed if the patient had an intestinal biopsy while on a gluten-containing diet. Review of the original histology slides, if available, may suffice to confirm the diagnosis. The length of time it takes to relapse with gluten challenge is quite variable. The gluten in 3–4 slices of whole wheat bread daily should be sufficient to produce damage in 2–4 weeks, although it can take longer for the full pattern of injury to occur. Some very sensitive patients may need a reduction of this dose to prevent severe symptoms. Patients who do not develop symptoms should be followed and biopsy delayed until symptoms or

endomysial antibodies appear, whichever comes sooner (131). Most patients relapse within 6 months although, in rare cases, it may take years to relapse.

Treatment

The mainstay of treatment of CD is lifelong adherence to a diet that excludes foods containing "gluten." In the context of CD, "gluten" encompasses the storage proteins from wheat, rye, barley, spelt, and kamut (Table 19–5).

Patients may have difficulty accepting that something as fundamental to their diet as wheat can injure them. Adolescents seem especially likely to be non-compliant with the dietary restrictions (132). The patient can be motivated with the expectation of what can often be a dramatic improvement in general well-being in addition to improvement of GI symptoms. Advanced age, obesity, cognitive impairment, or institutionalization should not detract from the decision to treat with a GFD, even though these situations may require special effort to succeed. An optimistic attitude on the part of the physician is crucial to the future success of the patient. Patients need to know that they cannot depend on their reactions to questionable foods as a measure of safety. Cheating on the diet should be actively discouraged. An active interest on the part of the clinician can improve compliance, as can the knowledge that follow-up blood tests can detect gross gluten ingestion. The past trend in many places to treat patients with a low-gluten diet ran the risk of significant damage and attendant complications (124, 133, 134). A GFD should result in a prompt and even dramatic improvement in symptoms. The recovery is more rapid and complete in children than adults (135). Resolution of symptoms may take 3–6 months, and complete healing of the intestine may take longer, especially in the elderly.

Detailed instruction from an experienced, well-informed dietitian is invaluable for most patients. It is essential that up-to-date materials are used (136). In the absence of dietary instruction, many patients unfortunately resort to books or the internet for information and may not fully understand important details. Inadvertent gluten intake or an overly restricted diet deficient in essential nutrients may be adopted. A CD support group can provide local information and emotional support to newly diagnosed patients.

Table 19–5.
Sources of Gluten in the Diet

Gluten-Containing Grains and Flours
Wheat
Rye
Malt
Barley
Triticale (wheat-rye hybrid)
Couscous
Kamut
Spelt
Semolina
Gluten-Containing Foods
Bread
Breaded foods
Cakes
Cookies
Crackers
Croutons
Pasta
Pizza
Stuffing
Toast
Commonly Overlooked Sources of Gluten
Beer
Broth
Brown rice syrup
Coating mixes
Caramel color
Cereal products
Catsup and mustard
Candy bars
Cheese spreads
Chip and dip mixes
"Cornflour" in Europe
Hydrolyzed vegetable protein
Hot chocolate mixes or cocoa
Imitation bacon or seafood Marinades
Instant coffee and tea, salad dressings
Modified food starch (starch in non-US foods)
Natural flavorings
Nondairy creamer
Non-fat processed food
Malt or malt flavoring
Processed meats and poultry
Some brands of ice cream
Sausage products
Sauces
Soup bases
Soy sauce
Stuffings
Thickeners
Tomato sauce
Unexpected Sources of Gluten
Medications (both prescription and OTC)
Glues, pastes and dry wall filler
Airborne flour
Communion wafers
Cross-contamination

Oat was once taught to be toxic for most CD patients, but recent controlled studies have shown that a moderate amount of a pure oat product did not impair healing of the intestine or cause a relapse in treated patients (137, 138). However, con-

tamination of commercial oat products with other grains may occur. Vigilance is needed on the part of the patient and physician if a decision is made to incorporate oats in the diet.

Hidden sources of gluten are frequently present in what seem to be safe foods (Table 19–5). Ingredient lists of gluten-free foods must be reviewed regularly for changes. It may be difficult to ascertain the exact grain source of ingredients because of production outsourcing. Even non-food items may be sources of trace gluten and can cause symptoms in more sensitive patients (Table 19–5) (139–141). Contamination of supposedly gluten-free products can also occur. Obtaining flour substitutes from reliable sources that cater to needs of CD patients is encouraged.

Lactose intolerance affects one half of celiacs at diagnosis but usually resolves with mucosal restoration (142). Temporarily limiting lactose ingestion or using lactase may be necessary. Intolerance to other non-gluten-containing foods may be reported (141). The mechanisms of these intolerances are not known but may diminish with healing of the gut.

Vitamin or mineral supplements are given to correct deficiencies of iron, folate, B12, calcium, and fat-soluble vitamins as needed. Marked osteopenia or even osteoporosis are very common in both men and women (143). Patients with decreased axial bone density should be advised to obtain at least 1200 mg of calcium and replacement doses of vitamin D. Higher doses may be needed if vitamin D deficiency is detected and to overcome malabsorption. Secondary hyperparathyroidism may occur but tetany is rare. Intensive, even parenteral, nutritional support and fluid replacement may be needed in very ill patients. Pancreatic enzyme supplementation may be useful in very malnourished patients, and this may accelerate weight return (144).

All patients should be followed up to ensure compliance with, and a response to, the GFD. All antibody levels diminish with the institution of a GFD, often within weeks; by 6 months both tTg-IgA and EMA-IgA may be undetectable (145). The gliadin antibody titers, however, often persist for a year or more into the GFD (146). Repeat testing is used to monitor the diet. However, a negative test is not entirely reliable as an indicator of low-level gluten ingestion (133, 134, 147–149). Improved absorption may cause patients to gain excess weight, increase absorption of medications such as thyroid replacement, and develop increased cholesterol.

Persistent or Recurring Symptoms

Patients with previously diagnosed CD who have persistent or recurrent diarrhea should undergo a careful dietary review and a check of their endomysial and gliadin antibodies (149, 150) (Fig. 19–7). If both are negative and it is less than 6 months since diagnosis, treatment with lactose-restricted diet, pancreatic supplements (especially if there are features of malabsorption), and antibiotics for bacterial overgrowth should be started. Colonic biopsies should be done to rule out coexisting microscopic colitis (151). If it has been longer than 6 months since diagnosis, it may be prudent to re-biopsy the small intestine to assess for improvement. If there was any doubt about the original diagnosis, other conditions should be considered, particularly Crohn's disease and small bowel ischemia (if post-prandial pain is a feature).

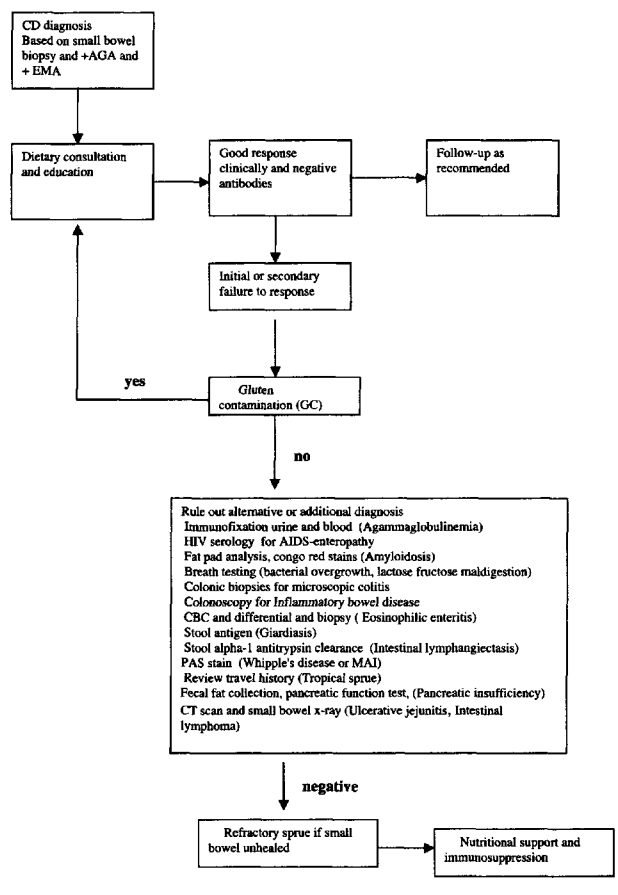

Figure 19–7. Flow chart for evaluating patients who either have not responded to a GFD or who relapse while on it. The precise work-up will be determined by the age of the patient, duration of symptoms, and severity of illness. (Adapted from Current Treatment Options in Gastroenterology 2002;5:27–38.)

Immunoelectrophoresis may detect immunodeficiency states.

Patients may develop other complications from inflammation in the gut. Benign strictures may cause proximal small bowel obstruction (152). Intussusception or ulceration may occur. Recurrent acute pancreatitis may result from inflammation of the papilla of Vater and require sphincterotomy (153).

Refractory Sprue

Refractory sprue is a combination of continued or recurrent severe malabsorption, progressive malnutrition (despite compliance with a GFD for more than 6 months), and intestinal villous atrophy on repeat biopsy. Usually tTG or EMA antibodies are negative. These patients are often elderly and in poor nutritional shape with multiple complications of malabsorption. They may have extensive ulcerative jejunoileitis with a high risk of perforation, obstruction, and transformation to lymphoma. Enteric protein loss may be marked. Rarely biopsies may reveal the deposition of a thickened layer of collagen in the epithelium. So-called "collagenous sprue" is very rare and may respond to immunosuppressives (154).

Enteropathy-associated T cell lymphoma should be carefully sought. CT scanning, small bowel radiography, and enteroscopy usually establishes the diagnosis. The presence of T cell clones on molecular analysis can indicate either a lymphoma or a variant of refractory sprue that is likely a prelymphomatous state (155, 156). Differentiation lies in the presence or absence of morphological evidence of malignant expansion. Even in the absence of lymphoma, this form of refractory sprue with T cell clones has a high mortality due to perforation, relentless malnutrition, and ultimately development of lymphoma (157). Absence of a T cell clone has a much better prognosis, with responses to steroids or immunosuppression likely. Parenteral nutritional support allows correction of the nutritional problems. Antibiotics, pancreatic enzyme supplements, and elemental diet may help (150, 158). The increasing armamentarium of immunomodulating drugs offers potent tools in reversing what, in these patients, has become a self-perpetuating process.

Mortality in Celiac Disease

The mortality in patients with CD was double that expected in a large Italian cohort, although this excess was not seen in a smaller population-based cohort from Minnesota (159, 160). Malignancies are a common cause of death in patients diagnosed later in life and those not compliant with the GFD. Patients diagnosed as children who are compliant with the GFD do not have an increased risk of malignancy. The otherwise rare fatal enteropathy-associated T cell lymphoma is the most common malignant cause of death in non-compliant CD patients. This lymphoma originates in the proximal small intestine and presents either with a return of malabsorptive symptoms or acutely with obstruction, perforation, or rarely, bleeding. Occasionally, patients present with painless lymph node enlargement or systemic symptoms of fevers, sweating, and weight loss. The development of hypoalbuminemia, anemia, recurrent steatorrhea, weight loss, fevers, and malaise in a previously stable patient should prompt a search for neoplasm. Therapy is standard multimodal chemotherapy. Few patients are fit for bone marrow transplantation. Those that present acutely with a surgical abdomen do better than the long-standing patients who have progressive malabsorption (161). In the author's experience, these patients are very prone to lethal infections during chemotherapy, possibly due to malnutrition and coexistent hyposplenism.

Adenocarcinoma of the small intestine is also more common in CD, although the mechanism is not known (162). Nasopharyngeal and esophageal cancer occur more commonly in CD than expected (124).

Screening Family Members

Consider screening at-risk family members, including siblings, children, and possibly parents, because at least 50% of patients have an undiagnosed family member with CD (163). Serologic screening tests could be used for asymptomatic relatives, but those with specific symptoms suggestive of CD should undergo interstitial biopsy. Children may have to be screened more than once. Of course, the family members must be ingesting gluten-containing foods to maximize the accuracy of the testing.

The Future of Celiac Disease

Is CD really an autoimmune disease or an immunologically based reaction to an exogenous protein? It contains features of both, but which-

ever it is, the damage is the result of "friendly fire." Recent advances have brought us to the threshold of unraveling the mechanisms of this common disorder. As our understanding of the mechanisms that lead to the injury of CD advances, so too will opportunities for blocking or modifying that response to gluten. It may be possible to prevent gluten sensitization or to reestablish gluten tolerance in the intestine in diagnosed CD. Potent immunosuppression may have some effect on CD but the risks of that cannot match the safety of the diet.

The diagnosis will be achieved, in many cases, with serology alone, obviating the need for biopsy in most subjects and bringing CD into the realm of the primary care doctor. Increased rates of detection, and hence, greater experience on the part of the diagnosing physician and treating dietitian will lead to a greater awareness of the disease and improve dietary management. Understanding the interaction between the environment and the immune system in CD may well provide insights into other diseases that also have an environmental origin or trigger.

Accurate and complete listing of ingredients in processed foods would make it easier for those affected to obtain a safe and interesting diet in our heavily processed food culture. Is there an alternative to gluten avoidance? Will it be possible to reduce the antigenicity of wheat for celiacs? It would be readily achievable if a single immunodominant motif was present that was not crucial to the qualities that make wheat so desirable as an ingredient for baking. The focus for nondietary treatment will be on modulating the immune responses to gliadin in the intestine. However, more likely to be successful are strategies to allow the development of immune tolerance or anergy to the antigen(s). Strategies focused on producing tolerance or desensitization to the immunodominant peptides of wheat, or on blocking gluten's interaction with the mucosa and innate immune system, have been, or will be, tested in animals (39, 164, 174).

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Exercise- and Pressure-Induced Syndromes

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The association of physical urticaria syndromes with the ingestion of food or other substances has been described in the literature. The relationship is controversial, although the body of evidence that supports its existence has grown over the last three decades. The concept implies that two or more subthreshold stimuli that cannot cause allergic mediator release when encountered individually can do so when combined in a temporal relationship. Such a combination of factors could include foods toward which a patient has developed specific antibodies and a physical stimulus such as exercise or pressure. The clinical manifestation of the cellular reaction is a syndrome ranging in severity from itching and hives to anaphylactic shock. Although the mechanism appears logical, it has neither been proved nor does it strictly comply with the classic concept of allergic sensitivity—that is, exposure to a single antigen, such as a specific food to which the individual has previously been sensitized, directly induces a clinical allergic reaction. The two physical urticaria syndromes in which the ingestion of food represents an important but "subthreshold" precipitating factor are exercise-induced anaphylaxis (EIA) and delayed pressure urticaria (DPU).

Exercise-Induced Anaphylaxis and Urticaria

The clinical syndrome of EIA and urticaria has been described in several comprehensive reviews (1–7). It is characterized by the onset of cutaneous erythema, itching, and urticaria during or shortly after exercise. Vascular collapse and/or upper airway obstruction can follow. Other symptoms may include angioedema, choking, headaches, nausea, and wheezing. Attacks may be precipitated by various types of exercise. The association of this syndrome with an underlying atopic history is not consistent, although atopy is clearly more common in this syndrome than in the general population (1). In some studies of these patients, the reactions have been reproduced in the laboratory (8). Elevated histamine levels are associated with the development of symptoms (2–4, 6) and elevated tryptase during an acute episode has been reported (9). Skin biopsies performed on patients with developing symptoms during a challenge confirm mast cell (MC) degranulation (10). The skin response to the nonspecific MC degranulator compound 48/80 increased in some patients with food-dependent exercise-induced anaphylaxis (FDEIA) after food ingestion and exercise, whereas challenges with food or exercise alone failed to reveal this increased responsiveness (11).

Maulitz and associates described the first case of EIA associated with food ingestion (12). This first case involved a runner who developed anaphylactic reactions when he ran within 8–12 hours of the ingestion of shellfish. The patient ran regularly, averaging 50–130 km per week. Over a 3-year period prior to diagnosis of the condition, the patient sustained approximately 10 bouts of transient facial flushing and edema, with diffuse urticaria and pruritis occurring during or immediately after exercise. Two of the reactions resulted

in almost complete upper airway obstruction requiring emergency therapy with epinephrine and antihistamines. The patient had no initial suspicion of allergic sensitivity to, or clinical reactions following, shellfish ingestion, but eventually the association was made. During further evaluation, he had positive immediate reactions with epicutaneous skin testing to clams, oysters, shrimp, and crab (all at a weight:volume ratio of 1:20), peanuts, trees, grasses, and weeds. Once the diagnosis was made and causative foods identified, avoidance of ingestion of these foods for at least 12 hours prior to exercise resulted in almost complete elimination of any further reactions.

It should be noted that the level of exertion required to cause an FDEIA reaction has not been determined and it varies from patient to patient. EIA is most commonly associated with aerobic exercise such as jogging, tennis, and cycling (13); however, even less intense physical activities such as yard work and shoveling snow have also been implicated (14). Biedermann described a case in which mild activity (ironing) after a meal containing pork induced edema, shortness of breath, and hypotension in a woman skin test-positive to pork (15).

Kidd and colleagues reported a series of four cases of EIA temporally related to the ingestion of food (16). The reactions occurred during or immediately after exercise and included urticaria, abdominal cramps, wheezes, dyspnea, angioedema, and pruritis. All of the attacks began with generalized tingling, itching, and warmth. In three patients, celery was implicated as the offending food. In the other patient, the reaction was induced by *any* food ingested within 2 hours before exercise. In another patient, the reaction was precipitated by the ingestion of food *after* exercise. Of the three patients whose reactions were precipitated by specific foods, all had positive skin tests to celery as well as other antigens. In the patient who developed reactions after eating any food prior to exercise, food skin tests were negative. It is interesting to note that celery is a member of the dill family (along with carrots). Two of the three patients with reactions to celery had skin reactivity to dill and carrot but did not exhibit clinical sensitivity after eating these foods. This suggests that celery may possess a unique antigen that predisposes to anaphylactic sensitivity. The authors emphasized that care must be taken to ensure that the food antigen used for skin tests is potent to avoid false negative results. Fresh celery used as a "puddle test" may be necessary to detect sensitivity.

Novey and colleagues (17) also reported a patient who developed anaphylaxis during exercise after eating. As with the patient described in the previous study, no specific foods were implicated. These cases fall into a subset of FDEIA recently termed "non-specific" FDEIA. Subsequent articles describing EIA in other patients have again implicated celery (18) and shellfish (19, 20), wheat (20–22), lentils (23), peaches (24), apples (25), grapes (26), eggs (20), hazelnuts (27), a cheese sandwich (8), and pork and beef (15).

Hanakawa and colleagues (28) reported a case of FDEIA in which the systemic allergic reaction depended on the amount of allergen ingested. A 24-year-old woman with EIA associated with wheat ingestion had positive radioallergosorbent test (RAST) class 2 for wheat and gluten, positive RAST (class 3) for rye, and positive skin prick tests (SPTs) for wheat, bread, gluten, and udon (Japanese wheat noodles). Challenge tests with bread were performed. Exercise following ingestion of 64 g, but not 45 g, of bread induced generalized urticaria. Challenge tests with udon also provoked urticaria in a dose-dependent manner: 200 g, but not 100 g or 150 g, elicited symptoms. They concluded that a negative challenge in patients suspected to have FDEIA may result from an insufficient amount of ingested food allergen (or subthreshold exercise). Another study with similar conclusions was performed by Aihara and associates (29). They reported a case of a 14-year-old boy with FDEIA diagnosed by provocation testing with the simultaneous ingestion of wheat and umeboshi (unripe plums pickled in brine and other ingredients, used in Japanese cooking), followed by exercise. Provocation tests with wheat or umeboshi alone failed to produce the transient increase in plasma histamine levels and drop in forced expiratory volume in 1 second (FEV_1) elicited by the combination of food allergens. Again, this could partly account for negative challenge tests in patients with a strong clinical history for FDEIA.

Kushimoto and Aoki (30) found that peptides from pepsin-digested gluten caused a positive SPT response more effectively than undigested gluten, whereas trypsin digestion of gluten resulted in a negative SPT, suggesting that ingestion of food allergens may result in increased allergenicity due to partial digestion.

Cross-reactivity of allergenic food components may also play an important role in FDEIA. Palosuo and associates (31) examined the sera of 23 adult patients with wheat-dependent EIA for cross-reactivity of wheat omega-5 gliadin with

other cereal proteins. They found that gamma-70 and gamma-35 secalins in rye and gamma-3 in barley cross-react with omega-5 gliadin. This suggests that rye and barley may also elicit symptoms in patients with wheat-specific EIA.

In a review of 167 cases of FDEIA in the Japanese literature since 1983, Harada and colleagues (32) described several characteristics of the disease, including a recent upward trend in the number of reports of FDEIA, male predominance, and teenagers accounting for more than half of the cases. Wheat was a more common cause than shrimp, although shrimp was most common in patients under the age of 20. Skin test and/or IgE RAST were positive in most cases, and 40% of cases revealed an atopic history. Patients undergoing provocation with aspirin plus food and/or exercise had a positive response. Seventeen cases were reported at recess or physical education class after lunch. These common trends again point out that FDEIA is linked to a type I allergic reaction. Aspirin may have a key role in FDEIA. Education about the natural course of FDEIA is crucial to its effective treatment.

Diagnosing an underlying food sensitivity in patients with EIA may prove difficult. The history, as noted in the initial case report (12), is the most important diagnostic tool. The best clue comes from the presence of intermittent or sporadic reactions superimposed on the baseline of consistent, uneventful exercise. Although a specific food is sometimes implicated as a coprecipitating factor (specific FDEIA), some patients suffer from EIA after any meal (nonspecific FDEIA) (33). Most cases of EIA, however, are labeled "idiopathic." A confounding factor may be the time lag between ingestion and reaction. This may extend to 12 hours, making the association with a specific food trigger extremely difficult. Skin testing is useful, because all cases of EIA with a specific food identified as a coprecipitating factor have SPTs to the implicated food. Routine skin test batteries miss some potential coprecipitating factors. Some patients in the "idiopathic" category may be reacting to unidentified ingestants, a combination of allergenic ingestants, or a cross-reacting ingestant. Romano and colleagues (33) stress the importance of both in vivo and in vitro testing to an extensive panel of foods. They studied 54 patients with suspected FDEIA. After detailed histories, the patients were subjected to SPT to 26 commercial food allergens, prick-plus-prick SPTs (P + P) with 15 fresh foods, and RASTs for 31 food allergens. Forty-eight patients had suspected a particular food in association with attacks; six could not recall a certain food. Fifty-two subjects were positive to at least one food; two had no positive results at all. All suspect foods were positive and each of the three tests revealed varying degrees of sensitivity, with positive results not discovered by the other tests. A detailed history, skin testing and RAST, and provocation testing is the ideal diagnostic combination for this unique disease.

Some types of EIA may represent variations of cholinergic urticaria. Patients with cholinergic urticaria are typically not atopic and symptoms are usually not dramatic, although anaphylaxis has been documented (2, 4). The classic triggers include exposure to heat, exercise, anxiety, and hot showers. As described by Kaplan and associates (4), symptoms in cholinergic urticaria with anaphylaxis occur following exercise to a certain work level. In some cases, protocols using regular graded exercise to carefully desensitize the patient, in conjunction with antihistamine therapy, have been useful.

In contrast to cholinergic urticaria with anaphylaxis, FDEIA symptoms cannot usually be predicted. It may be necessary to perform exercise challenge tests with and without ingestion of suspected food allergens to distinguish the two conditions. If a specific ingestant is identified, avoidance of exposure to this ingestant for at least 4 hours prior to exercise usually prevents an urticarial or anaphylactic reaction during or after exercise (33). In those patients who fall into the "idiopathic" category, however, management becomes more difficult. Exercise after a fast—especially first thing in the morning or on an empty stomach—is recommended. Antihistamines, taken either daily or half an hour before exercise may help blunt the attack. However, no data exist to suggest that this therapy can eliminate or prevent episodes. Zafirlukast, a leukotriene antagonist, has been shown to attenuate exercise-induced bronchoconstriction, but no studies have looked at this class of agents in FDEIA so far (34). Patients with a history of life-threatening reactions should avoid exercising alone and should carry self-injectable epinephrine (i.e., Epi-Pen). Emergency treatment at the time of the reaction may be lifesaving, although no confirmed deaths have been attributed to EIA.

Delayed Pressure Urticaria

DPU is another syndrome in which the association has been made between the ingestion of food and the development or exacerbation of a

physically-induced urticarial reaction (35–42). This unusual disease, which is more prevalent than previously appreciated, is characterized by the delayed onset of deep cutaneous swellings in areas exposed to prolonged pressure of variable intensity. The onset of the lesions can occur within 30 minutes to 9 hours of application of pressure from a variety of stimuli (e.g., for the feet, prolonged walking on a hard surface; for the shoulder, carrying luggage; and for the hands, hammering nails). The lesions usually peak 6–9 hours after pressure and may last as long as 36 hours. There may be a refractory period for development of new lesions in locations with recent urticaria (43). In 30%–90% of patients, DPU is associated with chronic "idiopathic" urticaria (38, 40, 42). Some patients develop "flu-like" symptoms (malaise, arthralgias, and fever) in association with the skin lesions. Almost half of DPU patients have an elevated erythrocyte sedimentation rate (ESR) and mild leukocytosis with or without eosinophilia. The systemic symptoms have been interpreted by some observers as evidence of proinflammatory cytokine release, including interleukin (IL)-1 (44). Usually the delayed pressure symptoms and reactivity parallel the activity of the chronic urticaria. The disease can persist up to 30 years (38). The condition often creates a significant functional disability, especially in individuals whose occupations require heavy physical labor, such as carpenters, construction workers, and auto mechanics.

DPU is best diagnosed by a thorough history. Many patients have been incorrectly diagnosed as having refractory angioedema in association with their chronic urticaria. The diagnosis can be confirmed using several tests (37–40). The simplest and most reliable test utilizes 15-lb of weight split into two sandbags connected by a thin strap. This device is then suspended over the shoulder for a period of 15 minutes while the patient is walking (28). The shoulder is examined 4–8 hours after challenge for the development of a deep, often painful, erythematous swelling. In most cases the test is positive initially. In patients with a good history and a negative initial test, a follow-up test at least 48 hours later may be positive. The test can be negative when the disease is quiescent or in remission.

The pathogenesis of DPU remains unclear. It was thought initially to be a manifestation of an Arthus phenomenon, but biopsies failed to reveal either immunoglobulins or complement in the vessel walls (37). Lesions can be induced by injection of compound 48/80 into the skin, suggesting that release of MC mediators may be important in lesion induction (45). Increased histamine levels have been shown in skin blisters above the lesions (40). Biopsy specimens reveal mild mononuclear perivascular infiltrates with some eosinophils and a small number of polymorphonuclear leukocytes (46). Fibrin deposition and edema among the collagen fibers at the pressure challenge site suggest a similarity between the lesions of DPU and those generated by the cutaneous late-phase reaction seen after allergen injection (47). Barlow (48) proposes a lower threshold in DPU patients to form wheals compared to control subjects. Hermes and colleagues noted endothelial cell up-regulation of tumor necrosis factor (TNF)-α and IL-3 in non-lesional skin of patients with DPU, and up-regulation of TNF-α production in perivascular cells (49). They suggest a role for these cytokines in the pathogenesis of DPU by an induction of subthreshold inflammation in endothelial cells of uninvolved skin. Kallikrein generation (40), leukotriene production (40), and cytokine release in lesions (44) have been hypothesized but, to date, not confirmed.

The pressure-induced lesions of most patients with DPU respond poorly to standard drugs used in the treatment of chronic urticaria and angioedema. The delayed pressure component often does not respond to conventional H1 antihistamines alone or to a combination of H1 and H2 antihistamines (37–39, 42). The urticarial lesions usually do respond to this therapy, hence the incorrect diagnosis of "refractory" angioedema. Some patients (38) respond partially to nonsteroidal anti-inflammatory agents (NSAIDs), but this has not been consistently seen in studies (42). Other patients have responded to cetirazine (50, 51). Sulfasalazine, at doses used in inflammatory bowel disease (IBD), was found to be an effective steroid-sparing agent for the angioedema in two patients with refractory DPU (52). No follow-up studies with larger patient populations have yet been conducted. Leukotriene antagonists have been shown effective in chronic "idiopathic" urticaria but are poorly studied in DPU (53–55). Berkun and colleagues (56) reported the first case of a patient with steroid-dependent DPU responding to montelukast, a leukotriene antagonist. The patient responded to 10 mg of montelukast daily within 1 week of its initiation; steroids were tapered and discontinued and there was no recurrence (pressure challenge tests were negative) for 20 months. Withdrawal of montelukast resulted in recurrence of urticaria within 3 days.

The only medications that consistently relieve the delayed pressure symptoms are corticosteroids (37–39, 42). Some patients require relatively high doses of these agents and prolonged therapy to remain functional and able to work.

The future of therapy for DPU is unclear. Agents such as antimalarials (57), hydroxychloroquine (58), cyclosporine (59), dapsone (60, 63), intravenous immunoglobulins (61), methotrexate (62), pentoxifylline (63), and stanozolol (64) have been used with some success in chronic idiopathic urticaria but have yet to be studied in DPU. Among the most promising of the immunomodulating medicines effective in skin are the new macrolide immunomodulators, including tacrolimus and pimecrolimus. Perhaps these will have a role in the treatment of DPU.

Specific causal factors for DPU are rarely identified; however, Davis and colleagues (65) identified specific food ingestion as an exacerbating factor. In this report, six selected patients with challenge-proved DPU were studied, all of whom required daily prednisone for symptomatic control. The patients either fasted, receiving only water, or were given a diet of unflavored Vivonex for a minimum of 48 hours. In five of the six patients, both spontaneous urticarial lesions and pressure-induced symptoms cleared after 24–48 hours of fasting. A control group of patients with chronic urticaria was treated in the same way, but none responded to the fast.

The mechanism by which food induces or exacerbates DPU remains unclear. On cutaneous testing to suspected foods, some patients developed late cutaneous reactions (after 6 hours) following a positive immediate wheal and flare, whereas other subjects experienced only a late induration 4–6 hours later. None of the patients, however, had IgE antibodies to foods demonstrable by RAST. Elimination diets excluding the offending food resulted in not only improvement of chronic urticaria but also loss of the positive delayed pressure response to provocative testing. Those patients who responded to the dietary elimination of the offending foods were eventually either withdrawn from corticosteroid or required significantly lower doses for control of symptoms. It is unclear from this article what percentage of patients with DPU has a food sensitivity as an underlying aggravating factor or when the evaluation for this condition should be initiated.

A subsequent report described two patients with DPU in whom lesions could be elicited when they had eaten normal food but not when they had been on at least 5 days of an elimination diet (66). Skin testing was not described, however. In studies conducted by Czarnetski and colleagues (39, 67), patients with positive cutaneous responses to foods failed to respond to elimination diets. In one report, the group presented 13 patients with DPU (67). All of the subjects received SPTs to a large battery of common allergens, including food extracts. Seven of these patients had positive early cutaneous reactions (15 minutes), and six developed positive late cutaneous reactions (after 6 hours). Two patients had only early cutaneous reactions, and four experienced only late cutaneous reactions to food allergens. None of the patients, however, showed any improvement on diets that eliminated those food antigens to which they developed a late cutaneous reaction. It is not clear whether any of the patients fasted for any prolonged period of time to exclude other allergens that were not part of the skin test battery.

Although a role for food ingestion in the causation of DPU has been suggested, it has not yet been well documented or proved. Nevertheless, given the high morbidity of DPU in some patients, including the potential requirement for long-term systemic corticosteroid therapy, it is a worthwhile effort to exclude ingestants as aggravating factors in almost any patient with significant DPU.

Pathophysiology

Clinical syndromes relating physical urticarias such as DPU and EIA to food ingestion are described in the literature. However, there is a paucity of well-controlled studies confirming food ingestion as a causal factor in these syndromes. The pathophysiologic mechanisms underlying the role of food ingestion in these diseases is not yet well defined. Thus, the existence of these syndromes remains controversial.

One could infer from the nature of these syndromes that they are caused by two or more subthreshold stimuli that individually are inadequate to produce mediator release from MCs or basophils, yet when combined in a temporal relationship can produce MC or basophil degranulation. Zuberbier and colleagues (68) describe the combination of two incomplete stimuli in a 43-year-old woman with angioedema resulting from a combination of nonspecific food intake and elevation of body temperature. Neither the food intake nor passive or active elevation of body temperature elicited angioedema. Once these incomplete stim-

uli were combined temporally, an allergic reaction ensued.

The clinical precedent creates a compelling argument. In a review article, Wong and colleagues (69) described several studies in which patients developed dermographism when treated with certain medications. They also described patients with exercise-induced and cold-induced dermographism. The cutaneous passive transfer of the dermographic response with serum—in some cases IgE—has also been described (70–72). Further studies by Moore-Robinson and Warin (73) noted a worsening of cold urticaria after exercise. Doeglas (74) noted a high incidence of aspirin sensitivity in patients with both chronic urticaria and physical urticarias, including cholinergic and pressure-induced urticaria. In a later paper, however, Moore-Robinson and Warin (75) could not implicate aspirin as a potentiating factor to physical challenges in their patients with dermographism, cold urticaria, and cholinergic urticaria. Murdoch and associates (76) demonstrated that certain dyes (azo and non-azo) and other additives, including butylated hydroxyanisole (BHT), sodium benzoate, and aspirin at pharmacologic levels, caused histamine release from leukocytes of both normal subjects and patients with urticaria, and they increased the spontaneous release from leukocytes of patients with urticaria. These studies suggest that certain factors may be able to cause mediator release and may also lower thresholds for the release of mediators from MCs or basophils without directly causing them to degranulate.

Clinically, the major task is to determine factors that alter MC releasability and, specifically, those that are present in each individual patient. Many endogenous peptides can release histamine from MCs both in vivo and in vitro (77). These peptides include substance P (78), vasoactive intestinal peptide (VIP) (79), calcitonin gene-related peptide (79), gastrin (80), pentagastrin (80), and endorphins (77). Release of these peptides occurs via various stimuli, including digestion, anxiety, pain, exercise, and local irritation. Any of these factors could be involved in physical urticarias or other allergic syndromes, including chronic urticaria, asthma, allergic rhinitis, and anaphylaxis. A study by Wallengren and colleagues (50) highlighted this mechanism by demonstrating increased levels of certain peptides in skin blisters from patients with urticaria and dermographia as compared to normal subjects. Three studies in patients with EIA and urticaria have demonstrated a decreased cutaneous MC releasability threshold during a reaction (11, 81, 82). Two of these investigations (11, 81) used compound 48/80; the other (82) used codeine (an MC degranulator used as a positive control in skin tests). EIA patients exhibited greater skin reactivity to these agents post-exercise. Control patients' skin test reactivity did not change (82).

The proposed mechanisms of food sensitivities in EIA and urticaria, although not proved, appear plausible. The pathophysiology of DPU related to food sensitivity, however, is much less clear. Histopathologically, the lesions of DPU resemble the late cutaneous IgE response (47) and are reproducible in affected patients by injection of compound 48/80 (45). The factors that alter MC releasability and predispose to a late-phase type of cutaneous reaction in patients with DPU have not been definitively identified (83). In the study described above relating food sensitivity to the clinical syndrome of DPU, specific IgE to the implicated food could not be demonstrated by RAST (65). Furthermore, in some patients the development of a late cutaneous reaction to certain foods was not preceded by an immediate wheal and flare reaction as was seen in the IgE-mediated late-phase cutaneous reactions. A later study noted similar skin test phenomena in these patients (67), but elimination of the foods that induced positive skin tests did not ameliorate the clinical syndrome. Thus, although some patients appear to have DPU from food sensitivity, IgE antibodies to foods are not demonstrable. Other factors, such as IgG4, may play important roles but have not yet been studied.

Conclusion

Increasing evidence suggests that in some physical urticarial syndromes—specifically, EIA and urticaria and DPU—food sensitivity or allergy and the subsequent ingestion of a specific food can either induce or exacerbate the clinical disease. Although this relationship remains controversial, the hypothesis is quite provocative. It suggests that the commonly accepted mechanism of direct mediator release from MCs and basophils that is induced by the binding of a food antigen to specific IgE on their surfaces is not the only form of food allergy. Other mechanisms may be more important, such as IgE antigen-antibody complex formation, interleukin generation, and/or low-affinity IgG receptor binding. These may serve to alter the threshold of mediator releasability of the MC and

are, most likely, insufficiently dense to independently alter the cell membrane enough to initiate the cascade of mediator release. The density may be adequate, however, to permit MC degranulation by the addition of other factors, especially physical stimuli or endogenous substances such as peptides or hormones, which would individually be inadequate to cause this release. This mechanism may explain some of the well-known variability and irreproducibility of clinical reactions and challenge testing to foods seen in some patients with credible histories of FDEIA or DPU.

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21

Occupational Reactions to Food Allergens

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US census data from the year 2000 showed that, of 135 million employed civilian individuals, about 6 million work in some aspect of the food preparation and service industry. In addition, up to 4 million Americans are employed in the farming sector (Table 21–1) (1). These workers can be exposed to a wide variety of substances that may lead to hypersensitivity diseases. Most sensitizing materials are food-derived protein allergens such as flour and shellfish. Nonfood agents may also induce allergic or immunologic diseases, e.g., honey bees, grain storage mites, antibiotics, thermophilic actinomycetes, and even rubber boots. It is well established that these materials can affect the skin, gastrointestinal (GI) tract, and respiratory system. In occupational exposure to food allergens/antigens, the routes of exposure are primarily through inhalation and contact, and they vary depending on agents and industries. The ensuing diseases include occupational rhinitis (OR), conjunctivitis, asthma, hypersensitivity pneumonitis (HP, or extrinsic allergic alveolitis), and occupational dermatitis.

Making a diagnosis of one of these occupational diseases can have significant social and economic impact on both the individual and the society as a whole, and should not be taken lightly. Diagnosing an occupational disease requires confirmation of the causal relationship between exposure at work and disease; although most cases are of new-onset diseases, this is not exclusive, e.g., the history of previous asthma does not exclude occupational asthma (OA). In the case of occupational dermatitis, the skin inflammation should improve while away from the workplace. In occupational lung diseases, unfortunately, symptoms may be slow to resolve or persist long after removal from the workplace. Each of these types of reactions will be discussed in greater detail below. Several examples of each of the aforementioned diseases in occupational settings have been chosen to illustrate important points.

Definitions

The diagnosis of OA involves demonstrating asthma that can be attributed to work. Criteria have been established by numerous groups for the purposes of epidemiology and clinical evaluation. For example, the American College of Chest Physicians established criteria that include a compatible history, reversible airflow limitation, airway hyperresponsiveness in the absence of airflow limitation, and objective demonstration of work-relatedness (2).

A similar definition, from Bernstein et al (3), is variable air-flow limitation and airway hyperresponsiveness due to causes and conditions that are attributable to a particular occupational environment and not to stimuli encountered outside the workplace. Asthma occurring at the workplace

Table 21–1.
Employed Civilians by Occupation: Year 2000—Census (For civilian noninstitutional population age 16 years and older.)

Occupation	*2000 Total Employed (×1000)*
Food preparation and service occupations	6327
Bartenders	365
Waiters and waitresses	1440
Cooks	2076
Food counter, fountain, and related occupations	357
Kitchen workers, food preparation	317
Waiters' and waitresses' assistants	670
Farming and fishing	3399
Farm operators and managers	1125
Other agricultural and related occupations	2115
Farm workers alone	768
Fishers, hunters, and trappers	51

Source: (1).

is not necessarily OA, and it is important, for medico-legal reasons, to draw this distinction. Asthma can be exacerbated at work by exercise or by exposure to irritants such as cold air, dust, or fumes in excessive quantity.

Reactive airways dysfunction syndrome (RADS), or irritant-induced asthma, is an occupational lung disease that occurs after acute high-level exposure to irritant gas, smoke, fumes, or vapors (4, 5). Unlike OA, which results from a previous sensitization to a substance, there is no latent period in RADS. RADS will not be discussed further in the context of food-induced occupational reactions, although it may be seen in this industry due to accidental exposure, such as ammonia spills from refrigeration systems.

OR is defined as episodic, work-related sneezing, nasal discharge, pruritis, and congestion, which contribute to distress, discomfort, and work inefficiency (6). OR is 2–3 times more frequent than OA, although it often coexists with OA. Rhinitis symptoms frequently precede the development of asthma in the work environment (7). In laboratory animal allergy, OR preceded the development of OA in 45% of subjects and occurred at the same time in 55%, but never developed subsequently (8).

HP, or extrinisic allergic alveolitis, is an immunologically mediated inflammatory disease involving the terminal airways of the lung associated with intense or repeated exposure to various inhaled allergens. The result of this exposure is initially a lymphocytic alveolitis followed by granuloma formation and eventually irreversible pulmonary fibrosis in the untreated patient (9, 10).

Traditionally, the term "contact dermatitis" has been used to describe any rash resulting from a substance touching the skin. Cutaneous manifestations of occupational exposure are generally divided into irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD) or a combination of ICD and ACD. ICD is diagnosed on the basis of history and clinical appearance. It is a non-immunologic form of dermatitis that, like RADS in the airways, does not require previous sensitization. In contrast, ACD is an immunologically mediated disorder that represents a form of type IV delayed hypersensitivity, and thus occurs following an acquired sensitivity to a given substance.

Occupational contact urticaria is an important occupational skin disorder among food industry workers, particularly cooks, bakers, caterers, and food handlers. Morphologically, it presents as an erythematous, papular, pruritic rash like that seen in classic hives; however, in this case it is associated with a specific occupational exposure. The mechanism is usually an IgE-mediated process.

Prevalence and Incidence

Generally, of all the occupational lung diseases in an industrialized nation, OA is the most common. The overall frequency of OA, according to numerous sources, has remained stable in the last 10 years although the causative agents may vary in frequency (11).

Determining prevalence or incidence of occupational diseases with any certainty is difficult, particularly in the food industry. Both employees and physicians tend to underreport health problems, and epidemiologic data on agriculture workers and food handlers remain scanty. However, as the importance of occupational lung disease has become more recognized, national databases have been established to monitor this data, including the SWORD and SHIELD in the United Kingdom, PROPULSE in Canada, and SENSOR in the US.

According to the CDC's National Center for Health Statistics in 1998, 10.6 million people (39 per 1000) had experienced an asthma attack or episode in the previous 12 months in the US and 5438 (0.05%) of these people died. The exact prevalence of OA is unknown, but epidemiological studies suggest that 6.7%–9% of all cases of adult asthma are attributable to occupational exposure (12, 13).

In those food-related industries in which the prevalence of OA is known, rates do not signifi-

cantly differ from those found in nonfood industries. For example, OA occurs in 3% (14) to 10% (15) of workers exposed to green coffee beans, 15% of snow crab processing workers (16), and 10%–30% of bakers (17–19).

OR occurs three times more frequently than asthma in the occupational setting. Its prevalence in subjects with OA is 76%–92% (7, 16). The prevalence of OR has been reported to be between 3% and 60%, depending on the exposure environment. In health care workers exposed to latex gloves, sensitization has been reported as high as 20%, with OR occurring in 9%–12% (20, 21). In seafood processing workers, the prevalence of OR ranges from 5%–22% (22), and nasal symptoms were reported by 24% of fish-food factory workers (23).

The incidence of HP is more difficult to determine because of the disease's generally low occurrence, problems with differential diagnosis, and the lack of prospective epidemiologic studies. Incidence also depends on exposure levels of the offending antigen and varies widely in different industries or even in areas of the same plant. For example, in one study it was estimated that the form of HP called farmer's lung affects less than 1%–6% of farmers (24). However, in a survey among 1054 farmers who grind moldy hay, the prevalence of farmer's lung was reported at 8.3%–11.4% (25). Farmer's lung on dairy farms in Wisconsin has been calculated to be 4.2 per 1000 farmers (26). Other studies have noted incidence rates for farmer's lung of 2.5–153.1 per 1000 farmers. In a survey of 200 pigeon breeders, 5% of breeders had findings consistent with bird-fancier's lung (27).

Most epidemiologic studies of dermatologic reactions in food-industry workers have included only subjects already diagnosed with occupational skin disease. Consequently, although types of skin reactions can be distinguished and many of the important etiologic agents can be identified, the true prevalence of disease remains difficult to determine. In a study of 1052 workers in the Finnish food industry, 17% were identified as having a skin disease (28). In that study, 8.5% of 541 female workers had occupational dermatitis, most commonly caused by fish, meat and vegetables. Of the 196 workers handling food, hand dermatitis was present in 15%. In a 5-year retrospective study, 3662 consecutive patients, including 180 food handlers, were patch-tested (29). In 91 (50.5%) of 180 subjects, dermatitis resulted from an occupational exposure, of which 25 (13.8%) of 180 were from exposure to meats or vegetables. Patch tests were positive in 59 (32.7%) of 180 patients. Hjorth and Roed-Petersen (30) evaluated 33 cases of occupational dermatitis occurring in restaurant kitchen workers. Metals, onions, and garlic were implicated most frequently in contact dermatitis; fish and shellfish were the major agents responsible for provoking contact urticaria. The same food allergens were also identified as the most important in a study of caterers (31). Table 21–2 lists allergens or irritants that may cause reactions in food handlers.

Using questionnaires, Smith estimated the mean annual incidence of skin conditions in the food manufacturing industry to be 2103 per 1 million employees per year, and 1414 per 1 million employees per year in the retail/catering industry (32).

Other data on occupational dermatitis comes from the EPIDERM, a voluntary surveillance system for occupational skin disease in the UK, and OPRA (Occupational Physicians Reporting Activity) surveillance plan, which have been collecting data on occupational skin diseases in the United Kingdom since 1993. The physicians that provided data for these studies report an annual incidence of occupational contact dermatitis of 12.9 per 100,000 (33).

Risk Factors

Both industrial and individual factors are associated with an increased risk of developing occupational hypersensitivity. The best studies have

Table 21–2.
Examples of Substances That Act as Irritants and/or Allergens in Causing Contact Dermatitis in Food Preparation Workers

Irritants	*Irritants or Allergens*	
Vegetables and fruit juices (contact urticaria)	Basil	Mugwort
Raw fish	Bay leaf	Mustard
Raw meats (benzylpenicilloyl polylysine)	Capers	Nutmeg, mace
Garlic	Caraway	Oregano
Onion	Cardamom	Paprika
Leeks, chives, shallots	Cayenne, chili pepper	Parsley
Spices	Cinnamon	Parsnip
Moisture	Clove	Pepper
Sugar	Coriander	Rosemary
Flour	Curry	Sage
Heat	Dill	Sesame
Soaps and detergents	Fennel	Star anise
Scouring pads	Ginger	Tarragon
	Laurus Nobilis	Thyme
	Lovage	Turmeric
	Mint, peppermint	

Adapted from (110).

been done in OA and rhinitis. Physicochemical properties of occupational agents; dose, duration, and route of exposure; allergenic potency; and industrial hygiene and engineering practices influence the potential of occupational agents to induce allergic disease. The level of exposure in different settings is clearly a major determinant for many occupational agents (19, 34–36).

Because only a small proportion of exposed workers develop occupational reactions, host factors clearly play an important role in disease development. These factors may include atopy, genetic predisposition, cigarette smoking, and possibly preexisting increased nonspecific bronchial responsiveness (NSBR).

Atopy

Atopic individuals have a personal or family history of hay fever, asthma, or atopic dermatitis (AD) and exhibit a greater tendency to develop sensitivity to environmental agents than do nonatopic subjects. Atopic individuals frequently show elevated total IgE levels. Nevertheless, history alone is not sufficient for the diagnosis of atopy, because identical symptoms can arise from allergic and non-allergic mechanisms. Skin prick testing (SPT) or radioallergosorbent tests (RASTs) are often used along with suggestive history to establish a diagnosis.

Although OA is frequently associated with increased production of specific IgE antibodies, atopy per se is not always associated with an increased incidence of OA. In general, the association between atopy and OA is found consistently in OA caused by high molecular weight (HMW) agents. However, the association is not high and other factors are equally likely to be important in the ultimate development of disease, such as the degree of exposure and concentration of the suspected agent.

Atopy appears to be an important factor in some occupational exposures, such as papain (37), flour (17), and green coffee beans (14); data on bakers remain controversial (38–40).

In some instances where the incidence of OA might be expected to be influenced by a worker's atopic status, such as in snow crab processing workers (16) and grain handlers (41), no relationship between atopy and development of disease has been discerned, although sensitization, as assessed by skin test, was related to atopy.

Although an association between sensitization to HMW agents and atopy has been observed in many food-related work environments, atopy and the development of OR have not been linked. Unlike OA, there is no higher incidence of HP disease in atopic subjects.

The role of atopy has not been clearly defined in the pathophysiology of occupational dermatoses. AD, in particular, may predispose workers to develop ICD; however, it does not appear to predispose to ACD. In a prospective follow-up study evaluating hand dermatitis in bakers, confectioners, and bakery shop assistants in order to determine risk factors, Bauer et al (42) noted that mild to moderate irritant contact dermatitis was the most frequent finding. Atopic individuals had a 3.9-fold relative risk of developing hand dermatitis. Total serum IgE was quantified but did not correlate with disease.

Genetics

Almost no information has been gathered on HLA type and its relationship to the development of OA, OR, HP, or occupational dermatitis, particularly those resulting from exposure to allergens in the food industry. With the results of the Human Genome Project, and interest in discovering the potential genetic basis of disease, it is anticipated that more data on OA will become available.

As with OA no specific genetic basis has been clearly identified for HP. The nature of the antigen, quantity of antigen inhaled, frequency of exposure, and host susceptibility are important. Camarena et al (43) looked at polymorphisms of the major histocompatibility complex (MHC) class II alleles in 44 patients with pigeon breeders' disease (a form of HP). An increase of one HLA-DRB1 allele and one HLA-DQB1 allele was noted when MHC typing was performed by polymerase chain reaction (PCR)-specific sequence oligonucleotide analysis; however, no specific association was found between the alleles in question and the development of HP.

Very little data exist on the genetic basis of occupational dermatitis. However, Holst studied ICD in monozygotic and dizygotic twins and found a high degree of concordance among monozygotic twins (44).

Smoking

The role of cigarette smoke, including exposure to secondhand smoke, in development, exacerbation, or pathogenesis of OA is not clear. Exposure to cigarette smoke increases bronchial

epithelial permeability (45), which could increase access of inhaled antigens to immunocompetent cells and evoke an immune response. Smoking may also inhibit cellular function so as to impair development of HP (46–48). A potential relationship between asthma, cigarette smoke, dust, aerosol, or vapor exposure appears intriguing, but epidemiologic studies in this area are limited. Smoking seems to be a risk factor for developing OA in several cases such as crab processing workers (16), workers exposed to green coffee beans or castor beans and grain dust.

Smokers exposed occupationally to green coffee bean or castor bean dust appear to be at higher risk for the development of occupationally induced allergies than similarly exposed nonsmokers (49). Furthermore, a significantly higher proportion of smokers appear among "sensitized" than "nonsensitized" coffee factory workers, and sensitization appears to progress more rapidly in smokers (50). Pulmonary effects of smoking and grain dust exposure are additive (51). These findings underscore the importance of imposing controls for smoking during data analysis.

HP is uncommon in smokers, unlike other pulmonary diseases, of which smoking increases frequency. Several studies have shown an underrepresentation of smokers among patients with HP. The strength of the association varies by study. Additionally, socioeconomic status may be a confounding factor. Nevertheless, once HP has started, smoking does not appear to be protective (52).

Bronchial Responsiveness

OA, at least in a worker still exposed and symptomatic, is usually associated with increased NSBR, as demonstrated by histamine or methacholine inhalation challenges. There is no evidence that increased NSBR is a risk factor for the development of OA (53).

Agents Associated With Allergic Occupational Diseases of Food Workers

Hundreds of agents are known to cause occupational rhinoconjunctivitis and OA. Most of these substances are chemicals, pharmaceuticals, wood dusts, and metals (54, 55); in addition, more than 50 agents encountered in food or food-related industries are known to induce OR and OA. In fact, the food industry accounts for the largest number of cases of OR (56). In some industries, such as coffee factories, OA is a well-recognized problem; in other types of work-places, only individual case reports have been reported. Agents encountered in food industries that are known to cause OR and OA are listed in Table 21–3. It is not possible to discuss in detail each agent, or even each group of agents, but specific examples will be given where appropriate. A wider-ranging list of airway sensitizing agents can be found in a review by van Kampen (57). Additionally, Siracusa et al (58) have a comprehensive list of agents specifically for OR.

Organic dust derived from bacteria, fungi, protozoa, plant and animal products, and simple chemicals can induce HP. A list of agents encountered in food industries that are known to induce HP are given in Table 21–4. Many of these materials are of fungal origin. Coffee dust has been omitted from this list because the single case of "coffee worker's lung" (59) was subsequently described as cryptogenic fibrosing alveolitis associated with rheumatoid arthritis (60).

A wide variety of foods, additives and flavorings, as well as materials used in food preparation, are known to induce several types of occupational skin disease. Table 21–5 lists etiologic agents, along with diagnoses. Some materials, such as seafood and garlic, commonly induce dermatitis, whereas others, including nonfood items such as betadine, are seldom reported to cause occupational skin disease.

Relationship of Sensitization Routes: Inhalation at the Workplace Versus Ingestion at Home

The relationship between sensitization by inhalation and symptoms following inhalation or ingestion of the same or a related antigen is intriguing. Exposure to food allergens typically occurs only via ingestion. Subjects that are sensitized to traditional food allergens by inhalation provide an opportunity to compare elements of the two exposure routes. Most food-related occupational allergens have not been shown to induce symptoms following ingestion by workers sensitized by inhalation. In some individuals, certain allergens can elicit symptoms following inhalation and ingestion: a spice factory worker who developed asthma following inhalation of garlic dust noted the immediate onset of wheezing after eat-

Table 21–3.
Materials Used in Food or Food-Related Industries That Are Known to Induce Occupational Asthma or Rhinitis

Agents	*Occupational Exposure*	*References*
Animal Products		
Sea Animals		
Prawn, crab, king crab, snow crab, lobster, oyster, clams	Seafood processing	16, 22, 142–149
Shrimp meal	Aquaculture	150
Fish meal, fish flour	Factory workers	151, 152
Mother of pearl	Button factory workers	153
Sea squirt	Oyster shuckers	154, 155
Seashells	Shell grinders	156
Trout	Processing workers	157
Farm Products		
Cows	Dairy farmers	158–160
Milk	Factory worker	161
Hogs, swine food	Hog farmers	162–164
Poultry	Poultry workers	165
Pheasants, quail, doves	Breeders	66
Eggs, egg lysozyme	Egg processor, bakery workers	166–171
Insects		
Poultry mites (*Ornithonyssus sylviarum*)	Poultry workers	172, 173
Grain storage mites (*Glycyphagus destructor*)	Grain workers	174–177
Honey bees	Beekeepers, honey processors	178–180
Bee-moth	Fish-bait breekers	181
Rice flower beetle	Rice flower workers	182
Enzymes		
Pepsin, trypsin, pancreatic enzymes	Pharmaceutical workers	183–186
Miscellaneous		
Spiramycin	Chick breeders	187
Pyrolysis products of polyvinyl chloride or label adhesives	Meat wrappers	188–194
Plants/Fungi		
Grains/flours		
Coffee	Coffee factory workers	172
Flour (wheat, rye)	Bakers, millers	195–197
Buckwheat, carob bean flour	Food workers	198–200
Rice	Rice millers	201
Soybeans, soybean lecithin	Agricultural workers	202, 203
Grain dust	Grain handlers	204–207
Spices/herbs		
Garlic	Factory workers, farmers	61, 208–210
Coriander, mace, ginger, paprika	Factory workers	211, 212
Cinnamon	Spice workers	213
Paprika plants	Greenhouse workers	214
Aromatic herbs	Butcher	215
Vegetables		
Green beans	Homemaker	216
Okra	Homemaker	217
Enzymes		
Fungal amylase, xylanase	Bakers	218–220
Bromelain, Papain	Factory workers	63, 99, 221–225
Miscellaneous		
Castor beans	Factory workers, dock workers	226
Tea, herbal tea	Tea factory workers, tea garden workers	227–231
Pollens	Sugarbeet workers	232
	Sunflower workers	65
	Grape growers	233
Pectin	Candy or jam makers	234, 235
Alkaline hydrolysis derivative of gluten	Bakers	236
Alternaria/Aspergillus spp.	Bakers	237
Colophony	Poultry venders	238
Hops	Brewery chemists	239
Devil's tongue (*Amorphophallus konjac*)	Food workers	240
Mushrooms	Soup manufacturers	241
	Growers	242
Verticillium albo-atrum	Greenhouse workers	243

ing garlic-containing foods (61). A provocative challenge with garlic aerosol immediately reduced the forced expiratory volume in 1 second (FEV_1) by 35%. An oral challenge with 1600 mg of garlic (in capsules) caused apprehension, flushing, and nausea within 10 minutes. Diarrhea, increased pulse rate, and a 21% reduction in FEV_1 occurred within 2 hours. In contrast to the immediate response to inhalation challenge and natural ingestion of garlic-containing foods, maximal symptoms were noted 2 hours after laboratory challenge, suggesting that inhalation of garlic vapors or absorption through the oral mucosa was necessary to produce an immediate response. Buckwheat (62), pineapple protease (63), snow crab (16), and honey/pollen (64, 65) have also been shown to produce allergic reactions following inhalation and ingestion by sensitive subjects.

Some individuals sensitized by inhalation to one occupational agent report symptoms following ingestion of a related antigen. A bird breeder developed OA following exposure to birds concomitant with an exquisite GI sensitivity to ingested chicken eggs. Her primary sensitization involved bird serum antigens, which cross-reacted with ingested egg yolk proteins (66). Butcher and colleagues (67) described an individual who developed and lost sensitivity to toluene diisocyanate vapor and ingested radishes, which contain isothiocyanates.

Table 21–4.
Etiology of Hypersensitivity Pneumonitis Occurring in Food and Food-Related Industries

Agent	*Source/exposure*	*Disorders*	*References*
Thermophilic Actinomycetes			
Faenia rectivirgula	Moldy hay	Farmers' lung	244, 245
Micropolyspora faeni	Moldy compost	Mushroom workers' lung	89
Thermoactinomyces sacchari	Moldy sugar cane	Bagassosis	246
T. vulgaris	Moldy compost	Mushroom workers' lung	88
	Moldy hay	Farmer's lung	247
T. viridis	Vineyards	Vineyard sprayers' lung	248
Fungi			
Aspergillus clavatus	Moldy barley/malt	Malt workers' lung	249–251
A. clavatus	Moldy cheese	Cheese workers' lung	252
A. flavus	Moldy corn	Farmers' lung	253
A. fumigatus	Vegetable compost		254
A. oryzae	Soy sauce brewer		255
Cladosporium	Moldy hay	Farmers' lung	247
Mucor stolonifer	Moldy paprika pods	Paprika slicers' disease	256
Penicillium sp.	Moldy hay	Farmers' lung	247
P. caseii, P. roqueforti	Cheese	Cheese workers' lung	257–259
Botrytis cinerea	Moldy grapes	Wine growers' lung	260
Insects			
Grain weevil (*Sitophilus grainarius*)	Infested wheat	Millers' lung	261, 262
Cheese mites (*Acarus siro*)	Cheese	Cheese workers' lung	263
Animal Products			
Duck proteins	Feathers	Duck fever	264
Chicken proteins	Chicken products	Feather pluckers' disease	265, 266
	Hen litter		267
Turkey proteins	Turkey products	Turkey handlers' disease	268
Goose proteins	Feathers		264
Bird proteins	Fishermen		269
Fish meal	Fishmeal workers		270
Miscellaneous			
Mushrooms	Spores	Mushroom workers' disease	271
Erwina herbicoa (*Enterobacter agglomerans*)	Contaminated grain	Grain workers' lung	272
Tea plants		Tea growers' lung	273
Oyster shells	Oyster shell dust		274

Pathophysiology of Occupational Allergies

Occupational Rhinitis

OR has been classified by Bardana (68) as annoyance, irritational, corrosive, or immunologic. Annoyance, irritational, and corrosive rhinitis have no immunologic or allergic basis. Immunologic or allergic rhinitis is usually IgE-mediated, although the exact mechanism is unknown for most low molecular weight (LMW) agents. Annoyance reactions occur from exposure to mild workplace irritants. These reactions are triggered by exposure to perfumes, air fresheners, and cooking odors. Irritational rhinitis is caused by inhalation of high concentrations of airborne chemicals. This reaction is often associated with a burning sensation. The proposed mechanism for this reaction is release of substance P and neuropeptides from nasal sensory nerves. Vasodilation and neurogenic inflammation results from substance P (56, 68, 69). Corrosive rhinitis occurs after exposure to high concentrations of chemical gases such as ammonia, chlorine, and organophosphides. Signs of systemic intoxication may also be present.

Immunologic or allergic OR can result from HMW or LMW allergens. HMW allergens are more sensitizing, especially in atopic workers, and they compose the majority of allergens in the food industry. Examples include flour, soybean dust, vegetable gums, and animal proteins. Guar gum, used as a thickener and gelling agent, is a common cause of OR in the food industry.

Occupational Asthma

The characteristic bronchial reaction observed in OA may result from pharmacologic, or type I, IgE-mediated mechanisms (70). Complex organic mixtures, such as grain dust, have numerous biological actions, which may or may not be pathogenic. Other agents induce OA by an as-yet-undefined mechanism.

Table 21–5.
Dermatitis in Food Processing and Food Service Workers

Industry	*Exposure*	*Diagnosis*	*References*
Agriculture			
Milk controllers, milk recorders, milkers	Bronopol, Kathon CG	Dermatitis	275
Milk testers	Chrome, dichromate		276, 277
Milk analyzers	Dichromate	Allergic contact dermatitis	278
Ewe milker		Dermatitis	279
Celery harvesters	Celery fungus (*Sclerotinia sclerotiorum*)	Phototoxic dermatitis	280, 281
Apple packers	Apples sprayed with ethoxyquin	Allergic contact dermatitis	282
Orange pickers	Omite-CR	Dermatitis	283
Grocery workers	Celery furanocoumarins		284, 285
Food Preparation			
Fish factory workers	Fish, mustard	Dermatitis, contact urticaria	286
Cooks	Mustard, rape	Dermatitis	287
Cooks	Garlic/onions	Dermatitis	288
Cooks	Paprika, curry	Contact dermatitis	289
Salad makers	Mustard	Dermatitis	290
Food workers	Cashew nuts (cardol)	Dermatitis	291
Sandwich makers	Codfish, plaice, chicken, onion, garlic	Dermatitis	30
Food workers	Lettuce	Dermatitis	292
Food workers	Lettuce, chickory, endive	Contact dermatitis	293
Bakers	Sodium metabisulfite	Contact dermatitis	293
	Persulfate	Contact dermatitis	294
	Cinnamon	Dermatitis	295
	Sorbic acid	Dermatitis	296
	Propyl gallate	Allergic contact dermatitis, dermatitis	297
	Dodecyl gallate	Dermatitis	298
	Chromium	Dermatitis	299
	Flour mite	Dermatitis	300
	Sugar mite	Dermatitis	301
	Karaya gum	Dermatitis	302
	Flour	Contact urticaria	300
Butchers/Poultry Processors			
Butchers	Rubber boots	Allergic contact dermatitis	303
Butchers	Knife handle	Dermatitis	304, 305
Butchers	Povidone-iodine	Allergic contact dermatitis	306
Slaughtermen	Blood (cow and pig), gut casings	Contact urticaria, eczema	307, 308
Butchers	Calf's liver, pig's gut, beef	Urticaria	309, 310, 311
Poultry workers	Various	Irritant allergic dermatitis, eczema	312
Chicken vaccinators	Antibiotics	Contact dermatitis	313
Seafood			
Fishmarket workers	Shrimp	Allergic contact urticaria	314
Caterers	Shrimp	Contact urticaria	315
Seafood processors	Prawns	Dermatitis	143
Crabs processors	Crabs	Urticaria, dermatitis	316
Oyster shuckers	Oysters	Dermatitis	317
Mussel processors	Mussels	Dermatitis	318
Food handlers	Fish and shellfish	Contact dermatitis	30
Food handlers	Cuttlefish	Contact dermatitis	319
Fishermen	Fish	Dermatitis	320
Fish workers	Fish	Contact urticaria	321
Cooks	Fish	Contact urticaria	322
Fishermen	Fish	Dermatoses	323
Caterers	Fish	Dermatitis	31
Trawlermen	Bryozoa	Dermatitis, eczema	324, 325
Fishermen	Rubber boots	Dermatitis	326
Fishnet repairers	Fishnets	Dermatitis	327
Miscellaneous			
Snackbar-meat producers	Penicillin residues	Dermatitis	328
Spice workers	Turmeric, cinnamon, cinnamic aldehyde	Allergic contact dermatitis	329, 330
Margarine manufacturers, workers	Octyl gallate	Eczema, dermatitis	326, 327
Peanut butter manufacturers	Octyl gallate	Dermatitis	331
Food workers	Sesame oil	Contact sensitivity	332
Food workers	Artichokes	Eczema	333
Confectioners	Cardamom	Allergic contact dermatitis	334
Cookie workers	"Thin mint" cookies	Eczema	335
Beekeepers	Propolis	Dermatitis	336, 337
Beekeepers	Beeswax, poplar resin	Dermatitis	338
Coconut climber	Coconut trees/coconuts	Dermatitis	339
Bartender	Citrus peel, geraniol citral	Allergic contact dermatitis	340

Pharmacologic Mechanism

The classic example of acute asthma caused by a pharmacologic mechanism occurs in farm workers exposed to organophosphate insecticides (71, 72). These chemicals irreversibly inactivate cholinesterase, which causes an accumulation of acetylcholine, with subsequent bronchospasm.

Immune Mechanisms

Many agents encountered in the workplace are antigenic or allergenic and elicit type I, IgE-mediated reactions in sensitized individuals. As with other agents inducing IgE-mediated OA, only a small proportion of exposed workers develop disease. A latent period, ranging from several weeks to years, precedes development of symptoms (73).

A common classification system is to divide the agents into HMW and LMW agents. In general, HMW agents act through an immune mechanism. Certain LMW agents cause production of IgE antibodies, whereas others act as haptens that must be conjugated to a carrier protein to be allergenic. LMW allergens cause disease through largely unknown mechanisms, although non-IgE-mediated and cell-dependent immunologic mechanisms appear to be important.

Asthma in Seafood Workers

The seafood industry is an example of a sector of the food industry that has continued to grow to meet world demands, and consequently has experienced greater exposures and corresponding disease. In 1999, the world's production of fish, crustaceans, and mollusks reached 126.2 million tons. Of this amount, 92.9 million tons was derived from capture fisheries and 33.3 million tons was from aquaculture. Seafood is one of the most highly traded commodities in the world market (74). The value of seafood production for 1999 was estimated to be US$125 billion. With the increase in production and consumption of seafood have come more allergic reactions in the occupational setting (75). The reported prevalence of OA due to seafood alone varies from 7% to 36% (76).

De Besche (77) published the first report of occupational allergy from seafood in a 1937 paper describing a fisherman with asthma, angioedema, and conjunctivitis. Since De Besche's time, seafood processing plants have become more technologically advanced with varying processing procedures. Crab, fish, mussel, and prawn processing cause an aerosolized protein exposure to which workers can become sensitized by inhalation (78). Table 21–6 lists possible exposures in the seafood industry.

Sensitization by inhalation frequently makes the respiratory tract a primary route of occupational exposure during seafood processing (79). Occupational exposure to crab has been extensively studied in the context of a range of allergic diseases, including asthma. A 1982 National Institute for Occupational Safety and Health (NIOSH) investigation concluded that during the crab processing season in Alaska, the monthly incidence of

Table 21–6.
Common Processing Techniques for Seafood Groups and Sources of Potential High-Risk Exposure to Seafood Products

Seafood Category	*Processing Techniques*	*Potential Sources of Exposure*
Crustaceans		
Crabs, lobsters, crawfish	Cooking (boiling or steaming), "tailing" lobsters, "cracking," butchering and degilling crabs, manual picking of meat, cutting, grinding, mincing, scrubbing and washing, cooling, crab leg blowing	Inhalation of wet aerosols from lobster "tailing," crab "cracking," butchering and degilling, boiling, scrubbing and washing, spraying, cutting, grinding, mincing, crab leg blowing, cleaning processing lines/tanks with pressurized water
Prawns	Heading, peeling, deveining, prawn "blowing" (water jets or compressed air)	Inhalation of wet aerosols from prawn "blowing"; dermal contact from unprotected handling of prawns, hand immersion in water containing extruded gut material
Mollusks		
Oysters, mussels, clams, scallops, abalone	Washing, oyster "shucking," shellfish depuration, chopping, dicing, slicing	Inhalation of wet aerosols from oyster "shucking," washing; dermal contact from unprotected handling of mollusks
Finfish		
Various species	Heading, degutting, skinning mincing, filleting, trimming, cooking (boiling or steaming), spice/batter application, frying, milling, bagging	Inhalation of wet aerosols from fish heading, degutting, boiling; inhalation of dry aerosols from fishmeal bagging; dermal contact from unprotected handling

Modified from (82).

new cases of asthma was 80 times that reported for the general population, controlling for age (80).

A 1998 survey was conducted with symptom questionnaires, spirometry, and serologic testing on 107 workers in a crab processing facility (81). In this study the incidence of asthma-like symptoms was 26%. The prevalence of asthma-like symptoms was noted to be 14% early in the crab season and 32% late in the season. At the end of the season, 4% met the criteria for an obstructive pattern by spirometry. Only 9% of the workers with asthma-like symptoms had elevated IgE antibody to crab. Small study group size and short duration of follow-up may have limited the study, but the observations are interesting nonetheless.

Although several studies have been conducted, further investigation into occupational exposure to seafood agents is necessary to better understand the health effects for seafood workers. These investigations should include characterization of aerosolized protein antigen, dose-response relations, exposure routes, temporal component to exposure, extent of antigen cross-reactivity, and host factors (82).

Hypersensitivity Pneumonitis

Occupations at risk of developing disease that are most commonly cited in the literature include farming, sugar cane harvesting, and mushroom packing. Mushroom worker's lung (MWL) is a good example of this disease.

Pathogenesis

The complete relationship between the immunologic response and environmental factors that lead to the development of HP have not been fully elucidated. One of the questions that remains unanswered is why only certain subjects in a group of similarly exposed subjects go on to develop HP. The mechanisms in HP appear to involve a Gell-Coombs type III and type IV hypersensitivity reaction. Many patients who develop HP report a recent viral respiratory infection; this may represent the disease itself or an exacerbating factor that leads to the development of disease.

The early neutrophilia that is seen in bronchoalveolar lavage (BAL) fluid occurs at 24–48 hours; this is then followed by lymphocytosis at 48–72 hours. Activated macrophages and multinucleated giant cells are also seen. Eventually, with ongoing exposure, non-caseating granulomas form, and finally fibrosis develops. A positive correlation between the percentage of lung neutrophils and the percentage of lung fibrosis has been demonstrated. The contribution of gram-negative endotoxin to the neutrophilic infiltrate is interesting because endotoxin can often be found in the same environment that supports the allergens in HP. Further studies are needed to elucidate the significance of endotoxin (83).

Proinflammatory cytokines such as macrophage inflammatory protein-1 (MIP-1) α and interleukin (IL)-8 are elevated in HP subjects during acute disease compared to controls. MIP-1α is a chemotactic factor for monocytes and macrophages as well as for T lymphocytes. Additionally, it has a role in Th0-to-Th1 differentiation. IL-8 is a chemotactic factor for T lymphocytes and neutrophils. Once subjects were treated, either with allergen avoidance or with medical therapy, decreased levels of both cytokines were observed in the HP subjects. Addition of anti-MIP-1α caused inhibition of CD8+ cytotoxic T lymphocyte (CTL) attraction (84–86).

At a cellular level, although bronchoalveolar MCs increase in HP, there has been no direct evidence of immediate hypersensitivity contributing to the development of disease. Further, IgE levels as well as eosinophil levels are normal in HP subjects.

Mushroom Worker's Lung

During the years 2000–2001, 264 growers in the US produced 853 million pounds of mushrooms valued at US$863 million (87). HP among mushroom workers was first reported in 1959 (88), and the term "mushroom worker's lung" (MWL) was coined in 1967 (89).

After an outbreak of seven cases of MWL between 1982 and 1985, a cross-sectional respiratory morbidity survey was conducted at the mushroom farm where the outbreak occurred. Other than the outbreak subjects, 20% of the more heavily exposed workers reported occasional symptoms consistent with MWL. No radiographic changes were noted; however, serologic tests showed that almost all workers, from different work areas on the farm, had been exposed to antigens that could potentially cause disease. Therefore, all workers on a mushroom farm should be educated about the signs and symptoms of MWL (90).

MWL is caused by a variety of antigens associated with cultivation of mushrooms—notably microorganisms and mushroom spores. The specific exposures depend on where an individual works in the operation, harvest conditions, and which mushroom species are involved. Most

cultivated mushrooms are grown in compost. During fermentation of compost, temperatures reach as high as 60°C (140°F), and growth of thermophilic organisms flourishes. Meanwhile, a growth medium is inoculated with mushroom spores; after growth begins, this material is transferred onto grain. The combination, called spawn, is mixed with fermented compost prior to seeding mushroom beds. High levels of thermophilic actinomycetes are liberated during the mixing process. Thermophilic organisms are the traditional source of MWL including *Thermomonospora sp., Streptomyces sp., Thermoactinomyces vulgaris,* and *Faenia rectivirgula* (91).

Mushroom spores themselves can also cause HP in sensitive individuals, and this is particularly true in more exotic mushrooms such as oyster and shiitake, which spore continuously and have become more popular in recent years. Most commercial mushrooms (*Agaricus sp.*) are harvested before sporulation; however, workers can be exposed to high spore levels if picking occurs after this stage. OA and occupational dermatitis have also been reported in mushroom growers (92, 93).

Occupational Dermatitis

Bakers can suffer from not only OA but a variety of skin diseases associated with occupational exposure to dough, flour, additives, and flavorings (Table 21–7). Most reactions are irritant rather than allergic, and result from continuous exposure to wet, sticky dough, sweetening agents, or flavorings. Irritant responses can be distinguished from allergic reactions by patch testing with putative agents. Flour itself can induce contact urticaria, and flour contaminants (e.g., mites) can induce occupational dermatoses in sensitive workers.

Table 21–7.
Additives Encountered by Bakers That Can Cause Skin Disease

Irritants	*Allergens*
Emulsifiers	Benzoyl peroxide
Acetic acid	Potassium bromate
Lactic acid	Cinnamon oil
Calcium acetate/sulfate	Limonen, oil of
Yeast	Balsam of Peru
Potassium iodide/bromate	p-Amino-azo-benzene
Potassium bicarbonate	Eugenol
Bleaching agents	Vanilla
Ascorbinic acid	Sorbic acid
	Karaya gum
	Ammonium persulfate
	Sodium metabisulfite

Diagnosis

History and Physical Examination

Individuals with suspected OA usually experience episodic dyspnea, chest tightness, cough and wheezing. Typically, symptoms are worse at work and improve over weekends or holidays. However, the relationship to work exposure may be masked by intermittent exposure, or by symptoms worsening at home in the evening or not improving over short periods such as weekends. Any questionnaire should include questions about not only the current job but also previous jobs. The history should identify whether symptoms began a short time after a job or workplace changed, if new materials or processes were introduced into the workplace, if agents with known asthma-inducing potential are used in the workplace, and if other workers exhibit similar symptoms. Usually, a latent period occurs between first exposure and development of symptoms; the length of this latency can range from weeks to more than 30 years (73). The occurrence of rhinitis, conjunctivitis, or skin rashes at work in a subject with asthma is surely suggestive of OA. As with all occupational diseases, a high index of suspicion is needed to make a diagnosis. However, a highly suggestive history of OA is not sufficient to confirm the diagnosis even in the hands of experts; the predictive value of a positive questionnaire is only 67%. Experts are generally better at excluding the diagnosis, with a negative predictive value (NPV) of 83% (94). Physical examination is nonspecific and does not confirm the diagnosis of asthma ("all that wheezes is not asthma") but may be helpful in excluding other conditions.

OR manifests as nasal congestion, itch, sneeze, and rhinorrhea with exposure to the work environment. Like other forms of occupational disease, symptoms typically improve with removal from the work environment. As in OA, the history of workplace exposure is extremely important. A medication history is equally important, because symptoms may be masked by certain medications. Symptoms initially felt to be related to the work environment may become prolonged or worsen after removal from the culprit environment with the overuse of certain medications. For example, rhinitis medicamentosa may develop as a result of chronic topical decongestant use for the treatment of OR. Physical findings in OR are nonspecific and similar to findings in rhinitis from nonoccupational causes.

The clinical presentation of HP is often classified as acute, subacute, or chronic. In the acute

presentation, flu-like symptoms including fever, chills, and cough often result in misdiagnosis as a bacterial or viral respiratory infection. The symptoms usually begin 4–12 hours after work exposure. The subacute form may have a more prolonged course of shortness of breath, weight loss, and fatigue. In chronic disease, the antigen exposure is not interrupted and the subject may go on to develop restrictive pulmonary disease that may not be reversible.

In the acute form of HP, physical examination reveals fine bilateral crackles. Occasionally, ronchi or wheezes can be detected, although asthma rarely constitutes a part of this disease syndrome. As with OA, history is important and disease must be temporally correlated with exposure.

In evaluating patients with occupational skin disease, physical examination is also important. The appearance helps to determine whether the dermatitis is endogenous (constitutional), contact, or a combination of the two. Secondary bacterial infections may also be involved, making morphology-based diagnosis more difficult. Distribution may suggest a probable cause. Approximately 90% of occupational dermatitis involves the hands, usually the backs and palmar surfaces of the wrists (95). When occupational disease is suspected, matching the location of the dermatitis and the exposure source becomes necessary. Actual or simulated workplace practices may aid in accomplishing this task.

In the differential diagnosis, contact dermatitis caused by non-occupational exposure and endogenous dermatitis need considering. Often occupational dermatitis is multifactorial, with irritants, allergens, endogenous factors, and secondary bacterial infection all causally involved. When taking the worker's history it is important to ask about other work aside from their primary occupation, as well as hobbies since they may have potential exposures at these sites. The worker should also be asked about treatments that have been attempted either by themselves or by medical personnel, because some of these treatments may be the actual cause of the problem or may exacerbate the current skin condition.

Laboratory Tests

Asthma/Rhinitis

When a subject with suspected OA is evaluated, the diagnosis of asthma needs to be objectively confirmed by demonstrating either reversible airways obstruction or increased NSBR, as assessed by methacholine or histamine inhalation challenge. Confirming the diagnosis of asthma does not, however, confirm the diagnosis of OA; the relationship between work exposure and asthma needs to be confirmed by other means, such as monitoring of peak expiratory flows (PEF) and NSBR at and off work or by specific inhalation challenges. However, the absence of increased NSBR in a subject who has been off work for some time (usually weeks, although a few days may be enough) does not exclude the diagnosis of OA; return to work or a specific challenge may then be associated with increased NSBR (96, 97). Alternatively, normal NSBR in a symptomatic worker still at work makes the diagnosis very unlikely (98).

Knowledge of the etiologic agent is important to understanding pathogenic mechanisms. Identification of the agent may ultimately lead to changes in the workplace environment and decreased incidence of disease. When the putative agent is antigenic, laboratory tests may help establish a diagnosis. Some of these tests can be performed at the workplace, but others must be conducted in a laboratory.

SPTs with common environmental antigens, including pollens, molds, and dusts, are used to identify atopic individuals. In addition, skin testing with specific occupational allergens may assist in establishing a diagnosis of OA or monitoring workplace populations. Positive skin tests are not themselves diagnostic of disease, rather they are indicative of exposure and sensitization. The lack of standardized skin test reagents makes it difficult to do skin testing with predictive reliability. Further, with most LMW agents skin test results are of little value. As with all skin testing, care must be exercised, particularly with allergens of extreme potency, such as bromelain and latex, which may induce systemic reactions (99).

Specific IgE levels can be assessed using RAST. Like SPTs, RASTs can be used to evaluate both individuals and populations. Although it is less sensitive than skin tests, RAST is more convenient for testing industrial populations. Serum can be collected during the worker's regular plant physical so that the employee does not have to be removed from the production line for testing, and a physician's presence is not required. RAST can also be used for retrospective studies as long as sera have been stored (100). RAST is also not diagnostic of disease but rather demonstrates potential sensitization.

As a noninvasive assessment of respiratory inflammation, sputum analysis has been proposed

in the diagnosis of OA. Lemière et al (101) have shown increased sputum eosinophils and sputum eosinophil cationic protein in subjects when at work compared with the periods out of work. Comparison of induced sputum in HMW and LMW agent-exposed workers showed that eosinophil percentages were higher in non-occupational asthmatics and asthmatics with HMW-induced asthma than in normal subjects and subjects with OA due to LMW agents (102). The clinical utility of these analyses remains to be determined.

As in OA, in making the diagnosis of OR, allergen-specific IgE should be measured if the test is available. The presence of allergen-specific IgE helps support the diagnosis of OR when the history is suggestive.

Hypersensitivity Pneumonitis

There are no pathopneumonic markers of disease in HP. As with all occupational diseases, a careful history focusing on work place exposures is essential along with the appropriate clinical symptoms. Reduction in symptoms when away from the workplace exposure is helpful in making a diagnosis. When the diagnosis is suspected and an inciting agent cannot be identified, a site visit may be needed.

Peripheral blood leukocytosis, with or without eosinophilia, also occurs (103). Chest X-rays (CXR) are usually consistent with a diffuse interstitial or alveolar filling process; occasionally findings suggest pulmonary edema in the acute phase. If episodes are infrequent, radiographs may be normal. Airspace consolidation, reticulonodular patterns, and interstitial fibrosis, which may be described as a honeycomb pattern, are seen in acute, subacute, and chronic disease respectively. A high resolution CT-scan is more sensitive than CXR or traditional CT for evaluation of parenchymal abnormalities and may show abnormalities when the CXR is normal. An example of the radiographic changes seen in HP is shown in Figure 21–1 (104).

Unlike the characteristic reversible obstructive pattern seen in asthma patients, HP subjects classically have a restrictive pattern. However, spirometry, like CXR, may be normal between attacks in HP prior to developing chronic disease. When changes are noted, they are typically restrictive defects with decreased lung volumes and diffusion capacity. Oxygen desaturation, particularly on exercise, may also be seen. Finally, a mixed obstructive/restrictive pattern is also frequently seen.

Precipitating antibodies against the offending antigen can be helpful in making the diagnosis, but studies have shown that between 3% and 50% of asymptomatic subjects may also have precipitans. False negatives may also occur because of problems with sera concentration, use of non-

Figure 21–1. High resolution CT scan in HP. A 39-year-old woman with HP presenting initially (A) with diffusely distributed centrilobular nodules and patchy ground-glass opacity on high resolution CT scan. Follow-up study at 6 years (B) showed progression of parenchymal changes, with honeycomb cysts, traction bronchiectasis, and bullae. Reproduced with permission from (104).

standardized commercial extracts, or because the test was done with the wrong antigen.

Immunoglobulins can be elevated, particularly IgG. IgM and IgA may also be elevated, but IgE is not usually elevated. Increases in erythrocyte sedimentation rate and C-reactive protein are secondary to the active inflammatory process. Skin testing for immediate hypersensitivity is of no value in diagnosing HP.

BAL shows variable cellular presentations. Classically, neutrophilia is seen within the first 48 hours of antigen challenge, and it is followed by lymphocytosis. The lymphocytosis may be of CD4+ or CD8+ T lymphocytes. The CD4 to CD8 ratio depends on the specific time course in the disease during which the BAL is performed. BAL CD4 to CD8 ratios have been variable among studies and among specific causative allergens of HP (105, 106). Aside from the type of allergen, the dose of the allergen and stage of disease may also affect the ratio.

When clinical history and laboratory data are not sufficient to make a diagnosis, a lung biopsy may be needed. Lung biopsy may be performed by transbronchial or thoracoscopic/open biopsy depending on the location and ability to obtain affected lung tissue. This also allows one to rule out infectious etiologies (107).

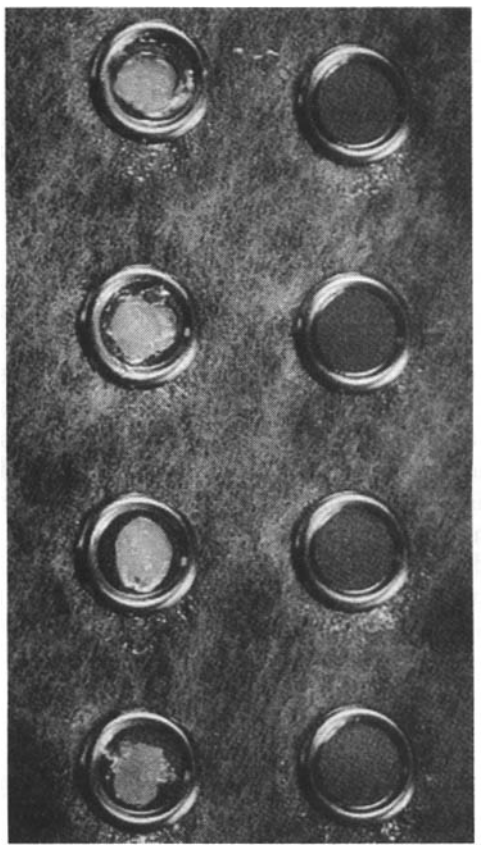

Figure 21–2. Finn Chamber.

Allergic Contact Dermatitis

In allergic contact dermatitis, the patch test which was first devised by Jadassohn in 1895 represents the only practical assay for diagnosis (108). A common commercially available patch test kit in the US is the TRUE Test (GlaxoSmith-Kline). However, in the case of food allergens, readymade patch testing is often not available. In these cases, one must prepare a personalized tray. If this is to be done, it is critical that the agents are prepared at concentrations that do not give an irritant effect (109–111).

The Finn chamber, shown in Figure 21–2, is an example of an apparatus used to perform patch testing with a variety of agents that the clinician could select and/or prepare. It is a common method of patch testing in which multiple 8-mm aluminum cups are filled with the test material and applied to the upper half of the back with an adhesive. The area that the chamber is to be applied to should be free of rash or large amounts of hair. The patch is affixed to the skin with tape. The patient is instructed not to shower during the period that the patch test is on. After 48–72 hours, the patch is removed and the underlying skin examined. The area should be examined on more than one occasion, including at 72 hours, 96 hours, and 1 week. Using only one reading can decrease accuracy and may cause difficulty in differentiating irritant from allergic responses. The interpretation of patch testing should be performed by individuals skilled in such procedures. As with all testing, false positives and false negatives are possible. False positives may occur because of use of irritant substances or because of a pressure reaction over the applied site. False negatives may be caused by material concentration, improper vehicles, or inappropriate reading times.

Monitoring Pulmonary Function

Pulmonary function testing is used in helping to make the diagnosis of occupational lung disease as well as for monitoring disease progression. To confirm work-aggravated asthma, monitoring of PEF has proven to be very useful with sensitivity

of 81%–100% and specificity of 74%–89%, compared to specific challenges (112–114).

Workers are asked to measure their PEF, and the best of 3 reproducible (± 20 L/min) attempts kept for analysis, ideally every 2 hours from awakening to bedtime, or at least 4 times per day and when symptomatic. PEF meters offer the advantages of being cheap, portable, and readily available. However, PEF measurements are effort-dependent, and compliance has been shown to be poor, especially when workers are seeking compensation (115, 116). Although PEFs are a less reliable way to assess change in airway caliber, monitoring of FEV_1 using portable devices has not proved more reliable (117). When monitoring of PEF is done, it is important to keep medication at a minimum, using short-acting β2-agonists on demand only and, if they are taken, keeping the dose of inhaled steroids or theophylline constant (118).

Monitoring of FEV_1 before and after work shifts is not adequately sensitive or specific (119). Monitoring of NSBR coupled with monitoring of PEF may be useful in certain cases, because NSBR may decrease upon return to work and improve when taken off work. Figure 21–3 illustrates monitoring of PEF and histamine PC20 (the provocative concentration of histamine inducing a 20% fall in FEV_1) in a snow-crab processing worker with OA. When there is discrepancy between monitoring of PEF and NSBR, specific inhalation challenges either in the laboratory or at work may allow better accuracy of the diagnosis. Although monitoring of PEF (and NSBR) can confirm the diagnosis of OA, it does not allow the identification of the offending agents.

Specific Challenge

Traditionally, challenges with food allergens are performed by ingestion. To simulate industrial exposures, however, inhalation challenges must be performed. They are indicated if previous in-

Figure 21–3. Monitoring of histamine bronchial responsiveness and PEF in a crab processing worker. The upper panel illustrates the variation in PEF before and upon return to work in a crab processing worker. Squares represent days at work. Upon return to work there is a large variation in PEF associated with symptoms requiring two puffs of albuterol taken as needed (closed diamonds). PEF continued to fluctuate following work withdrawal for a few days. The lower panel illustrates the change in PC20 (provocative concentration of histamine inducing a 20% fall in FEV_1), which decreased significantly upon return to work, while baseline FEV_1 had not changed significantly when the subject was seen in the clinic. Return to baseline took almost 1 year. Adapted with permission from (16).

vestigation with monitoring of PEF (and NSBR) was dubious or impossible, e.g., subject is unable to return to work, or identification of the offending agent is required. These tests can be done either in the laboratory or at work, although the latter is less well controlled. They are safe when performed by trained personnel under the close supervision of an expert physician, and they offer the advantage of rapid diagnosis. Challenge testing in this manner should only be performed in a controlled setting that has the resources to handle medical emergencies.

Specific inhalation challenges done in the laboratory are the gold standard for diagnosis of OA and identification of the etiologic agent (2, 120, 121). Nevertheless, false positive (especially in unstable asthma) and false negative reactions (due to loss of specific bronchial reactivity, using the wrong agent, or taking medication prior to the challenge) may still occur. Although these tests are not well standardized, guidelines have been developed and should be followed (2, 120, 121). Subjects should reduce or stop their medication according to standard recommendations, and the stability of the asthma should be assessed on a control day. The FEV_1 is the best index to monitor the bronchial response because PEF is less reliable, especially during the late asthmatic response (122, 123), and it should be monitored for at least 7–8 hours after the end of exposure. In certain cases, challenge at work with similar monitoring of FEV_1 may also confirm the diagnosis of OA, especially when the offending agent is unknown.

Nasal challenge is necessary to secure the diagnosis of OR, but is not widely used. Nasal challenges are time consuming and not standardized. Although many methods of objective assessment of the nasal physiologic response to challenge are available, most are cumbersome and impractical. Acoustic rhinometry uses a piezoelectric spark to generate a three-dimensional image of the nasal passages, allowing measurement of nasal volume and cross-sectional area. This measurement can be used rapidly and repeatedly in nasal challenges (124).

Respiratory response patterns seen in individuals with OA or HP resulting from exposure to food antigens do not differ from those observed in subjects with allergic lung disease due to exposure to common environmental or other occupational antigens (125). The most common types of asthmatic responses following exposure with HMW agents are immediate (65%), late, and dual (22%) (125). Figure 21–4 illustrates these responses in sensitized snow-crab processing workers. In the immediate response, a decline in FEV_1 occurs within minutes of exposure, reaches a peak within 20–30 minutes, and resolves within 1–2 hours. In late reactions, the FEV_1 decline starts 3–4 hours after exposure and is maximal between 6 and 10 hours. Dual responses are a combination of immediate and late responses. In some cases, a pattern of recurrent nocturnal asthma has been described with falls in FEV_1 occurring at approximately the same time on successive nights following a single

Figure 21–4. Specific inhalation challenges in crab processing workers. The top panel illustrates the change in FEV_1 in a worker presenting an immediate type of asthmatic reaction, after a 5-minute exposure in the work place. The middle panel illustrates a late asthmatic reaction occurring about 2 hours after the 125 minutes of exposure in the workplace, with full recovery at the end of the day post-albuterol (S). The lower panel illustrates a dual asthmatic response following the inhalation of crab boiling water in the laboratory. Adapted with permission from (16).

exposure (126); the latter is probably due to increased NSBR. Atypical patterns have also been described but are rarely seen with HMW agents.

Specific inhalation challenges have limited value in most HP patients, with the possible exception of some patients with acute disease. When these challenges are performed, baseline PFTs are conducted, then exposure is done progressively and in a closed environment, using either nebulized extracts of suspected antigens, or exposing the subject to the suspected agent in the same way as at work. The lack of standardized extracts creates difficulties in nebulizing a standard amount of extract for challenge testing (127). The subject's symptoms and PFTs are followed serially by looking for clinical (fever) and spirometric changes previously described for acute disease, which are more easily characterized than the symptoms in chronic disease. Monitoring of CBC is also useful. Aside from a controlled chamber challenge, another consideration in subjects with acute disease is a re-exposure challenge to the suspected workplace.

Prognosis

While OA was considered a self-limited disease, many studies have shown that this is not the case. Most studies have indeed shown that the majority of workers are still symptomatic or have abnormal pulmonary function after they left work (128, 129). No study has been performed strictly in workers in the food industry, except follow-up studies on individuals with OA who are employed as snow-crab workers (130), but it is likely that it is similar to other industries (131). In snow-crab workers taken off work, improvement of FEV_1 reaches a plateau after 1 year and improvement of NSBR seems to plateau at 2 years; similarly, there is a concurrent reduction in specific IgE antibodies, which does not seem to reach a plateau. The factors most important to duration of symptoms after work withdrawal are duration of exposure after onset of symptoms, total duration of exposure, degree of impairment in FEV_1, and degree of bronchial hyperresponsiveness at diagnosis (37, 130, 131). Although some patients may continue to suffer from OA after removal from the work environment, the best prognosis results from early diagnosis and early removal from the exposure environment (68).

In other types of OA, a similar pattern of improvement has been shown (128). Individuals who continue to work are thus at risk to develop irreversible disease, stressing the importance of early removal from exposure.

Although NSBR usually improves with work withdrawal, most workers exhibit persistent specific bronchial responsiveness if rechallenged with the agent responsible for their OA, even after several years off work (132).

The socioeconomic consequences of OA are not negligible (133) and vary among countries according to the compensation systems and retraining programs. This stresses the importance of proper diagnosis. In Quebec, where workers are no longer exposed to their offending agent once the diagnosis is made, about one third of subjects find an adequate job with the same employer and one third find a different job with another employer. Only 8% of subjects remain unemployed after 2 years of follow-up. Quality of life of subjects with OA in the same province is slightly, though significantly, less satisfactory than that of subjects with common asthma of comparable severity. In other countries, the situation is less favorable, the number of subjects still unemployed varying between 25% and 69% (134, 135). Many workers must stay in the same environment, which may worsen their asthma. Moscato et al (129) showed that subjects with OA who stayed at work needed more medication than those who ceased to be exposed.

The clinical prognosis for individuals with HP primarily depends on the amount of damage at the time of diagnosis and the ability of the individual to avoid contact with the etiologic agent, although this may not affect pulmonary function tests and CXRs (136, 137). When HP is diagnosed early and ongoing exposure with the antigen is avoided, the outcomes are generally good, and clinical, radiographic, and pulmonary function return to baseline. Most of the recovery should occur within several months. If the patient still has changes after 6 months away from the exposure, the changes are likely to be permanent. With delays in diagnosis and treatment, subjects may progress to the chronic form of the disease that may lead to irreversible changes as well. Patients may also go on to develop symptoms of asthma or emphysema. As for diagnosis, no pathognomonic prognostic markers exist for HP. Lung function may continue to decline despite removal from the inciting agent at the acute stage. In particular, there was continued decline in diffusion capacity for carbon monoxide and total lung capacity (TLC) over several years. If the subject does progress to

the chronic fibrosis stage, he or she may go on to respiratory failure and death, or right-sided heart failure.

The majority of individuals with contact dermatitis have an excellent prognosis, provided that exposure to the allergen is eliminated. If an employee cannot change jobs, dermatitis can become chronic. Chronic dermatitis can also occur in some subjects despite the apparent elimination of allergen exposure. This condition is particularly troublesome in industrial settings and may reflect complex exposures, mixed disease, or endogenous or irritant dermatitis.

Prevention and Treatment

The best "treatment" of allergic occupational disease is prevention. Reduction of exposure levels is the only way to reduce significantly the incidence of respiratory symptoms among workers, although some individuals may still be sensitized at very low levels. This reduction in exposure may be achieved by enclosing the responsible process, improving ventilation and personal protection devices, modifying the process by encapsulating the agent, etc. Although threshold limit values have been established to prevent exposure to irritant levels of many agents, limit values that can prevent sensitization are not known for most agents (36). However, once an individual has developed clinical evidence of OA, asthmatic responses will occur at minute exposure levels, usually less than any industrial plant can maintain.

Pre-employment screening and periodic health monitoring with education of workers about risk of disease and ways to reduce exposure have been suggested for prevention of allergic respiratory disease. Questions arise over which tests are appropriate. SPTs with specific allergens may be useful for monitoring, although positive responses do not necessarily correlate with disease and, as in atopy, do not predict adequately who will develop OA (138). Furthermore, human rights laws would not allow pre-screening to exclude subjects from being hired. However, monitoring of SPTs for specific allergens during work in high-risk industries may be useful, allowing reallocation of sensitized individuals to a low-exposure environment and reducing their risk of developing clinical diseases (139).

Once OA or HP has been diagnosed, the worker should be removed permanently from further exposure to the offending agent to prevent further deterioration and improve prognosis. Although OR and/or conjunctivitis may precede OA (7), there is no information on the level of risk of OA in workers with OR. In such subjects, removal of exposure will improve the symptoms, but simple treatment with H_1 antagonists or inhaled corticosteroids may be enough to control the symptoms and allow the worker to continue the job, preferably in a much lower exposure environment.

Furthermore, when cases of HP are discovered in an occupational environment, it is important to follow non-affected workers also, because they too may eventually develop symptoms or disease. For example, when HP caused by inhalation of mollusk shell dust was discovered among employees in a factory, evaluation of the health status of the other factory employees revealed functional declines in the subjects originally unaffected, despite attempts at improving the occupational environment (140).

Aside from removal of the inciting agent, the specific treatment of OA is the same as non-occupational asthma. In more severe cases of HP, systemic corticosteroids may be needed, although only with careful monitoring of X-rays, pulmonary function tests, and clinical symptoms. The subject should have slow tapering of the steroids after clinical improvement, as rapid tapering may cause relapse. Although steroids improve the acute symptoms, there is concern that steroid treated patients may be at higher risk of disease relapse (141).

As with respiratory disease, drug treatment of occupational dermatoses produce only temporary benefit unless the individual receives no further exposure. Specifically, workers with less than 10% skin involvement are treated with topical steroids and those with more extensive involvement may be treated with oral steroids. The steroids should be tapered, because abrupt termination can cause a flare of skin symptoms. Protective measures that reduce skin contact, such as appropriate clothing and gloves, may be used if avoidance is impossible. It should not be automatically assumed that such devices are impervious to all materials. Workers have better outcomes of their occupational dermatitis when they receive hands-on instruction in the measures needed to improve the dermatitis. However, if these measures do not improve or resolve the dermatitis, the worker should be withdrawn from exposure.

Conclusion

Exposure to a wide variety of food-derived and food-associated materials encountered in the

workplace is associated with development of OA, HP, rhinitis/conjunctivitis, and dermatitis in sensitized individuals. The number of causative agents will undoubtedly continue to rise as new agents are introduced into the workplace and as physician awareness of these conditions continues to grow. Little is known about the prevalence and incidence, importance of host factors, treatment, or prognosis of the occupational diseases resulting from exposure to these antigens. As the number of individuals employed in the food industry grows, the need for this type of information will increase significantly.

The examples described in this chapter are but a few of the wide array of food-associated occupational hypersensitivity reactions. New agents causing occupational allergies are being reported. With globalization of world markets and a continuing increase of individuals employed in the food industry, it is essential that the clinician keep abreast of new reactions when diagnosing a new or unusual occupational reaction. For example, genetically modified (GM) crops may contain novel proteins to which no prior human exposure has occurred. Although most efforts at food safety analysis are directed at ingestion of foods developed through biotechnology by consumers, it is possible that such novel proteins could cause occupationally related allergic reactions in food workers. Although this is unlikely because of the low expression levels of such proteins, the possibility must be considered whenever occupational reactions occur in industries that grow or process foods developed by biotechnology or that use ingredients that have been similarly altered.

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Part 3

Adverse Reactions to Food Additives

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22

Asthma and Food Additives

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Introduction

Food additives are substances added to food products for coloring, flavoring, nutrient, antimicrobial, and other purposes. Because additives are typically minor ingredients in food, the intake of additives by consumers is usually small. An estimated 23%–67% of asthmatics perceive that food additives exacerbate their asthma (1–3). The actual prevalence rate of food additive-induced asthma exacerbations reported by various double-blind, placebo-controlled (DBPC) studies is less than 5% (1, 4, 5). Because the current therapy for food-additive induced asthma is avoidance or elimination of inciting agents (6), a correct diagnosis is imperative to avoid unnecessary dietary restriction (1). Sulfites, monosodium glutamate (MSG), and tartrazine are three substances frequently implicated in food additive-induced asthma. They will be discussed in detail in this chapter.

Evaluating Asthma Studies

A variety of data are available implicating sulfites, MSG, and tartrazine in asthma exacerbations. Because many of the studies evaluating these substances are poorly designed, one must read the literature critically to determine whether a true link exists between a particular food additive and asthma.

Well-designed studies evaluate asthmatic subjects with stable lung function at baseline. If the subjects have wide variability in peak expiratory flow rate (PEFR) or forced expiratory volume in one second (FEV_1) at baseline, how would one determine whether the variability seen during the study is truly related to the challenge substance or merely a reflection of poor asthma control? Asthma medications that are allowed or disallowed during the study are key. If medications are to be discontinued, the timing of this in relation to the challenge must be carefully evaluated. For example, in studies of sulfite-induced asthma, antiasthma and antiallergy medications that can inhibit a response to sulfites must be withheld before a challenge. Typically, β_2-agonists are typically withheld the day of the challenge, and cromolyn sodium or antihistamines are withheld 24 hours prior to the challenge. Asthma controller medications, such as theophylline and inhaled or oral corticosteroids, may be continued because they do not interfere with a response to sulfites and in fact contribute to the stability of a patient's baseline lung function.

If rescue medication is allowed in a study, the timing of administration of the medication in relation to the challenge must be critiqued. For instance, if a rescue medication was given within 3 hours of a challenge, and a decline in lung function was seen 6 hours after challenge, the decline in lung function is more likely due to waning β_2-agonist effect than to bronchoconstrictive properties of a challenge substance. Consistent timing of challenges is important to exclude confounding effects due to physiologic diurnal variability in PEFR. To eliminate observer bias, challenges should be double-blinded and placebo-controlled.

The method of administration of challenge substance may influence study results. For example, some sulfite-sensitive asthmatics respond to oral capsule challenges, whereas others respond only to challenge with acidic sulfite solutions. The

route of administration chosen in diagnostic challenges should be tailored to a patient's history and presentation.

The reliability of the outcome measure used in a study is another key aspect in defining a study's quality. The flow-volume loop obtained with spirometry is precise and reproducible, while PEFR is more variable. Criteria used to define positive challenges should also be considered in evaluating a study's quality.

Duration of subject evaluation following a challenge is also important. Determining when reactions linked to the challenge substance are most likely to occur helps determine the length of time subjects should be observed. Reproducibility of results is another quality of a well-designed study.

The criteria outlined above are used in the remaining sections of this chapter to present and evaluate data regarding a potential link between asthma and the food additives sulfites, MSG, and tartrazine.

Sulfites

Sulfiting agents have been used in foods for hundreds, possibly thousands, of years (7). The ancient Romans and Egyptians may have been the first to use sulfites as food ingredients. To sanitize wine vessels, they burned sulfur to create sulfur dioxide (SO_2) (8, 9). The first recorded use of sulfites as a food preservative was in 1664 (7). By the 1880s, sulfites were widely used in the US. Sulfite salts came into use in the 1920s for the manufacture of wine and beer. Although sulfites are often added to foods, they also occur naturally in certain foods (e.g., mushrooms, Parmesan cheese).

Adding sulfites to foods serves many purposes. Sulfites act as antioxidants that inhibit enzymatic browning of fresh fruits, vegetables, shrimp, and raw potatoes (10). Non-enzymatic browning of dehydrated potatoes and other vegetables, dehydrated fruits, white grape juice, frozen lemon and lime juice, and some types of vinegar is also prevented by sulfites. The broad-spectrum antimicrobial action of sulfites is useful in fermentation processes such as wine making and corn wet milling, as well as for sanitizing food containers or fermentation equipment in the food and beverage industry (9, 10). Other uses of sulfites in foods include bleaching of maraschino cherries and hominy, and dough conditioning for frozen pie and pizza crusts (7). The antioxidant property of sulfites is also used in certain pharmaceutical agents, including medications used to treat allergic diseases and asthma (Table 22–1).

Common forms of sulfites used as food or drug additives include SO_2, inorganic sulfite salts, sodium or potassium metabisulfite ($Na_2S_2O_5$ or $K_2S_2O_5$), sodium or potassium bisulfite ($NaHSO_3$ or $KHSO_3$), and sodium or potassium sulfite ($Na_2S_2O_3$ or $K_2S_2O_3$). Sulfites can react with a variety of food constituents, including sugars, proteins, amino acids, lipids, starch, other complex carbohydrates, and vitamins (9). Dissociable forms of bound sulfite can serve as reservoirs of "free" sulfites. Irreversibly bound sulfites are removed permanently from the pool of free sulfites that may exist in foods (7–9).

The form of sulfite present in foods is affected by pH. For example, a low pH favors H_2SO_3, intermediate pH (4.0) favors HSO_3^-, and high pH favors SO_3^{2-} (8). At neutral pH, sulfites are the main forms of sulfiting agents. In solution, especially at an acid pH (saliva, gastric juice) and in the presence of heat, as in the stomach, sulfites are readily transformed into bisulfite and sulfurous acid (10). These substances may then be volatilized as SO_2, which has been implicated in causing bronchoconstriction (11).

The estimated prevalence of sulfite sensitivity in adult asthmatics is approximately 5% (12–14), with a higher prevalence in steroid-dependent asthmatics (15). Using a combination of capsule and neutral sulfite solution challenges, Bush and colleagues (15) (Table 22–2) evaluated the largest

Table 22–1.
Some Antiasthma Medications that May Contain Sulfites

Generic Name	*Preparation*	*Sulfite Contained*	*Amount of Sulfite*
Epinephrine	Solution	Sodium metabisulfite	0.1–1 mg/mL 1:1000 solution
Isoproterenol HCl	Intravenous preparation	Sodium metabisulfite	1.0 mg/mL 1:5000 solution
Dexamethasone	Injectable	Sodium metabisulfite	1 mg/mL
Prednisolone Sodium Phosphate	Intravenous preparation	Sodium metabisulfite	1 mg/mL

Table 22–2.
Studies Evaluating Sulfite-Induced Asthma

Reference	*Consistent time*	*H/O Sulfite-Induced Asthma*	*Status of Anti-asthma Meds*	*Type*	*Placebo Order*	*Dur Obs (hrs)*	*Outcome Measure*	*Dose Free Sulfite*	*Re-challenged*	*# Positive Response/ # Challenged*
31	Unknown	14-Yes	Withheld BD and Na Cr for 8 h prior; CS continued	O	No	0.1	$FEV_1 > 12\%$	100 ppm Soln	No	8/14
					NA					
29	Yes	4-Yes	All meds Continued (theo, adrenergic drugs; ICS)	S	Yes	0.5	FEV_1	1–50 mg capsule	1	4/4
					First					
30	Unknown	29	Unknown	S	Yes	12	PEFR > 20% confirm w/ FEV_1	Capsule 25–100 mg	No	0/29
					First					
								Solution 5–50 mg		19/29
15	Unknown	203 83-SD 120-NSD	Withheld SABA and Cr Na 8 h prior; antihist 12 h prior	S	Yes	0.5	FEV_1 >20%	1–200 mg capsule or solution	Yes	21/203 16/83 SD 5/120 NSD
					Random					
				D						4/12 3/7 SD 1/5 NSD
27	Yes	16-Yes	Withheld SABA 8 h prior;Cr Na, ICS, antichol for 12 h prior; LABA and short act antihist for 24 h; Theo for 3 d prior	D	Yes	1	$FEV_1 > 15\%$	1.9–300 ppm wine		3/16
					Random					
		10		S						2/10
28	Yes	24-Yes	Withheld SABA 8 h prior;Cr Na, ICS, antichol for 12 h prior; LABA and short act antihist for 24 h; Theo for 3 d prior	S	?	1	$FEV_1 > 15\%$	300 ppm wine	Yes	4/24
		4-Yes		D	Random	1		20–300 ppm wine		4/4 to 300 ppm
		12-Yes			Random	0.5		20–750 ppm wine		2/12

Abbreviations: antichol, anticholinergic; antihist, antihistamine; BD, bronchodilator; CS, corticosteroids; D, double-blind; FEV_1, forced expiratory volume in 1 minute; H/O, history of; ICS, inhaled corticosteroids; LABA, long-acting beta-agonist; Na Cr; sodium cromolyn; NSD, non-steroid dependent; O, open; obs, observation; PEFR, peak expiratory flow rate; S, single-blind; SABA, short-acting beta agonist; SD, steroid dependent; theo, theophylline.

cohort of asthmatics to date for sulfite-sensitive asthma. The subjects (n = 203) were separated on the basis of whether or not they required oral or inhaled steroids to control their asthma. The sulfite challenge was performed in two phases. Initially, a single-blind challenge was performed. If the single-blind challenge was positive (20% or greater decrease in FEV_1 from baseline), a double-blind challenge followed. In the single-blind challenge, 16 (19%) of 83 steroid-dependent asthmatics had a positive response, whereas only 5 (4.2%) of 120 non-steroid dependent asthmatics had a positive response. When these results were confirmed with double-blind challenges, 3 (43%) of 7 steroid-dependent asthmatics and 1 (20%) of 5 non-steroid dependent asthmatics had a positive response. Based on the double-blind challenge results, the estimated prevalence of sulfite sensitivity in their non-steroid dependent asthmatics is 0.8%. In the steroid-dependent asthmatics, the prevalence was much higher (8.4%). This study demonstrates that the prevalence of sulfite sensitivity in the asthmatic population as a whole is less than 3.9%, and that steroid-dependent asthmatics are at most risk (15).

The largest group of sulfite-sensitive asthmatics are individuals who respond to ingestion of acidic sulfite solutions (8). Among these patients, some react to acidic sulfite solution challenge and others do not, a phenomenon perhaps explained by variable inhalation of SO_2 (16).

The mechanism by which sulfites induce asthma symptoms has not yet been fully elucidated. Various hypotheses have been proposed to explain the induction of bronchoconstriction by sulfites: a cholinergic reflex mechanism, an IgE-mediated mechanism, or deficiency of sulfite oxidase. The cholinergic reflex mechanism suggests that inhaled SO_2 (16), such as might occur when swallowing an acidic sulfited beverage, acts on irritant receptors in the lung (17). Although the nature of these irritant receptors is poorly understood, the cholinergic reflex hypothesis is supported by the fact that an asthmatic response to sulfites in sensitive individuals can be blocked by the administration of anticholinergic drugs such as inhaled atropine, or doxepin, an antihistamine with anticholinergic properties (7, 9, 11, 18).

Another proposed mechanism for sulfite sensitivity in asthmatics is IgE mediated (7, 8, 11). This mechanism has not yet been proven (8), but it is supported by the presence of positive skin prick tests (SPTs) (19–22) to sulfite and by sulfite-sensitive anaphylaxis in certain individuals (7, 23, 24).

Sulfite oxidase deficiency has also been proposed as an explanation for sulfite sensitivity in asthmatics (9–11). Sulfite oxidase metabolizes sulfite (SO_3) to inactive sulfate (SO_4), and a decrease in sulfite oxidase activity has been seen in skin fibroblasts of sulfite-sensitive asthmatics compared with controls (10).

Although sulfite-induced asthma is typically triggered by the oral ingestion of a sulfited food, beverage, or drug, inhalation of SO_2 can also be a trigger. Several factors are important in determining the likelihood of an adverse reaction: the nature of the food, the level and form of residual sulfite in the substance, and sensitivity of the patient. Sulfite-sensitive asthmatics are most likely to respond to free sulfites. However, the degree of sensitivity these patients have to the various forms of reversibly and irreversibly bound forms of sulfites has yet to be elucidated.

The levels of sulfiting agents in foods are usually expressed as SO_2 equivalents (8) because sulfite salts can release SO_2 under some assay conditions. Two methods have been used to measure sulfites in foods: the Ripper method (25), which detects "free" SO_2 (undissociated H_2SO_3, HSO_3^-, SO_3^{2-}); and the Monier-Williams method (26), which measures "total" SO_2. Sulfites measured by the Monier-Williams method include the same substances detected by the Ripper method plus some combined forms of sulfites (8). Drawbacks of these methods include the following: 1) some non-sulfite substances may interfere with the analyses; 2) some hazardous combined forms of sulfites may not be measured under the assay conditions; and 3) some combined forms that are not hazardous may be detected by these assays (8).

In the US, total daily per capita intake of sulfites in foods is approximately 6 mg of SO_2. The threshold response to challenges with sulfites in sensitive asthmatics is typically between 12 mg and 30 mg SO_2 equivalents (20 mg and 50 mg potassium metabisulfite, respectively).

The levels of sulfites in foods vary (see Table 24–5), reflected in the fact that sulfited foods and drugs do not always induce an asthmatic response in sensitive individuals. Levels of sulfites in foods are typically expressed as parts per million (ppm); 1 part per million equals 1 μg/g. Frozen dough and jellies typically contain less than 10 ppm. Fresh shrimp, pickles, and fresh mushrooms contain up to 60 ppm. Dried potatoes, wine vinegar, and

maraschino cherries contain up to 100 ppm, while the highest levels (up to 1000 ppm) are contained in dried fruits and lemon, lime, grape, and sauerkraut juices (7). Food processing and preparation may decrease sulfite levels. Therefore, the amounts of sulfite used initially to treat foods will not necessarily reflect residue levels after processing, storage, and preparation (8). Food processing also differs in various countries, so caution must be used in interpreting reports from other countries that implicate sulfites in eliciting asthma symptoms.

The clinical syndrome incited in sulfite-sensitive asthmatics consists of bronchoconstriction. The consequences of sulfite-induced asthma can be quite severe; several deaths have been attributed to sulfite exposure in sensitive individuals (9). Sulfite-induced asthma is complex and may involve several co-dependent mechanisms. Studies discussed in this chapter that evaluate sulfite-induced asthma are summarized in Table 22–2. In a DBPC trial, Vally and colleagues (27) evaluated 16 asthmatics with a history of wine-induced asthma. Patients were challenged with low-sulfite red and white wines or wine placebo drinks. Lung functions including FEV_1, PEFR, and forced expiratory flow between 25% and 75% of the vital capacity (FEF25–75) were evaluated before challenge and at 5, 10, 15, 30, and 60 minutes after challenge. Only three subjects reacted to one or more of the challenges, defined by a $> 15\%$ decline in FEV_1: one patient reacted to the low-sulfite red wine challenge and the other two patients to wine challenge and to one of the placebo challenges. Ten subjects were also given a single-blind challenge with high-sulfite white wine. Only two of the ten subjects exhibited a clear reaction.

In a more recent study, Vally and colleagues (28) (Table 22–2) attempted to define the dose-response characteristics of wine-sensitive asthmatics. No placebo response was seen in this study, and a significant difference was found in response to high-sulfite (300 ppm) compared with sulfite-free ($\sim$ 20 ppm) wine. The maximum response occurred within 15 minutes of wine consumption, and the drop in FEV_1 ranged from 15.1% to 45.7%. The timing of this response correlates with the typical history of rapid onset of asthma symptoms after wine ingestion and supports the hypothesis that sulfite-induced asthma occurs via cholinergic pathways in the airway.

Stevenson and colleagues (29) (Table 22–2) performed single-blind, placebo-controlled oral challenges in four steroid-dependent asthmatics. All had substantial decreases in FEV_1 (23%–49%). The challenge was repeated in one patient, and the results were reproduced. SPT was also performed in this study. In all patients, skin tests to sulfites were negative, indicating that IgE may not be involved.

A study by Towns and Mellis (30) (Table 22–2) indicates that ingestion of sulfited solutions is more likely to precipitate asthma attacks than is ingestion of encapsulated sulfites. Freedman and colleagues (31) (Table 22–2) evaluated 14 asthmatics with a history of reaction after ingesting a sulfited orange drink. A challenge with $Na_2S_2O_5$ in citric acid solution calculated to contain 100 ppm SO_2 was performed without placebo control or blinding. Eight patients demonstrated a 12% or greater drop in FEV_1 (12%–57%). The asthmatic responses seen in the Towns and Mellis (30) and Freedman (31) studies were most likely caused by inhalation of volatilized SO_2. Inhaling as little as 1 ppm SO_2 has been demonstrated to cause bronchoconstriction in asthmatics (17). In doses of 1–50 ppm, 99% of inhaled SO_2 is absorbed by the upper airway. The resulting bronchospasm may be initiated by stimulation of superficial afferent nerve endings in the larynx or tracheobronchial tree and then mediated by parasympathetic pathways in the bronchi (8, 17).

Although the precise mechanism has yet to be elucidated, the bronchoconstriction caused by exposure to sulfites in sensitive asthmatics can be severe and potentially life threatening. Therefore, accurate diagnosis is imperative. But because history does not always correlate with a positive challenge (15), history alone is insufficient for the diagnosis of sulfite-induced asthma. SPTs and serologic tests are also not reliable in the diagnosis of sulfite-induced asthma (8, 9). The diagnostic tool with the highest reproducibility is a DBPC challenge (11). However, there is no standardized procedure for challenging with sulfiting agents. Patients may be challenged with capsules, neutral solutions, or acidic solutions of metabisulfite. A capsule challenge may be preferred, as most exposures are to sulfites in bound form in foods rather than to sulfites in free form, such as in lettuce. Variable thresholds for bronchospastic responses have been seen, from 5 mg to 200 mg of encapsulated metabisulfite (8). The inhalation route is the most likely to provoke asthma. A challenge with sulfites in solution is optimal for patients who have reacted to beverages such as sul-

fited wines. In patients with a history of response to particular foods, food challenges are used diagnostically (8). Challenges, therefore, can be tailored to a patient's history of reaction (see Tables 24–1 and 24–2).

Challenges should be conducted very carefully, with equipment and expertise available to treat severe bronchospastic or anaphylactic reactions. Because certain drugs can inhibit the response to sulfites, anti-asthma and anti-allergy medications, such as β_2-agonists, cromolyn, and antihistamines, should be withheld before challenges (10). β_2-agonists are typically withheld the day of the challenge, while cromolyn and antihistamines are withheld at least 24 hours prior to the challenge. Theophylline and corticosteroids (inhaled and oral) can be continued, as these drugs do not interfere with sulfite-induced reactions.

Typically, if a single-blind challenge is positive, the results should be confirmed with a double-blind challenge. Randomization of administration of active and placebo challenges should be done, possibly with a third challenge day, to avoid an order effect of challenge. An order effect of challenge has been seen in patients who receive placebo on the first day and do not react but do react on subsequent challenge days regardless of whether they receive placebo or active challenge.

Given the diagnosis of sulfite-induced asthma with an appropriately performed challenge study and the establishment of a threshold dose of sulfite that provokes asthma, treatment is strict avoidance of sulfite treated foods and drugs, especially those containing greater than 100 ppm SO_2 equivalents. The US Food and Drug Administration (FDA) and the US Bureau of Alcohol, Tobacco, and Firearms require that foods and alcoholic beverages containing greater than 10 ppm total SO_2, determined by the Monier-Williams method (26), be labeled (9). Unlabeled sulfited foods still exist in restaurants, although the use of sulfites in fresh foods such as fruits and vegetables in salad bars was banned by the FDA in 1985 (7). The FDA allows residue levels of sulfites in shrimp, which are used to prevent enzymatic browning or black-spot formation. Imported table grapes are treated with sulfites to inhibit mold growth, but the US Environmental Protection Agency requires that imported grapes be detained at their port of entry until sulfite residues are no longer detected. Potatoes are still sulfited, so patients with sulfite-sensitive asthma should avoid all potatoes in restaurants except those baked with intact skins. Sulfite-sensitive asthmatics should avoid sulfite-containing pharmaceutical agents. Pharmaceutical corporations have eliminated the use of sulfites in many products used for the treatment of asthmatics, although epinephrine contains sulfites as antioxidants because there is no alternative antioxidant agent. The positive effects of the epinephrine overwhelmingly outweigh any negative effects of sulfites, so epinephrine should not be withheld from sulfite-sensitive asthmatics.(9)

Complete avoidance of sulfites is difficult, and reactions can be severe. Management of reactions includes administration of β_2-agonist medications or cromolyn sodium (18) via meter-dose inhalers, nebulized atropine, oral doxepin, and self-administered epinephrine for severe episodes of sulfite-induced asthma.

Monosodium Glutamate

Just as sulfites have been linked to asthma exacerbations in sulfite-sensitive asthmatics, MSG has been implicated in the precipitation of asthma flares. Unlike sulfites, however, there is little data to confirm that MSG causes bronchospasm.

MSG is a sodium salt of the non-essential amino acid L-glutamic acid. MSG occurs naturally in many foods, including vegetable proteins such as those in cereals, leguminous plants, tomatoes, mushrooms, and in animal products such as fermented cheese (Parmesan, Roquefort) (32). MSG exists in free form and bound in proteins (1) and is used as a flavor enhancer in many packaged foods (33). The average daily intake of MSG in Western countries is 0.3–1 g (34). In the US, the average daily intake of MSG is 0.2–0.5 g. As much as 4–6 g might be ingested in a highly seasoned restaurant meal (35).

Because MSG is perceived as the food chemical most likely to cause bronchoconstriction, it is the fourth most frequently avoided food item. However, the role of MSG in exacerbating asthma has not been firmly established. Levels of MSG precipitating any adverse event are much higher than the usual dietary exposure (2.5–3 g vs 0.2–0.5 g daily exposure) and occur in the absence of food (34, 36–39).

Allen and colleagues (35) (Table 22–3) published a paper in 1987 reporting that MSG invokes asthma symptoms. Thirty-two asthmatic patients with a history of MSG-induced asthmatic reactions were evaluated via single-blind, placebo-controlled oral challenge with MSG. PEFRs were followed hourly for 14 hours after oral challenge. A positive challenge was defined as a > 20% drop

Table 22–3.
Review of Studies on MSG and Asthma

Reference	*Consistent time*	*H/O MSG-Induced Asthma*	*Status of Anti-asthma Meds*	*Type*	*Placebo Order*	*Dur Obs (hrs)*	*Outcome Measure*	*Oral Dose MSG (g)*	*# Positive Response/ # Challenged*
35	No	32-Yes	Morning theo stopped; SABA given to all w/i 3 h; CS continued	S	Yes First	14	PEFR > 20%	0.5–5	13/32
32	Unknown	30 total 8-Yes	CS stopped 21 days prior; theo stopped 3 days prior	S	Yes First	8	PEFR	2.5	2/30
40	Yes	12-Yes	No BD for 12 h prior	D	Yes Random	4	$FEV_1 > 10\%$	25 mg/kg	0/12
1	Unknown	12-Yes	Continued BD and anti-inflam meds; SABA withheld 4 h prior	D	Yes Random	12 (4 h at home)	$FEV_1 > 15\%$	1–5	0/12
41	Yes	30-Yes 70-No	Continued usual asthma meds	S	Yes First	12	$FEV_1 > 20\%$	2.5	0/100

in PEFR from baseline on the challenge day. There were many possible confounding aspects of this study's design. The outcome measure used was PEFR, an effort-dependent measure, rather than the more precise flow-volume loop of spirometry. Morning PEFRs were reported as stable despite significant day-to-day variability. Patients were given placebo on day 1 of the study and then challenged with MSG on days 2 and 3, augmenting the lack of daily controller medications such as theophylline, which were stopped just prior to commencement of the study. Some patients were allowed to have rescue medication within 3 hours of initial challenge, such that the reported decline in lung function 6 hours after challenge was more likely due to waning effects of β_2-agonist rather than to bronchoconstrictive effects of MSG. Timing of the challenges was inconsistent; to eliminate confounding factors of the physiologic diurnal variability in PEFR, all patients should have been challenged at a consistent time. The results of this study were also not reproduced; a non-blinded challenge was repeated in only one patient.

Another study by Moneret-Vautrin (32) (Table 22–3) evaluated 30 asthmatic patients via a single-blinded, placebo-controlled oral challenge with MSG. Again, PEFR were monitored as the outcome measure. Although 2 of the 30 patients were reported to have "moderate semi-late bronchospasm" to 2.5 mg of orally administered MSG, both patients had significant variability in PEFRs during placebo challenges. Thus, it is impossible to discern whether the > 15% variability in PEFR 6–8 hours after oral challenge with MSG reflects the wide baseline variability or a true correlation with MSG ingestion.

Using oral challenges of 1.5 g of MSG in 12 asthmatic patients, Schwartzstein and colleagues (40) (Table 22–3) found no changes in FEV_1 that were statistically different from placebo. A strength of the study is that individual subjects were challenged at the same time of day. But the number of patients evaluated was small, and subjects were only evaluated for 2 hours after challenge, rather than 12 hours or more as other studies have done. And because this was an outpatient study, the patients' diets could not be accurately evaluated for MSG content. Despite its flaws, this study does suggest that in the usual quantities found in food, MSG is unlikely to induce bronchoconstriction.

Another outpatient study evaluated 12 asthmatics, all of whom had a history of asthma exacerbation with MSG ingestion (Table 22–3) (1). This study was a DBPC evaluation for MSG-induced bronchial hyperresponsiveness. Methacholine challenge was performed before and after oral challenge with MSG. The results of this study were completely negative. This study involved a small number of subjects, was outpatient so did not allow for direct supervision of MSG content in the diet, and patients were directly monitored for only 4 hours after challenge. Nevertheless, MSG-induced asthma was not demonstrated in this group of adult asthmatics with prior history of asthma symptoms precipitated by MSG.

In a more recent single-blind, placebo-controlled study, Woessner and colleagues (41) (Table 22–3) evaluated 100 asthmatic patients, 30 of whom reported prior asthma exacerbations with MSG exposure. Subjects were given 2.5 g of MSG, and FEV_1 was measured at hourly intervals and after the onset of symptoms for 12 hours. No significant drop in FEV_1 occurred, and no patients developed asthma symptoms.

In contrast to the perception that MSG-induced asthma exists, well-designed studies with oral challenges of MSG have not demonstrated changes in FEV_1 or symptoms of asthma. Thus, a report from the United States Life Sciences Research Institute published in 1995 (42) states that evidence is limited that patients with asthma are at increased risk to have adverse effects from MSG than the general population. The US FDA also has recognized MSG as "generally safe" (34).

When patients are concerned that a reaction may be occurring to MSG, an oral challenge can be performed (Table 22–4) (41). Maintenance asthma medications should be continued. An initial single-blind, placebo-controlled challenge should be done. Woessner and colleagues (41) used 5 placebo capsules containing 500 mg of sucrose each. FEV_1 should be monitored hourly. If the FEV_1 changes by more than 10%, the patient has failed the placebo challenge. If the FEV_1 is stable (change of less than 10%), a second placebo challenge should be performed and FEV_1 monitored hourly. In Woessner's study (41), the duration of the placebo challenge day was 12 hours.

If patients "pass" the placebo challenge day with less than 10% variability in FEV_1, a single-blind challenge with MSG should be performed. MSG is given in five 500 mg capsules, totaling 2.5 g. FEV_1 is monitored hourly for a total of 12 hours. Five placebo capsules should be given at the 6 hour point to maintain a sequence similar to the placebo challenge day. A positive response is defined as a drop in FEV_1 of > 20%. If patients have a positive response to a single-blind challenge, a double-blind challenge should be performed.

Table 22–4.
Protocol for MSG Oral Challenge (41)

Continue maintenance asthma medications.
Perform an initial single-blind placebo challenge.
- Administer five placebo capsules of 500 mg of sucrose each.
- Monitor FEV_1 hourly.
- Failure of placebo challenge is a change in FEV_1 > 10%.
- If FEV_1 remains stable, perform a second placebo challenge, monitoring FEV_1 hourly.
- Total duration of placebo day is 12 hours.

If patients pass the placebo challenge day, perform single-blind challenge with MSG.
- Give 5 capsules MSG totaling 2.5 g.
- Monitor FEV_1 hourly for total of 12 hours.
- Six hours after MSG administered, administer five placebo capsules to maintain a sequence similar to the placebo challenge day.
- Positive response is FEV_1 drop > 20%.

Tartrazine

Besides flavorants such as MSG, synthetic colorants are often added to foods. One such example is the azo dye tartrazine, also known as FD&C Yellow #5. As with MSG, many of the studies that have been performed have design flaws, and no well-designed study has corroborated claims that tartrazine provokes asthma exacerbations (Table 22–5).

In 1967, Samter and Beers (43) (Table 22–5) published data from a double-blind tartrazine challenge in 80 patients. Patients were given 25 mg tartrazine in aqueous solution. Only 3 of the 80 subjects developed bronchoconstriction. This early paper evaluating the correlation between tartrazine and asthma exacerbation has many possible problems: lack of placebo control, no objective measures of bronchoconstriction, and no data regarding current medications and whether these were continued during the challenge. The challenges were not repeated to evaluate for reproducibility.

In a study that did include a randomized placebo control, Juhlin and colleagues (44) (Table 22–5) evaluated seven patients with a history of aspirin sensitivity. The patients developed urticaria, asthma, or non-specific symptoms with aspirin exposure. Patients were challenged with a 1 mg dose of tartrazine administered orally in a single-blind fashion. All medications were withheld for 3 days prior to the challenges. Two of the seven patients had symptoms of asthma after oral challenge with tartrazine. This paper, however, lacks objective measures of lung function at baseline and after challenge. Controller medications were disallowed for 3 days prior to the challenges. Therefore, the asthma symptoms that occurred following tartrazine challenge may have reflected poorer asthma control as a result of discontinuing usual medications rather than a reaction to tartrazine.

Stenius and Lemola (45) (Table 22–5) also performed tartrazine challenges with placebo control, but the placebo was administered first in each case, risking an order effect of challenge. An oral challenge with 0.1 mg, 1 mg, or 10 mg of tartrazine was administered to 114 patients. Oral prednisone was continued if the dose were 10 mg or less. Antihistamines were withheld for 48 hours and bronchodilator for 6 hours prior to the challenge. PEFR was monitored 40 minutes after a dose was administered. A drop of 20% or greater was considered positive. Twenty-five of the 114 subjects met

Table 22–5.
Summary of Studies on Tartrazine and Asthma

Reference	*H/O ASA sens asthma*	*Status of Anti-asthma Meds*	*Type*	*Placebo Order*	*Oral Dose Tartrazine (mg)*	*# Positive Response/ # Challenged*
43	40-Yes 40-No	D	Unknown	Unlikely	25 in aqueous solution	3/80
44	7	Withheld	S	NA Yes	1 (solution)	2/7
45	114-Unknown 25-Yes	Withheld BD 6 hr prior; Pred = or < 10 mg cont	S	Random Yes First	0.1–10	25/114 12/25
47	5-Yes 49-No	Withheld theo; continued BD	D	Yes Random	25	0/5 0/49
48	44 Total	Withheld am BD Continued am BD	O D	Yes Unknown	2.5–2.0	7/44 0/7
46	44-Yes 233-No	Withheld BD and antihist 6–12 h prior	D	Yes Random	1–50	11/44 0/233
49	156-Yes	BD withheld 1 h prior; Theo withheld 8 h prior	O/D	Yes-D Unknown	1–25 (solution)	4/156 with DBPC
50	194-Yes 43-No	BD, Na Cr, antihist withheld; theo, CS, LABD continued	S/D	Yes Interspersed	25–50	0/194 0/43

the criteria for a positive challenge. The authors did not include baseline PEFR data on these patients. Thus, the changes seen in PEFR during the study may reflect baseline variability in the subjects and be unrelated to the tartrazine challenge. Without the baseline data, no conclusion can be drawn regarding correlation between tartrazine and PEFR changes. Challenges were not repeated to establish reproducibility.

Spirometry, rather than PEFR, was used as the objective measure of lung function in a study by Spector and colleagues (46) (Table 22–5), which evaluated 277 patients for asthma symptoms induced by tartrazine, 44 of whom had aspirin sensitivity. Bronchodilators and antihistamines were withheld for 6–12 hours prior to testing. Corticosteroids were continued. Patients were challenged with tartrazine in oral doses ranging from 1 mg to 50 mg. This study was placebo-controlled, randomized, and double-blinded. Spirometry was measured after challenge every 30 minutes for 4 hours. A positive reaction was defined as a 20% or greater fall in FEV_1. Eleven patients had positive responses. No patients who were aspirin tolerant had positive tartrazine challenges. The authors conclude that the patients with aspirin sensitivity "may well have as yet undiagnosed tartrazine idiosyncrasy." While this study used spirometry as the outcome measure, was randomized, double-blinded and placebo-controlled, it does not include information regarding the patients' lung functions at baseline. The study lacks information regarding the timing of challenges, which may have been inconsistent. Given the incomplete data presented in the paper, it is impossible to make a clear correlation between aspirin sensitivity and reactions to tartrazine.

Vedanthan and colleagues (47) (Table 22–5) evaluated 54 children with asthma for tartrazine and aspirin sensitivity. Bronchospastic reaction was defined as decline in FEV_1 of $> 20\%$. The criteria were sensitive enough to detect five aspirin-sensitive patients during the oral aspirin challenges, but none of the patients reacted to tartrazine.

Weber et al (48) (Table 22–5) found 7 (16%) of 44 asthmatic patients with a $\geq 20\%$ drop in FEV_1 within 4 hours of oral challenge with tartrazine after withholding morning bronchodilators. During repeat challenges in which morning bronchodilators were administered, FEV_1 declined by less than 20%. The authors attributed the drop in FEV_1 in the initial challenge to withholding of morning bronchodilators rather than a true effect of tartrazine.

To avoid increased airway instability due to waning β_2-agonist, Virchow and colleagues (49) (Table 22–5) allowed the morning bronchodilator to be given within 1 hour of oral tartrazine challenge in patients on chronic bronchodilator therapy. In the majority of subjects, however, bronchodilator and aminophylline were withheld for 8

hours prior to the test. The report does not comment on whether other controller medications were continued. In 156 patients, open tartrazine challenges were administered orally in increasing doses from 1 mg to 50 mg at consistent times. Spirometry was measured every 30 minutes following a challenge. A positive response was defined as a $\geq 25\%$ change in FEV_1. If no change was seen within 1 hour of a dose, the next larger dose of tartrazine was administered. If an open challenge were positive, the challenge was repeated in DBPC fashion. Four (2.6%) of the 156 patients tested had positive responses. Although the initial challenge was open, this study did repeat the challenges in DBPC fashion. Baseline lung function data were not included in the paper. It is difficult to interpret the positive responses as truly related to tartrazine without information regarding overall asthma control at baseline in these subjects.

In a total of 194 aspirin-sensitive patients evaluated for tartrazine sensitivity by oral challenge, Stevenson et al (50) (Table 22–5) did not demonstrate cross-sensitivity between aspirin and tartrazine. The authors concluded that reports of tartrazine-induced bronchospasm represent spontaneous asthma coincidentally associated with ingestion of tartrazine, rather than bronchospasm caused by tartrazine. In this study, usual medications were continued during the challenges, excepting β-agonists, cromolyn, and antihistamines. The challenge initially was single-blinded. Placebo was administered first. If FEV_1s were unstable during placebo challenge, the tartrazine challenge was not administered. A "conditionally positive" test involved a drop in FEV_1 of 25% or greater. A double-blind challenge was performed for those subjects with "conditionally positive" single-blind challenges. None of the subjects had a positive reaction when double-blind challenge was performed.

If a patient is concerned about reactions to tartrazine, an oral challenge can be performed (Table 22–6) (50). An initial challenge should involve hourly FEV_1 monitoring throughout the challenge. Placebo should be administered first. If FEV_1 remains stable after 3 hours, 25 mg of tartrazine can be given. If after another 3 hours FEV_1 is still stable, 50 mg of tartrazine can be administered. A "conditionally positive" test consists of an FEV_1 drop of 25% or more after the 25 mg or 50 mg dose of tartrazine.

Table 22–6.
Protocol for Tartrazine Oral Challenge (50)

Initial challenge
- Administer placebo first.
- Monitor FEV_1 hourly.
- If FEV_1 stable after 3 hours, administer 25 mg tartrazine.
- If FEV_1 stable after 3 hours, administer 50 mg tartrazine.
- A "conditionally positive" test consists of FEV_1 drop of 25% or more after the 25 or 50 mg dose tartrazine

Double-blind challenge
- Begin with a full day of placebo challenge using three doses of placebo administered 3 hours apart.
- Monitor FEV_1 hourly.
- On the following day, follow protocol for initial challenge using suspected provoking dose of tartrazine and two placebos.

When the initial challenge is positive, a double-blind challenge should be done in the fashion described above, using the suspected provoking dose of tartrazine and two placebos. This double-blind challenge should be preceded by a full day of challenge using three doses of placebo administered 3 hours apart. FEV_1 should be monitored hourly throughout the placebo challenge and active challenge.

Conclusions

Despite the fact that a multitude of food additives exist, only a few are commonly implicated in asthma: sulfites, MSG, and tartrazine. Of these three, only sulfites have been found to convincingly incite bronchoconstriction in sulfite-sensitive asthmatics, who should avoid sulfite exposure. In contrast, due to the lack of evidence in well-designed studies linking MSG and tartrazine to asthma exacerbation, asthmatic patients need not avoid exposure to MSG or tartrazine if a DBPC challenge is negative.

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23

Urticaria, Angioedema, and Anaphylaxis Provoked by Food and Drug Additives

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Many agents are added to the foods that we consume (1); the number ranges from 2000 to 20,000. These substances include preservatives, stabilizers, conditioners, thickeners, colorings, flavorings, sweeteners, and antioxidants. Despite the multitude of additives known, only a surprisingly small number have been associated with hypersensitivity reactions.

A number of investigators have suggested that the incidence of urticaria, angioedema, and anaphylaxis related to the ingestion of food additives is relatively common. This apparent misconception is based on several poorly controlled studies, mostly reported before 1990. Emerging evidence appears to contradict this notion, suggesting that the incidence of such reactions is relatively rare.

Table 23–1 lists the food and drug additives that may be associated with adverse reactions. In this chapter, these additives are discussed in detail as they relate to urticaria and angioedema, as well as to anaphylaxis or anaphylactoid reactions.

General Considerations and Descriptions of Some Additives

A brief overview of selected additives follows (2). For additional information, the reader is referred to Chapters 22 through 28 in this text.

Food Dyes

Dyes approved under the Food, Drug and Cosmetic (FD & C) Act are coal tar derivatives, the best known of which is tartrazine (FD & C Yellow No. 5). In addition to tartrazine, the group of azo dyes includes ponceau (FD & C Red No. 4) and sunset yellow (FD & C Yellow No. 6). Amaranth (FD & C Red No. 5) was banned from use in the US in 1975 because of claims related to carcinogenicity. Non-azo dyes include brilliant blue (FD & C Blue No. 1), erythrosine (FD & C Red No. 3), and indigotine (FD & C Blue No. 2).

Sulfites

Sulfites and the burning of sulfur-containing coal have been used for centuries to preserve food. In addition, sulfiting agents (including sulfur dioxide and sodium or potassium sulfite, bisulfite, or metabisulfite) are used by the fermentation industry to sanitize containers and to inhibit the growth of undesirable microorganisms. Sulfites act as potent antioxidants, which explains their widespread use in foods as preventives against oxidative discoloration (browning) and as fresheners. Many packaged foods, including fresh and frozen cellophane-wrapped fruits and vegetables, processed grain foods (crackers and cookies), and citrus-flavored beverages, may contain sulfites.

Table 23–1.
Additives Associated with Adverse Reactions

SYNTHETIC DYES
FD & C dyes
Azo dyes
Tartrazine (FD & C Yellow No. 5)
Sunset yellow (FD & C Yellow No. 6)
Ponceau (FD & C Red No. 4)
Amaranth (FD & C Red No. 2)
Non-azo dyes
Brilliant blue (FD & C Blue No. 1)
Erythrosine (FD & C Red No. 3)
Indigotin (FD & C Blue No. 2)
Parabens
Parahydroxybenzoic acid
Methyl-, ethyl-, butyl-, and propyl paraben
Sodium benzoate
Butylated hydroxyanisole (BHA)
Butylated hydroxy toluene (BHT)
Nitrates/nitrites
Monosodium glutamate (MSG)
Sulfites
Sulfur dioxide
Sodium sulfite
Sodium/potassium bisulfite
Sodium/potassium metabisulfite
Aspartame (NutraSweet)
Isosulfan blue (medical diagnostic agent)
NATURAL DYES
Annatto
Carmine

The highest levels, however, occur in potatoes (any peeled variety), dried fruits (apricots and white raisins), and possibly shrimp and other seafood, which may be sprayed after unloading on the dock. Sulfites are listed as ingredients in prepared and packaged foods or drinks that contain at least 10 parts per million (ppm) SO_2 equivalents. In 1986, the FDA banned the use of sulfites on foods marketed as "fresh".

Parabens

Parabens are aliphatic esters of para-hydroxybenzoic acid; they include methyl-, ethyl-, propyl, and butyl parabens. Sodium benzoate is a closely related substance usually reported to cross-react with these compounds. These agents are widely used as preservatives in both foods and drugs, and are well recognized as causes of severe contact dermatitis.

Monosodium Glutamate

Glutamic acid is a nonessential dicarboxylic amino acid that constitutes 20% of dietary protein. Glutamate occurs naturally in some foods in significant amounts: 100 g of Camembert cheese, for example, contains as much as 1 g of monosodium glutamate (MSG). The greatest exposure to MSG, however, occurs through its role as a flavor enhancer. Manufacturers and restaurateurs add MSG to a wide variety of foods. About 75 years ago a Japanese chemist established that MSG produces the flavor-enhancing properties of seaweed, a traditional component of Japanese cooking. Large amounts of MSG are commonly added to Chinese, Japanese, and other Southeast Asian cooking. As much as 6 g of MSG may be ingested in a highly seasoned oriental meal, and a single bowl of wonton soup may contain 2.5 g of MSG. MSG may be found in manufactured meat and chicken products. It has been reported to provoke, within hours of eating, a syndrome characterized by headache, a burning sensation along the back of the neck, chest tightness, nausea, and sweating. Recently, a trend toward reducing MSG use in Asian cooking has emerged, likely in response to consumer dissatisfaction related to the occurrence of the syndrome.

Aspartame

Aspartame (NutraSweet) is a dipeptide composed of aspartic acid and the methyl ester of phenylalanine. This popular low-calorie artificial sweetener is 180 times sweeter than sucrose.

Butylated Hydroxyanisole and Butylated Hydroxytoluene

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are antioxidants used in cereal and other grain products.

Nitrates/Nitrites

Nitrates and nitrites are widely used preservatives. Their popularity stems from both flavoring and coloring attributes. These agents are found mostly in processed meats such as frankfurters and salami (3).

Annatto

Annatto dye is an orange-yellow food coloring extracted from the seeds of the tree *Bixa orellana*, a large fast-growing shrub cultivated in the tropics. It is frequently used in cereals, beverages, cheese, and snack foods.

Carmine

Carmine (or cochineal extract) is a biologically derived red colorant derived from the dried bodies of female cochineal insects (*Dactyloopius coccus costa*). These insects reside as parasites on the prickly pear cactus (*Nopalea coccinelliferna).* It is commonly used in cosmetics, textiles, and foods. It is responsible for giving the liqueur Campari its characteristic color. It is often designated E 120.

Isosulfan Blue

Isosulfan blue (ISB; Lymphazurin 1%, US Surgical Corporation) is an isomer of the triphenylmethane dye patent blue. It is a contrast agent for the delineation of lymphatic vessels. Following subcutaneous administration, this dye binds to interstitial proteins in lymphatic vessels, imparting a bright blue appearance that makes the lymphatics more readily discernable from surrounding tissue. ISB is indicated as an adjunct to lymphangiography, assessing lymph node response to therapeutic modalities and for visualization of the lymphatic system draining the region of injection (4).

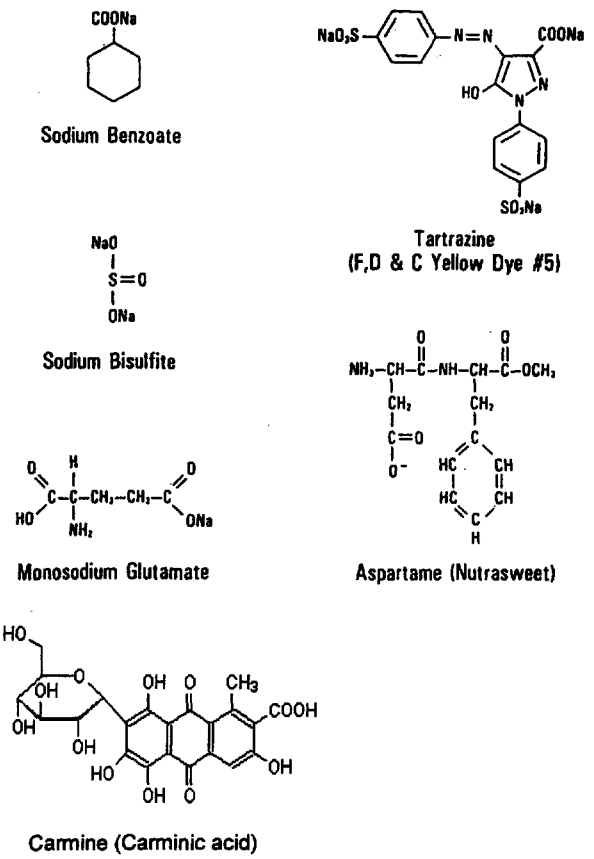

Figure 23–1. Chemical structures of some common food/drug additives.

Mechanism of Additive-Induced Urticaria, Angioedema, and Anaphylaxis

To date, the mechanisms underlying additive-induced urticaria, angioedema, and anaphylaxis have not yet been elucidated. It seems reasonable to postulate, however, that multiple mechanisms are responsible for these adverse reactions given the heterogeneity of chemical structures found among these additives (Fig. 23–1). Natural food colorants (e.g., annatto and carmine) are derived from proteins with molecular weights consistent with common food allergens.

Immediate (IgE-Mediated) Hypersensitivity

Naturally derived food colorings such as annatto and carmine contain proteins recognized in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) by gel bands that appear in the 10–100 kilodalton (kDa) range. Therefore, these colorants can be expected to potentially elicit IgE-mediated responses in some atopic individuals. Synthetic additives would have to act as haptens to create a response mediated by IgE. Only a few reports have suggested IgE-mediated reactions to synthetic additives, notably to sulfites and parabens. Instead, the overwhelming majority of these reactions are not of the immediate hypersensitivity type. In fact, many cases of additive-provoked urticaria occur as late as 24 hours after challenge, arguing against an IgE-mediated mechanism.

Evidence for an IgE-mediated mechanism as the cause for identified anaphylactic episodes associated with carmine colored foods derives from studies that have demonstrated positive skin prick tests (SPTs), a positive Prausnitz-Küstner (PK) test, a positive basophil histamine release assay, IgE (radioallergosorbent test) RAST studies, and SDS-PAGE with IgE immunoblot (5–9). Chung et al (10) identified in minced cochineal insects several protein SDS-PAGE bands of 23–88 kDa. The sera from three patients with episodic urticaria/angioedema/anaphylaxis occurring 3–5 hours after ingestion of foods containing carmine recog-

nized these bands on immunoblot. This reactivity was inhibited by carmine. Patient reactivity to specific bands varied. Commercial carmine appears to retain proteinaceous material from the source insects. These insect-derived proteins (possibly complexed with carminic acid) are responsible for IgE-mediated carmine allergy.

Nish et al (11) reported one case of annatto dye-induced anaphylaxis. SPTs to annatto were strongly positive in the case with negative control results. SDS-PAGE demonstrated two bands in the range of 50 kDa. Immunoblotting showed patient IgE-specific for one of these bands and controls showed no binding. Residual or contaminating seed protein was the likely responsible antigen in this rare case. Revan et al (12) reported their experience with annatto at the University of Michigan Allergy Clinic. They found 9 (12%) of 77 atopic patients were SPT-positive to liquid undiluted annatto. However, only two of these nine subjects had symptomatic annatto allergy: one patient with a 4+ SPT had a history of annatto-induced anaphylaxis, and another with a 3+ SPT had angioedema. Only one SPT-positive reactor was challenged (2+) and was negative. The negative predictive value (NPV) of SPT in this cohort was 100%; however, the positive predictive value (PPV) was low (22%). Perhaps the undiluted extract was too potent to differentiate between true reactors and an irritant response. Double-blind placebo-controlled (DBPC) challenges will be needed to confirm these results.

In 1976, Prenner and Stevens reported an anaphylactic reaction occurring after the ingestion of food sprayed with sodium bisulfite (13). Minutes after eating lunch at a restaurant, a 50-year-old male experienced generalized urticaria and pruritus, swelling of the tongue, difficulty swallowing, and tightness in his chest. He responded promptly to treatment with subcutaneous epinephrine. Subsequently, the patient's SPT and an intradermal test gave positive results (with negative controls). The authors were able to demonstrate PK transfer to a nonatopic subject. Yang and associates (14) also described one patient with a history of sulfite-provoked anaphylaxis. A borderline result was obtained via intradermal skin test, followed by a positive response to single-blind oral provocation challenge with 5 mg of potassium metabisulfite. This patient's cutaneous reactivity was also passively transferred via the PK reaction. However, Yang's group was unable to elicit positive responses to challenges in nine patients with histories of hives related to eating restaurant food. In addition, Sokol and Hydick (15) reported a case of sulfite-induced anaphylaxis that provided evidence for a specific IgE-mediated mechanism. Despite these isolated reports, IgE-mediated immediate hypersensitivity reactions to sulfites (possibly via a hapten mechanism) appear to occur only rarely.

Studies of neutrophil chemotactic factor of anaphylaxis have failed to demonstrate an increase in this mast cell (MC) mediator post-challenge in subjects with negative metabisulfite skin tests, suggesting that MC degranulation is not associated with non-IgE-mediated sulfite reactions (16). Cromolyn pretreatment did not ablate an urticarial reaction in an individual sensitive to potassium metabisulfite (17). In the overwhelming majority of cases, the mechanisms behind sulfite-provoked urticaria, angioedema, and anaphylaxis (or anaphylactoid reactions) remain unknown.

At least three cases of apparent IgE-mediated, paraben-induced urticaria and angioedema have been reported (18, 19). All of these cases concerned reactions to benzoates used as pharmaceutical preservatives. The three patients had positive skin test responses to parabens, but negative results when exposed to the drugs themselves minus the paraben preservatives. These subjects, however, could tolerate oral benzoates in their diets without reactions. Macy et al (20) recently studied a series of 287 patients who underwent immediate hypersensitivity skin test to methylparaben-preserved local anesthetics. Only three patients had positive skin tests. These three individuals underwent skin testing as well as provocative dose testing to 0.1% methylparaben in addition to local anesthetic without preservative. All three reacted definitively to the methylparaben, suggesting that methylparaben is a potential cause for local immediate hypersensitivity reactions previously attributed to the local anesthetics themselves.

Delayed (Type IV) Hypersensitivity

Another suggested mechanism focuses on delayed hypersensitivity. Studies in this area have been few in number and often poorly designed. Warrington and coworkers (21) measured the release of a T lymphocyte-derived leukocyte-migration inhibition factor in response to incubation with tartrazine, sodium benzoate, and aspirin (acetylsalicylic acid) in vitro using peripheral blood mononuclear cells from patients with chronic urticaria, with or without associated additive or as-

pirin sensitivity. Significant production of the inhibitory factor occurred in response to tartrazine and sodium benzoate in individuals with chronic additive-induced urticaria. The groups of patients studied (four patients per group) exhibited sensitivity to tartrazine, sodium benzoate, and aspirin as determined either by response to elimination diet alone or by challenge-proved sensitivity. In this study, the potential for false-positive reactions on the basis of response to diet alone created a problem. Essentially no details of the challenge procedure were given.

Valverde and associates (22) studied in vitro lymphocyte stimulation in 258 patients with chronic urticaria, angioedema, or both, using a series of food extracts and additives that included tartrazine, benzoic acid, and aspirin. They found positive stimulation (using the lymphocyte transformation test) to additives in 18% of subjects. After the patients were placed on a diet that excluded the offending additives, 62% had total remission of symptoms and 22% had partial remission. The investigators concluded that this response to diet lent credence to the lymphocyte transformation test as an in vitro diagnostic test for chronic urticaria and angioedema related to food additives. No provocation challenges were performed in this study, however. No conclusions regarding the presence or absence of a delayed-type hypersensitivity mechanism in additive-provoked urticaria can be made from the studies described above. It seems reasonable to conclude that a reaction occurring between 30 minutes and 6 hours (most reactions began within the first 6 hours) is not typical of a type IV mechanism.

Cyclooxygenase, Aspirin, and Tartrazine

The subject of tartrazine sensitivity remains controversial. Many claims of cross-reactivity between aspirin and tartrazine have been made; estimates of its incidence based on earlier studies range from 21% to 100% (23–27). In a DBPC study of tartrazine sensitivity in urticaria patients that utilized objective reaction criteria (and withheld antihistamines for 72 hours prior to challenge), only 1 (4.2%) of 24 patients experienced urticaria after challenge with 50 mg of tartrazine (28). When challenged with 975 mg of aspirin, this patient did not react, suggesting that cross-reactivity between aspirin and tartrazine may not occur. An earlier DBPC crossover challenge with 0.22 mg of tartrazine found sensitivity in 3 (8%) of 38 patients with chronic urticaria and 2 (20%) of 10 patients with aspirin intolerance (26). This dose of tartrazine is similar to that used to color medication tablets, but remains far less than that typically encountered in the diet. The report did not mention, however, whether antihistamines were withheld during the challenges.

No convincing evidence has been found to prove that tartrazine inhibits the enzyme cyclooxygenase (in the arachidonic acid cascade), an often-suggested mechanism for aspirin sensitivity. In addition, tartrazine (Fig. 23–1) and acetylsalicylic acid have dissimilar chemical structures. The mechanism of tartrazine sensitivity has not been well studied and remains unknown.

Neurologically Mediated Hypersensitivity

Considerable evidence exists that MSG has both neuroexcitatory and neurotoxic effects in animals (29) and humans (30). Neurologically mediated urticarias have been previously described (31). Several factors—including heat, exercise, and stress—may induce cholinergic urticaria. This mechanism represents only a theoretical basis for MSG-induced urticaria, possibly via release of cutaneous neuropeptides.

Anticoagulation

In 1986 Zimmerman and Czarnetzki (32) sought to disprove claims by earlier investigators that changes in the bleeding time play an important role in diagnosing anaphylactoid reactions to aspirin, other nonsteroidal anti-inflammatory drugs (NSAIDs), and food additives. They measured bleeding time, pro-thrombin time, and partial thromboplastin times in 10 patients with histories of anaphylactoid reactions to these drugs and various food additives. Challenges were not placebo-controlled, nor were they blinded. Nevertheless, the investigators found no correlation between patients' reactions and the aforementioned coagulation parameters.

Conclusion

Thus, aside from several case reports describing IgE-mediated reactions to sulfites and parabens, the majority of synthetic additive-induced urticaria, angioedema, and anaphylactic reactions involve mechanisms that have not been elucidated.

This stands in contradistinction to the natural food additives carmine and annatto, which show definite IgE binding to residual source protein antigens.

Food Additive Challenge Studies in Patients with Urticaria/Angioedema

Patient Selection

Selection of patients for study may include three types of subjects: 1) all available patients with chronic urticaria (or only those with chronic idiopathic urticaria); 2) patients with histories suggestive of food additive-provoked urticaria; or 3) patients who have responded to a diet free of commonly implicated additives. The percentage of positive reactors will depend on the group selected. This variability adds more confusion to the already difficult task of comparing results from differing studies.

Activity of Urticaria at the Time of Study

The relative degree of activity or inactivity of urticaria or angioedema at the time of challenge appears to affect the ability to obtain cutaneous responses to food additives. Challenges performed on patients with active urticaria are more likely to yield false-positive results. Challenges performed on patients whose urticaria is in remission, on the other hand, are more likely to yield false-negative results. In a study by Mathison and colleagues (33), only 1 of 15 patients whose urticaria was in remission experienced a reaction to aspirin, whereas 7 of 10 patients with active urticaria reacted to aspirin. These challenges were performed using objective reaction criteria, and the reactions observed were then compared with a baseline observation.

Medications

Several studies made no reference to whether medications—particularly antihistamines—were continued or withheld during challenges. The following caveats must be considered when interpreting such challenge studies. 1) Discontinuation of antihistamines immediately before or within 24 hours of challenge often generates more false-positive results. 2) Continuation of antihistamines during challenges may block milder additive-induced cutaneous responses and, therefore, give more false-negative results. 3) Subjects become increasingly likely to experience breakthrough urticaria as the interval from the last antihistamine dose to the "positive challenge" increases. Such results would be even more confusing if placebo-controlled challenges preceded additive challenges.

Reaction Criteria

Often no period of baseline observation is made by the investigators for comparison with reaction data. Most challenge studies performed to date have employed a loosely defined and rather subjective means to define urticarial responses. The reaction criteria could simply consist of "clear signs of urticaria developing within 24 hours." The studies by Stevenson et al (28) and Mathison et al (33), in contrast, utilized an objective system of scoring urticarial responses.

Placebo Controls

The use of placebo-controlled studies in additive challenges is desirable because studies without them are difficult to interpret when assessing positive urticarial challenge responses. Nevertheless, a surprising number of reported additive challenge studies do not employ placebo controls. Even in many placebo-controlled studies, the placebo is always the first challenge, followed by aspirin, and finally by an additive. Thus, a spontaneous flare of urticaria was least likely to coincide with the first placebo challenge. We also question the validity of having only a single placebo in challenge studies that test large numbers of additives. Clearly, a need exists for multiple placebos and randomization of placebo usage in the order of challenges.

Blinding

Among the most important features of any protocol for food additive challenge is a double-blind challenge, because urticaria may be exacerbated by emotional stress. In addition, it is necessary to eliminate observer bias given the subjective nature of positive responses. Open challenges are useful tools for ruling out additive-associated reactions. Positive challenge responses, in contrast, need double-blinded confirmation before they can be accepted as "true positives."

Multiple Additive Challenges in Patients with Chronic Urticaria

Examples of Studies with Less Stringent Design Criteria

One of the earliest additive challenge studies in patients with chronic urticaria was reported by Doeglas (34). Seven (30.4%) subjects reacted to tartrazine and "four or five" (17.4% or 22.7%) reacted to sodium benzoate. Placebo-controlled challenges were not performed. Thune and Granholt (35) reported that 20 (21%) of 96 patients reacted to tartrazine, 13 (15%) of 86 reacted to sunset yellow, 5 (71%) of 7 reacted to parabens, and 6 (13%) of 47 reacted to BHA and BHT. Furthermore, in the group of patients with chronic idiopathic urticaria, 62 (62%) of the 100 patients challenged reacted to at least 1 of the 22 different agents used. The challenges were not placebo-controlled, however, so any conclusions about the incidence of reactions to a particular agent derived from this study would be difficult to support.

In a study of 330 patients with recurrent urticaria, Juhlin (36) performed single-blind challenges using multiple additives and a single placebo, which always preceded the additive challenge. He found that one or more positive reactions occurred in 102 (31%) of patients tested. Reaction criteria were relatively subjective in this study. In fact, 109 (33%) of patients had reactions judged to be "uncertain" because, as the author stated, "Judging whether a reaction is positive or negative is not always easy." Furthermore, if patients reacted to the lactose placebo, retesting involved a wheat starch placebo. Questionable reactors were retested. If the repeat test gave a positive result, the first test was assumed to be positive as well; the same logic applied for negative retesting.

Supramaniam and Warner (37) described 24 of 43 children as reacting to one or more additives used in their double-blind challenge study. No baseline observation period was established, however, and only one placebo was interspersed among the nine additives used for challenge. Furthermore, no mention was made about whether antihistamines were withheld prior to or during challenges.

In 1985, Genton and coworkers (38) performed single-blind additive challenges on 17 patients with chronic urticaria or angioedema. The patients were placed on a 14-day elimination diet (free of food additives) before challenges and medications were discontinued at the beginning of the diet. Of the 17 patients in the study, 15 reacted to at least one of the six additives used for challenge.

Examples of Studies with More Stringent Design Criteria

In 1988, Ortolani and associates (39) reported 396 patients with recurrent chronic urticaria and angioedema; this report was a follow-up to a study performed in 1984 (40). DBPC oral food provocations were performed on patients that had experienced significant remissions while following an elimination diet. The diet was maintained, but medications were discontinued during challenges. The report did not describe the timing of discontinuation of medications. On the basis of history alone, 179 patients were considered for an elimination diet for suspected food or food additive intolerance; only 135 patients ultimately participated in the study. Only 8 (9.2%) of 87 patients who had significantly improved on the diet after 2 weeks gave positive responses to food challenges. Of the 79 patients with negative responses to food challenge, 72 underwent DBPC, oral food additive provocations. Twelve (17%) of these patients experienced positive responses to challenges with one or more additives. Many of these patients naturally reacted to two or three additives. Five (31%) of the 16 patients with positive responses to aspirin challenges gave positive responses to additive challenges; four of these subjects tested positive to sodium salicylate.

The similarity in chemical structure observed between aspirin and sodium salicylate supports the finding of cross-reactivity between them. They differ in that sodium salicylate is a "non-acetylated" salicylate. The doses used (> 400 mg) in the sodium salicylate challenge, however, far exceed the levels encountered in most conventional diets. Considering that the proposed mechanisms for reactions to additives such as tartrazine, sodium benzoate, and sulfites differ so dramatically, skepticism about the validity of the positive challenge results in this study is warranted. Furthermore, although it is important in assessing food sensitivity, a patient's history is usually a poor indicator of a possible additive hypersensitivity, because patients are usually unaware of all additives that they consume daily.

Hannuksela and Lahti (41) challenged 44 chronic urticaria patients with several food additives, including sodium metabisulfite, BHA or BHT, beta-carotene, and benzoic acid in a prospective, DBPC study. Only 1 (9.1%) of the 44 patients had a positive response to challenge, reacting positively to benzoic acid. Another patient also reacted to the placebo challenge. All medications were discontinued 72 hours before the first chal-

lenge and during the study. Patients were not placed on an additive-free diet prior to the challenge. The challenge dose of metabisulfite was quite low—only 9 mg. Similarly, Kellett and associates noted that approximately 10% of 44 chronic idiopathic urticaria patients reacted to benzoates, tartrazine, or both, but 10% of the subjects reacted to placebo challenges (42).

Elimination Diet Studies

An alternative strategy for investigating additive-induced urticaria involves the elimination of all additives from the diet and the observation of its effects on hives. Unfortunately, there are no reported blinded or placebo-controlled studies of this nature. In uncontrolled studies, Ros and coworkers (43) reported an additive-free diet to be "completely helpful" in 24% of patients with chronic urticaria; 57% of patients were deemed "much improved," and 19% were "slightly better" or experienced no change in their urticaria. Rudzki and associates (44) reported that 50 (32%) of 158 patients responded to a diet that eliminated salicylates, benzoates, and azo dyes. These studies did not address the question of which, if any, additives constituted the cause of the problem.

Gibson and Clancy (45) found that 54 (71%) of 76 patients who underwent a 2-week, additive-free diet "responded." They then challenged the responders with individual additives. Although the challenges were controlled, the patients always received the placebo first. No mention was made of whether the challenges were blinded. A diet that eliminated the offending additive was then continued for 6 to 18 months, followed by repeat challenge. All three patients who initially responded positively to tartrazine challenge had negative results upon rechallenge, as did one of the four patients with initially positive responses to benzoate challenges. Thus, despite this approach, the incidence of additive sensitivity in urticaria remains unknown.

Reports of Single Additive Challenge Studies

Sulfites

The reports by Prenner and Stevens (13) and Yang et al (14) discussed earlier presented single cases of sulfite-provoked anaphylaxis and gave skin test and PK transfer evidence to suggest that an IgE-mediated mechanism played a role in these reactions. In addition, Yang et al (14) performed a single-blind oral challenge. Their patient responded positively to a challenge with 5 mg of potassium metabisulfite.

In 1980, Clayton and Busse (46) described a nonatopic female who developed generalized urticaria that progressed to life-threatening anaphylaxis within 15 minutes of drinking wine. Her symptoms were not reproduced by ingestion of other alcoholic beverages. This case may have involved sulfite-provoked urticaria and anaphylaxis.

Habenicht and coworkers (47) described two patients who experienced several episodes of urticaria and angioedema after consuming restaurant meals. Only one of these individuals underwent a single-blind oral challenge with potassium metabisulfite. Generalized urticarial lesions developed in this patient within 15 minutes of receiving the 25 mg challenge dose. No placebo challenge was performed. Avoidance of potential sulfite sources apparently resolved this patient's recurrent symptoms.

Schwartz reported two patients with restaurant-related symptoms who underwent oral challenges with metabisulfite (48). Both subjects had symptoms temporally related to ingestion of salads: weakness, a feeling of dissociation from the body, dizziness, borderline hypotension, and bradycardia. These signs and symptoms are more consistent with vasovagal reactions than with anaphylaxis. One report has described a patient who received less than 2 mL of procaine (Novocaine) with epinephrine administered subcutaneously by her dentist (49). Within several minutes, she developed flushing, a sense of warmth, and pruritus, followed by scattered urticaria, dyspnea, and anxiety. Skin tests of various local anesthetics and sulfite proved negative. Thirty minutes after receiving a single-blind, oral dose of 10 mg of sodium bisulfite, she developed "a sense of fullness in her head, nasal congestion, and a pruritic erythematous blotchy eruption." No respiratory symptoms developed, and the investigators did not observe any pulmonary function test abnormalities. This patient was able to tolerate local anesthetics without epinephrine. Importantly, this patient did not describe a history of food-related symptoms. Furthermore, the usual dose of aqueous epinephrine (adrenalin) contains only 0.3 mg of sulfite, and local anesthetics contain only as much as 2 mg/mL of sulfite. Thus, the usual doses—even in the most sensitive persons—would not provoke reactions. The mechanism of this patient's reaction cannot be definitively linked to sulfite

and likely was a vasomotor response to the effects of epinephrine.

A DBPC challenge that reproduced urticaria after challenge with 25 mg of potassium metabisulfite was reported by Belchi-Hernandez et al (17). Skin tests were negative in this subject.

Two reports have demonstrated the inability to provoke reactions to sulfites in patients with idiopathic anaphylaxis, some of whom had histories of restaurant-associated symptoms (50, 51). In a study describing food-related skin testing in 102 patients with idiopathic anaphylaxis, only one patient was found to have metabisulfite sensitivity (52). In addition, the authors performed sulfite-ingestion challenges in 25 patients with chronic idiopathic urticaria and angioedema without a reaction (unpublished observations). At present, sulfite-induced urticaria, angioedema, or anaphylaxis appears to be a rare phenomenon.

Acute urticaria associated with leukocytoclastic vasculitis and eosinophilia was induced by a single placebo-controlled challenge with 50 mg sodium bisulfite in a subject suffering from recurrent urticaria and angioedema of unclear etiology. Blinded challenges were performed during a symptom-free period, followed by biopsy confirmation of the leukocytoclasis. Conscious avoidance of sulfites reduced the frequency of subsequent reactions dramatically (53).

Tartrazine/Azo Dyes

Murdoch et al (54) found at least 2 (8.3%) of 24 patients who developed hives after ingesting a panel of four azo dyes, including tartrazine. As previously indicated, Stevenson et al (28) found that only 1 (4.2%) of 24 aspirin-sensitive subjects undergoing double-blind challenge with 50 mg tartrazine developed urticaria. It appears, therefore, that tartrazine and other azo dyes rarely induce urticaria. The tartrazine-sensitive individual identified in Stevenson's study did not react to a blinded challenge with doses of aspirin of as much as 975 mg, suggesting a lack of cross-reactivity between tartrazine and aspirin.

Aspartame

Two cases of aspartame-provoked urticaria and angioedema have been reported. In these individuals, their hives emerged only after aspartame's 1983 approval as a sweetener in carbonated beverages. Both patients reported the onset of urticaria within 1 hour of ingesting aspartame-sweetened soft drinks. DBPC challenges reproduced urticaria with doses of aspartame (25–75 mg) that fall below the amount contained in typical 12-ounce cans (100–150 mg) (55).

In a well-publicized multicenter, randomized, placebo-controlled crossover study, Geha et al (56) challenged 21 subjects with histories of a temporal (minutes to hours) association between aspartame ingestion and urticaria/angioedema. These subjects were identified after an extensive recruiting process spanning 4 years. Only four urticarial reactions were observed—two following aspartame consumption and two following placebo ingestion. Doses ranged as high as 600 mg of aspartame.

BHA and BHT

In a DBPC study, Goodman et al (57) challenged two patients with chronic idiopathic urticaria who experienced remissions after following dye- and preservative-elimination diets. Both patients noted significant exacerbations of their urticaria after challenge with BHA and BHT. Subsequent avoidance of foods containing these antioxidants resulted in marked abatement of the frequency, severity, and duration of urticaria episodes. Long-term follow-up revealed urticarial flares after dietary indiscretion, but an otherwise quiescent disease.

Monosodium Glutamate

Squire described a 50-year-old man with recurrent angioedema of the face and extremities that was related to a history involving ingestion of a soup containing MSG (58). A single-blind, placebo-controlled challenge with the soup base resulted in "a sensation of imminent swelling" within a few hours, with visible angioedema emerging 24 hours after the challenge. In a graded challenge with only MSG, angioedema occurred 16 hours after challenge with a dose of 250 mg. Avoidance of MSG led to extended remission. Details of the challenge were not reported, nor did the author mention whether medications were withheld during challenges.

Nitrates/Nitrites

Hawkins et al (59) reported a single case of recurrent anaphylaxis occurring after eating take-

out food. DBPC capsule challenge with 25 mg each of sodium nitrates and sodium nitrite resulted in an acute anaphylactic reaction with hypotension within 15 minutes of the active challenge.

Food Additive Sensitivity in Chronic Idiopathic Urticaria/Angioedema

Malamin and Kalimo (7) performed prick and scratch skin tests on 91 individuals with chronic idiopathic urticaria/angioedema (CIUA), utilizing a panel of 18 food additives and preservatives. A positive response was defined as a wheal greater than or equal to the size of the histamine control. Sixty-four (26%) subjects had at least one positive skin test as compared with 25 (10%) of 247 nonurticaria control subjects. Ten of the 24 CIUA patients with positive skin tests underwent oral provocation with the additives that gave the positive skin test results. Details of the challenge procedure were not provided. Only one patient reacted, experiencing an urticarial reaction to benzoic acid. The activity level of the patient's prechallenge urticaria was not noted.

At Scripps Clinic and Research Foundation, patients with CIUA are undergoing single-blinded challenges with a panel of additives. Amounts of each additive are listed in Table 23–2. Positive reactors are confirmed with DBPC challenges. To date, no true positive reactors have been identified among more than 100 patients (60, and unpublished data). From these data, we can conclude with 95% confidence limits that sensitivity to any of the 11 food and drug additives in patients with CIUA is less than 1%.

Volonakis and colleagues (61) performed an extensive analysis of etiologic factors in 226 children with chronic urticaria. Elimination of food additives and DBPC challenges performed with a panel of four additives (tartrazine, sodium benzoate, nitrates, and sorbic acid) plus aspirin resulted in an overall incidence of 6 (2.6%) of the 226 cases attributable to these additives. Half of these patients (3 of 226, or 1.3%) reacted to aspirin (a known exacerbator of chronic urticaria), and the remaining 3 subjects (1.3%) reacted to tartrazine. No benzoate, nitrate, or sorbic acid reactions occurred among their subjects.

Table 23–2.
Suggested Maximum Doses for Additives Used in Challenge Protocols

Yellow Dyes #5 and #6: 50 mg
Sulfites: 100 mg
MSG: 2.5 g
Aspartame: 150 mg
Parabens/benzoates: 100 mg
BHA/BHT: 250 mg
Nitrates/nitrites: 50 mg

Food and Drug Additive Skin Test and Case Report Studies

Carmine

Several cases of carmine-induced urticaria, angioedema, and anaphylaxis have been described (5, 8–10). These have followed the initial case reports of carmine-induced anaphylaxis by Kagi et al (62) and Beaudouin et al (63). The food products implicated include Campari-Orange liqueur, Yoplait brand custard style strawberry-banana yogurt, imitation crab meat, Good Humor SnoFruit popsicle, and ruby red grapefruit juice. SPTs were positive with undiluted carmine in the history-positive patients and negative in control subjects. Contact urticaria associated with carmine-colored cosmetics have also been reported (10). Specific challenges have not been performed with carmine in any of the above reports, with the exception of Baldwin et al (8), whose patient showed negative oral challenges to each of the other components of the Good Humor SnoFruit popsicle. As noted above, these collaborators at the University of Michigan have demonstrated convincingly that an IgE-mediated mechanism is responsible for these reactions.

Annatto

Case reports of anaphylactic reactions to annatto dye have been documented. Revan et al (12) describe one patient with anaphylaxis (4+ SPT with undiluted extract) and one with angioedema (3+ SPT with undiluted extract) after ingesting annatto-containing foods. Neither patient was challenged.

Parabens/Benzoate

Macy's local anesthetic analysis noted above strongly suggests that methylparaben is a cause of local immediate hypersensitivity reactions previously attributed to the local anesthetics themselves (20). One isolated case report of sodium

benzoate induced anaphylaxis was reported by Michils (6).

Isosulfan Blue

Askenazi et al (64) recently reported three cases of anaphylactic shock to ISB dye used as a lymphatic contrast agent. All three patients reacted within 30 minutes of subcutaneous injection of ISB and all three had positive ISB skin tests. All of the 10 control subjects had negative skin tests to ISB. Two patients had elevated tryptase levels, indicating MC degranulation. All three patients were re-exposed to latex and the other perioperative concomitant medications after their reactions to ISB and demonstrated tolerance to them. Previous retrospective analyses demonstrate an acute allergic reaction rate of 1.1%–2.0% (65, 66).

Nitrates

Asero (67) recently reported a case of chronic generalized pruritis without skin eruption that disappeared on an additive-free diet. DBPC challenge with multiple additives resulted in symptom reproducibility within 60 minutes of the 10 mg sodium nitrate challenge. The patient did not react to seven other additives and multiple placebos.

Recommendations for Food Additive Challenge Protocols in Patients with Urticaria, Angioedema, and/or Anaphylaxis

A review of the literature on food and drug additive challenges in patients with urticaria suggests that more rigorously conducted studies are needed. With the use of more objective criteria and stringent design, more meaningful conclusions may be drawn regarding the true incidence of food additive-induced urticaria, angioedema, and anaphylaxis. Our recommendations for future additive challenge protocols in patients with chronic or acute urticaria/angioedema are presented in the following sections.

Patient Selection

In view of the ubiquitous and frequent dietary exposure to food and drug additives, the study population should be selected from patients with chronic "idiopathic" urticaria or angioedema, unless the study is intended to examine another defined subgroup of patients with acute or intermittent urticaria, angioedema, and/or anaphylaxis (e.g., patients with a convincingly positive acute history or patients responsive to an elimination diet). The diagnosis of chronic idiopathic urticaria or angioedema should be made in subjects with recurrent urticaria of at least 6 weeks' duration without identifiable cause. In addition, appropriate challenges should be conducted to ascertain any physical urticarias. After a negative workup, a patient's urticaria may then be considered idiopathic (68).

Activity of Urticaria

Chronic urticaria should preferably be in an active phase (e.g., some lesions should have appeared within 1 month prior to challenge), as additives may not only provoke urticaria de novo, but also exacerbate ongoing urticaria, as is true with aspirin (33). For patients with an intermittent and/or acute anaphylactic history associated with an additive, challenges should not be conducted for at least 2 weeks time after the last acute reaction.

Medications

Antihistamines should be withheld for 3–5 days prior to the challenges, if possible. For patients with intractable chronic symptoms, antihistamines should be tapered to the minimal effective dose. Although corticosteroids are not first-line treatment for chronic urticaria/angioedema, when necessary their use should also be tapered to the minimal effective dose.

Diet

Patients should be placed on a diet free of all additives included in the challenge protocol at least 1 week prior to challenge.

Reaction Criteria

Reaction criteria should be as objective as possible. The "rule of nines" used for assessing thermal burns provides a useful method for estimating skin surface area. On each of the 11 divided areas of the body, the investigator assigns a score of 0–4, then derives a total score (0–44 points). A positive urticarial response may be defined as either an absolute increase in the total score of 9 points or an

increase of more than 300% from the baseline score determined immediately before challenge. A positive angioedema response may be defined as a relative increase in size of more than 50% in the body part affected.

Baseline Observation

Prior to any challenges, skin scores should be recorded at the same intervals during a baseline period of observation as during challenges. The appropriate length of the baseline observation period depends on factors such as the activity of the patient's urticaria, the interval of time between discontinuation of antihistamines and the challenges, and the length of the challenge protocol.

In general, one day of pure observation with skin scoring should be followed by one day of single-blind placebo challenge with skin scoring, except perhaps in patients who are completely free of hives at challenge (in this instance, one day of placebo challenge should be sufficient). Skin scores on those 2 days should not vary by more than 3 points or 30% (whichever is greater) before proceeding to additive and further placebo challenges.

Placebo Controls

Placebo challenge should be conducted in a randomized fashion. Ideally, at least an equal number of placebo and active challenges should be undertaken. Screening open challenges may be performed without placebo. Here, a negative result does not require further confirmation, but positive reactors must undergo a placebo-controlled protocol, preferably double-blinded.

Blinding

Confirmatory challenges should preferably be conducted in a double-blind manner. Coded opaque capsules will serve for this purpose. The code should not be broken until the completion of all challenges. Screening challenges may be performed open or single-blinded. Any "positive challenges" should be confirmed with a double-blind protocol.

Additive Doses

The additive doses used in challenge protocols should reflect natural exposure to each agent. Suggested limits for some common additives are listed in Table 23–2. Starting doses should be individualized on the basis of the patient's history, but usually consist of 1/100 of the maximum dose. Challenges must be performed with informed consent and in a setting where severe reactions may be appropriately treated.

Conclusion

Unfortunately, only a small number of well-designed clinical studies have been conducted in the area of additive-provoked urticaria, angioedema, and anaphylaxis. The incidence of such reactions remains unknown, although it appears to be a relatively rare phenomenon despite claims in earlier (pre-1990) additive literature. Natural additives (carmine and annatto) contain source proteins capable of inducing direct IgE-mediated immediate hypersensitivity reactions. The case for similar immediate hypersensitivity reactions is less compelling when the synthetic additive group is analyzed. A relatively small number of case reports describing IgE-mediated reactions to sulfites and parabens exist, and the mechanisms responsible for such reactions are unclear at present.

It is now well accepted that many cases of CIUA have an autoimmune basis, as demonstrated by the presence of autoantibodies directed against the IgE receptor and/or IgE itself (68). CIUA is frequently associated with other autoimmune syndromes, most notably thyroid autoimmunity (68). Studies attempting to link causation and/or exacerbation of this condition by food or drug additives have been poorly designed. Emerging evidence appears to refute the earlier notion that these additives are frequently associated with chronic urticaria. Guidelines for conducting additive challenges in CIUA as well as in episodic urticaria/angioedema patients are reviewed in the text.

Although rare, IgE-mediated paraben reactions can confound the diagnostic evaluation of local anesthetic allergy, given the use of this preservative in multi-dose vials of these medications.

Finally, given the anticipated widespread usage of ISB as a lymphangiography contrast agent, the incidence of reported hypersensitivity reactions to this agent may escalate in the future.

Further well-designed trials addressing additive-provoked urticaria, angioedema, and anaphylaxis are needed before more definitive practice parameters can evolve.

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Sulfites

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Sulfites or sulfiting agents include sulfur dioxide (SO_2), sulfurous acid (H_2SO_3), and any of several inorganic sulfite salts that may liberate SO_2 under their conditions of use. The inorganic sulfite salts include sodium and potassium metabisulfite ($Na_2S_2O_5$, $K_2S_2O_5$), sodium and potassium bisulfite ($NaHSO_3$, $KHSO_3$), and sodium and potassium sulfite (Na_2SO_3, K_2SO_3). Sulfites have a long history of use as food ingredients, although potassium sulfite and sulfurous acid are not permitted for use in foods in the US (1). Sulfites occur naturally in many foods, especially fermented foods such as wines (1, 2). In addition, sulfites have long been used as ingredients in pharmaceuticals (3, 4).

Over the past 20 years, questions have arisen about the safety of sulfites in foods and drugs. These concerns were first voiced following independent observations in 1981 by Allen in Australia and Stevenson and Simon in the US, of the role of sulfites in triggering asthmatic reactions in some sensitive individuals (5–7). Although it is now apparent that sulfite sensitivity affects only a small subgroup of the asthmatic population (7–9), concerns remain because sulfite-induced asthma can be severe—even life-threatening—in some sensitive individuals.

As a consequence of the concerns related to sulfite-induced asthma, the use of sulfites in foods and drugs has changed considerably over the years. Sulfites have been replaced in some products; and the search for effective alternatives continues; in addition, levels of sulfites have been reduced in other products. Federal regulations have further restricted the use of sulfites in certain food products in the US. Nevertheless, the sulfite-sensitive individual must stay alert to avoid inadvertent exposure to sulfites.

Clinical Manifestations of Sulfite Sensitivity

A host of adverse reactions have been attributed to sulfiting agents, as reported to the US Food and Drug Administration (FDA) (10). The effects include diarrhea, abdominal pain and cramping, nausea and vomiting, urticaria, pruritis, localized angioedema, difficulty in swallowing, faintness, headache, chest pain, loss of consciousness, "change in body temperature," "change in heart rate," and nonspecific rashes. In most instances, diagnostic challenges were not undertaken to confirm the reported adverse reaction. For normal individuals, exposure to sulfiting agents appears to pose little risk. Toxicity studies in normal volunteers showed that ingestion of 400 mg of sulfite daily for 25 days had no adverse effect (11).

Nonasthmatic Responses to Sulfites

Various authors have suggested adverse reactions involving several organ systems, but for the most part these effects have not been substantiated by double-blind, placebo-controlled (DBPC) provocation studies. Schmidt et al (12) posited that sulfiting agents may have caused the appearance of a cardiac arrhythmia in a patient given intravenous (IV) dexamethasone. This relationship was never confirmed by appropriate challenge, however. Hallaby and Mattocks (13) attributed central nervous system (CNS) toxicity to the absorption of sodium bisulfite from peritoneal dialysis solutions. Wang et al (14) described eight patients who developed chronic neurological defects after receiving an epidural anesthetic agent that contained sodium

bisulfite as a preservative. Using an animal model, they demonstrated that the sulfiting agent produced a similar defect. Whether the clinical manifestation in humans was directly attributable to the bisulfite ingredient is unknown. In a preliminary report, Flaherty et al (15) presented a patient who appeared to have hepatotoxicity as manifested by changes in liver function tests following challenge with potassium metabisulfite.

Other adverse reactions suggestive of a hypersensitivity response have been observed in nonatopic individuals. Epstein (16) described a patient who developed contact sensitivity through exposure to sulfiting agents used in a restaurant; the patient's condition was later confirmed by appropriate patch testing. Belchi-Hernandez et al (17) reported a single case of sulfite-induced urticaria induced by ingestion of sulfited foods and beverages. The role of sulfites was confirmed by DBPC challenge. However, the toxicological mechanism involved in this reaction was not elucidated. Two patients have been reported (18) who presumably experienced urticaria and angioedema after ingesting sulfiting agents. On open challenge with potassium metabisulfite, one of the individuals experienced generalized urticaria. The study was not repeated using a double-blind procedure. Another individual described in the literature reportedly developed urticaria after administration of sulfited medications (19), although the reaction was not confirmed by challenge. Huang and Frazier (20) presented an individual who developed palmar and plantar pruritis, generalized urticaria, laryngeal edema, and severe abdominal pain with fulminant diarrhea after ingesting sulfiting agents. In a controlled challenge with a local anesthetic containing 0.9 μg of sodium metabisulfite, the patient experienced palmar pruritis but no generalized urticaria.

Role of Sulfites in Anaphylaxis

Anaphylaxis-like events have been described in several individuals, although appropriate confirmatory testing was performed in only some instances. Prenner and Stevens (21) described a non-asthmatic individual who developed urticaria, pruritis, and angioedema after eating sulfited foods in a restaurant. A single-blind challenge with no placebo controls was conducted with sodium metabisulfite. Some of the symptoms (nausea, coughing, erythema of the patient's skin) were reproduced by this challenge. Clayton and Busse (22) reported a patient who developed anaphylaxis after ingesting wine. An open challenge with wine reproduced the patient's symptoms of urticaria, angioedema, and hypotension. Although this patient represents a possible case of sulfite sensitivity, specific testing with sulfites was not conducted, nor was any association with sulfiting agents in wine recognized at that time.

Sokol and Hydick (23) identified a single case of sulfite-induced anaphylaxis presenting with urticaria, angioedema, nasal congestion, and nasal polyp swelling that was later confirmed by multiple, single-blind, placebo-controlled oral challenge trials. The patient, who had a history of similar food-related reactions, also produced a positive skin test to sulfite, and histamine could be released from her basophils following incubation with sulfites. Yang et al (24) described three patients with systemic anaphylactic symptoms (rhinorrhea with asthma in one; urticaria with asthma in the second; asthma only in the third) confirmed by sulfite challenge. These three patients had positive skin tests to sulfites, and two of the three had positive Prausnitz-Küstner (PK) tests. One individual subsequently died, allegedly after ingestion of sulfited food.

In addition, systemic adverse reactions have been attributed to IV and inhalation administration of sulfiting agents contained in pharmaceutical products. While receiving bronchodilator therapy with isoetharine, an asthmatic subject developed acute respiratory failure that required mechanical ventilation (25). The patient subsequently experienced erythematous flushing with urticaria upon IV administration of metaclopramide that contained a sulfiting agent. In placebo-controlled oral provocation with sodium metabisulfite, this patient developed flushing without urticaria, as well as a significant decrease in pulmonary function. Jamieson et al (26) performed inhalation challenge in a patient with presumed sulfite sensitivity. They observed intense pruritis, tingling of the mouth, nausea, chest tightness, and a feeling of impending doom. No placebo challenge was undertaken, however.

Schwartz (27) described two nonasthmatic subjects who developed abdominal distress and hypotension associated with oral challenge with potassium metabisulfite. Placebo-controlled challenges proved negative, however. Wuthrich (28) conducted single-blind, placebo-controlled challenges with sodium bisulfite in 245 patients with suspected sulfite sensitivity. Fifty-seven (15%) of the challenges were positive including 17 patients

with urticaria/angioedema, 7 with rhinitis, and 5 with local anesthetic reactions. Wuthrich et al (29) reported a case of acute intermittent urticaria with an associated vasculitis due to sulfites based on a placebo-controlled, single-blind challenge.

Studies have been undertaken to determine whether sulfiting agent sensitivity frequently causes idiopathic anaphylaxis or chronic idiopathic urticaria (30–33). Sonin and Patterson (30) conducted sodium metabisulfite challenges on 12 individuals with idiopathic anaphylaxis, nine of whom reported episodes associated with restaurant meals. None of the patients responded to the challenge. One additional patient with chronic idiopathic urticaria (CIU) and restaurant-associated symptoms was also challenged; this individual also failed to react to the challenge. Meggs et al (31) studied 25 patients with idiopathic anaphylaxis. Two of the individuals reacted on single-blind challenge; after repeating the sulfite and placebo challenge, one of these patients was subsequently found not to be sulfite-sensitive. Another individual appeared to react on repeated challenge and not to placebo. However, institution of a sulfite-free diet had no effect on this patient's subsequent episodes. Furthermore, studies by Meggs' group in eight individuals with systemic mastocytosis failed to demonstrate any reactions to sulfite challenge. In a preliminary report on 65 adults with CIU, none reacted to sulfites when appropriately challenged (32). Using a rigorous blinded, placebo-controlled trial and objective criteria for positive reactions, Simon (33) was unable to demonstrate a positive reaction to encapsulated metabisulfite (200 mg maximum dose) in 75 patients with chronic urticaria and/or anaphylaxis with a history suggestive of sulfite sensitivity.

Thus, although many adverse reactions have been ascribed to sulfiting agents, the risk appears to be rather low for the non-atopic, nonasthmatic subject. Properly performed DBPC challenges will be necessary to confirm whether sulfite sensitivity was responsible for suspected adverse reactions.

The Role of Sulfiting Agents in Asthma

Although sulfiting agents play a very limited and somewhat controversial role in the causation of nonasthmatic adverse reactions, their role in bronchospasm and severe asthma is better established. Kochen (34) was among the first to suggest that ingestion of sulfited food can cause bronchospasm. He described a child with mild asthma who repeatedly experienced coughing, shortness of breath, and wheezing when exposed to dehydrated fruits treated with sulfur dioxide that were packaged in hermetically sealed plastic bags. No direct challenge studies were conducted to confirm this observation, however. Single-dose, open challenges without placebo control performed in a group of asthmatics by Freedman (35, 36) suggested that sulfiting agents could trigger asthma. Eight of 14 subjects with a history of wheezing following consumption of sulfited orange drinks were shown to experience changes in pulmonary function upon administration of an acidic solution containing 100 ppm of sodium metabisulfite.

The role of sulfite sensitivity in asthma became more widely recognized after reports of Stevenson and Simon (6) and Baker et al (5). The initial studies of Stevenson and Simon (6) demonstrated that placebo-controlled oral challenges with potassium metabisulfite could produce significant changes in pulmonary function in certain asthmatics. Their first subjects had steroid-dependent asthma. In addition to their asthmatic response, these individuals experienced flushing, tingling, and faintness following sulfite challenges. A study in two steroid-dependent asthmatics showed that oral ingestion and IV administration of sulfites could cause bronchoconstriction to the point of respiratory arrest (5). In a preliminary study, Baker and Allen (37) reported a spectrum of asthmatic responses to sulfite, including an immediate decline in pulmonary function occurring 1–5 minutes after ingestion of an acidic metabisulfite solution, and a delayed response 20–30 minutes after ingestion of solid foods treated with sulfiting agents. Other patients responded to parenteral medications containing sulfiting agents administered through an IV route. In addition, some patients were believed to have chronic asthma caused by continuous ingestion of sulfited foods. Although anecdotal and clinical oral challenges support the observation that sulfites can provoke acute bronchoconstriction (both the immediate and delayed responses), data to support the continuous response have not been forthcoming.

Exposure to sulfiting agents may occur through ingestion and other routes. Sulfur dioxide generated from sulfited foods and drugs may be inhaled. Werth (38) described an asthmatic individual who developed wheezing, flushing, and diaphoresis upon inhaling the vapors released from a bag of dried apricots. The patient did not respond to ingested metabisulfite in capsule form, but reacted to inhalation of nebulized metabisulfite in dis-

tilled water. Reports have described several patients who suffered paradoxical responses to the inhalation of bronchodilator solutions. Koepke et al (39, 40) demonstrated that sodium bisulfite used as a preservative in bronchodilator solutions was capable of producing bronchoconstriction. Other studies from this group (41) confirmed that the concentration of metabisulfite contained in bronchodilator solutions could potentially generate 0.8–1.2 ppm of SO_2. Four of 10 subjects who tested negative to a capsule challenge with metabisulfite reacted upon inhalation, whereas 10 nonasthmatic controls did not respond.

In addition to sulfiting agents administered intravenously, orally, or via inhalation, patients may respond to the topical application of sulfiting agents. Schwartz and Sher (42) reported an individual who experienced a 25% decrease in forced expiratory volume in 1 second (FEV_1) after application of one drop of a 0.75 mg/mL potassium metabisulfite solution to the eye. This patient had previously experienced episodes of bronchoconstriction from the use of eye drops containing sulfite preservatives for the treatment of glaucoma.

Asthmatic subjects may develop bronchoconstriction in response to a wide variety of stimuli. Interestingly, a patient has been described (43) who failed to respond to typical triggers of bronchoconstriction, including inhalation of methacholine and cold air hyperventilation, but who nevertheless experienced increased airway resistance and decreased specific airway conductance following oral challenge with potassium metabisulfite. The significance of this response remains unknown, as no changes in other parameters of pulmonary function, including FEV_1, were observed.

The potential for fatal reactions from sulfite exposure has been confirmed (10, 24, 44). In many instances, individuals who supposedly died from an adverse reaction to sulfite had not undergone appropriate diagnostic challenges. Nonetheless, competent investigators observed that severe bronchoconstriction, hypotension, and loss of consciousness can occur, demonstrating the potential for fatal reactions in some subjects—particularly those with steroid-dependent asthma.

Prevalence

Adult Populations

The prevalence of adverse reactions to sulfiting agents is not precisely known. Although attempts have been made to establish the prevalence of sulfite sensitivity in asthmatic subjects, the nature of the population studied and use of several different challenge methods in these studies has resulted in some uncertainty regarding the prevalence estimates. Simon et al (9) examined the prevalence of sensitivity to ingested metabisulfite in a group of 61 adult asthmatics. None indicated a history of sulfite sensitivity. After challenges were conducted with potassium metabisulfite capsules and solutions, a placebo-controlled challenge was used to confirm positive responses. Five (8.2%) of 61 patients experienced a 25% or greater decline in FEV_1 upon challenge.

Koepke and Selner (45) conducted open challenges with sodium metabisulfite in 15 adults with a history of asthma after ingestion of sulfited foods and beverages. One (7%) of 15 patients showed a 28% decline in FEV_1; no confirmatory challenge was conducted. In a larger study by Buckley et al (46), 134 patients underwent single-blind challenges with potassium metabisulfite capsules. Of these subjects, 6 (4.5%) were suspected of having sulfite sensitivity. In these studies, the population consisted of a large proportion of steroid-dependent asthma patients being treated at major referral centers, although sulfite sensitivity was diagnosed in several mild asthmatics as well (7). Thus, the prevalence estimated from these studies may not be applicable to the asthma population as a whole. Wuthrich (28) challenged 87 suspected sulfite-sensitive asthmatics (SSAs) with capsules containing sodium bisulfite (5–200 mg doses). Fifteen (17.2%) of 87 asthmatics reacted to these sulfite challenges, but the proportion of steroid-dependent asthmatics in this study population was not determined. Because subjects were selected for suspected sulfite sensitivity, the results of this study cannot be used to assess the prevalence of sulfite sensitivity in the overall population of asthmatics.

In the largest study conducted to date, Bush et al (8) conducted capsule and neutral solution sulfite challenges in 203 adult asthmatics. None was selected based on a history of sulfite sensitivity. Of these patients, 120 were not receiving corticosteroids, and 83 were steroid-dependent. Of the non-steroid-dependent group, only one experienced a 20% or greater decline in FEV_1 after single-blind and confirmatory double-blind challenge. The steroid-dependent asthma group had a higher response rate, estimated at approximately 8.4%. The prevalence in the asthmatic population as a whole was less than 3.9%, with steroid-dependent asthmatic patients appearing to face the greatest risk.

Pediatric Population

Limited studies have been conducted in children. Towns and Mellis (47) evaluated 29 children aged 5.5–14 years with moderate to severe asthma. Seven subjects had a history suggestive of sulfite sensitivity. Challenges were conducted with placebo on one day and with sequential administration of sodium metabisulfite in capsule and solution form on a second day. Nineteen (66%) of the subjects showed a decrease in the peak expiratory flow rate (PEFR) varying from 23% to 72%, while PEFRs with placebo were either unaffected or dropped less than 19%. While a 20% decline in PEFR was viewed as a positive response, 19 (66%) of these children were considered to be sulfite-sensitive. Subsequently, the patients were instructed to avoid sulfited food for 3 months, but no overall significant improvement appeared in the patients' asthma as a result of this avoidance diet.

Friedman and Easton (48) studied 51 children aged 5–17 years. Eighteen (36%) of them showed a 20% or greater decrease in FEV_1 when provoked with potassium metabisulfite in an acidic solution, although placebo challenges in these individuals showed only one responder. No differences in steroid use were noted between responders and non-responders. Steinman et al (49) evaluated 37 asthmatic children and determined that 8 (22%) responded to double-blind challenges of sulfited apple juice with a 20% or greater decline in FEV_1. An additional eight children were considered to experience a reaction to sulfite when the criterion for a positive reaction was changed to a 10% or greater decrease in FEV_1. In contrast, a study by Boner et al (50) determined that only 4 (7%) of 56 asthmatic children responded to single-blind challenges with sulfite in capsules and/or solutions. Furthermore, the sulfite-sensitive individuals displayed no additional change in bronchial reactivity as assessed by methacholine challenges conducted after sulfite reactions. In this study, a positive response was defined as a 20% decline in FEV_1.

Whether sulfite sensitivity really occurs more frequently in children has yet to be definitively established. Difference in challenge procedures (capsule vs acidic beverage solutions) may account for the observed differences. Nonetheless, the overall prevalence of sulfiting agent sensitivity—particularly in adult asthmatics—is small but significant. Steroid dependency appears to be a coincident risk factor, particularly in adult asthmatics.

Mechanisms

The mechanisms of sulfiting agent sensitivity remain unknown. Depending on the route of exposure, a number of possible mechanisms have been hypothesized. Asthmatics experience significant bronchoconstriction upon inhalation of less than 1.0 ppm of SO_2 (51). Fine and coworkers (52) demonstrated that bronchoconstriction developed in asthmatics who inhaled SO_2 and bisulfite (HSO_3^-), but not sulfite (SO_3^{2-}). Alteration of airway pH itself did not cause bronchoconstriction. Thus, asthmatics may respond to forms of sulfite that depend on pH and the ionic species. Some asthmatics also respond to either oral or inhalation challenge with sulfite, although inhalation appears more apt to produce a bronchoconstrictive response (53). However, inhalation of SO_2 or various sulfites may not be the total explanation. Field et al (54) challenged 15 individuals with increasing concentrations of SO_2 gas or a metabisulfite solution. All 15 subjects reacted to the metabisulfite solution, and 14 of the 15 reacted to inhaled SO_2 with a 20% or greater drop in FEV_1. The investigators concluded that the generation of SO_2 gas cannot fully explain sulfite-induced asthma (54).

Considerable variability has been noted in the response to capsule and acidic beverage challenges with sulfiting agents (55). When challenged on repeated occasions, the same group of individuals may not consistently respond with bronchoconstriction. This variability may provide some clues to understanding of the mechanism of sulfite-induced asthma.

Inhalation During Swallowing

In a study of 10 SSA subjects, Delohery et al (56) demonstrated that all of the subjects reacted to an acidic metabisulfite solution when it was administered as a mouthwash or swallowed. However, none of these subjects reacted when the metabisulfite was instilled through a nasogastric tube. These same individuals did not respond with changes in pulmonary function when they held their breath while swallowing the solution. A control group of 10 non-SSAs showed no response to the mouthwash or swallowing challenge. The authors hypothesized that some individuals respond to these forms of challenge because they inhale SO_2 when they swallow.

Linkage with Airway Hyperreactivity

Because asthmatics respond to various stimuli (airway irritants) at concentrations lower than do normal individuals (i.e., they exhibit airway hyperresponsiveness), attempts have been made to link sulfite sensitivity with airway responsiveness to histamine and methacholine. Such an association has not been established (50, 56). For example, Australian investigators (56) were unable to demonstrate a relationship between the degree of airway responsiveness to inhaled histamine and the presence of sulfite sensitivity.

In human studies, attempts to block the effect of metabisulfite by agents such as inhaled lysine aspirin, inhaled indomethacin, and inhaled sodium salicylate demonstrated a slight protective effect, suggesting a possible role of prostaglandins in the mechanism of sulfite sensitivity (57). Further, leukotriene receptor antagonists attenuate SO_2-induced bronchoconstriction, implying that leukotriene release may also be involved (58). Administration of the neutral endopeptidase inhibitor, thiorphan, was shown to enhance the airway response to inhaled sodium metabisulfite challenge in normal individuals (59). This study suggests that tachykinins may play a role in metabisulfite-induced bronchoconstriction (59). This mechanism was also supported by observations in guinea pigs that capsaicin-sensitive sensory nerves are involved in sulfite-induced bronchoconstriction (60). Inhaled magnesium sulfate also has been shown to mildly inhibit inhaled metabisulfite-induced bronchoconstriction, but the mechanism is not known (61).

Refractoriness has been demonstrated to a number of indirect bronchoconstrictor stimuli including metabisulfite. The generation of nitric oxide (NO) as a possible explanation for the refractoriness has been investigated in asthmatic subjects undergoing inhaled metabisulfite challenge (62). Blockage of NO had no effect on either the response to metabisulfite per se or the refractory process, suggesting that NO is not involved in metabisulfite-induced bronchoconstriction.

Other animal models demonstrated that application of sodium metabisulfite to trachea of anesthetized sheep increased local blood flow and vascular permeability and induced epithelial damage (63). Sulfite-induced bronchoconstriction in sheep may also involve stimulation of bradykinin B_2-receptors, which may subsequently activate cholinergic reflex mechanisms (64).

Our group attempted to induce sulfite sensitivity in a group of 16 asthmatic subjects (unpublished). After the provocative dose of methacholine producing a 20% decrease in FEV_1 was established, a sulfite challenge using an acidic sulfite solution was instigated to identify any sulfite sensitivity. Three (19%) of the 16 subjects reacted to the sulfiting agent with a 20% or greater decrease in FEV_1. One week after this challenge, the patients underwent bronchial challenge with an antigen to which they exhibited sensitivity. The following day, the patients returned for a repeat methacholine challenge, followed by a second sulfite challenge 24 hours later. After the antigen challenge, only one additional subject showed a response to sulfiting agent that had not been present before antigen challenge. No significant increase was observed in airway response to methacholine. Thus, this study did not link airway hyperreactivity and sulfite sensitivity. Similar negative results were obtained in a study of asthmatic children (50).

Cholinergic Reflux

Because SO_2 may produce bronchoconstriction through cholinergic reflex mechanisms, preliminary studies have examined the effect of atropine and other anticholinergic agents (65). Inhalation of atropine blocked the airway response to sulfiting agents in three of five subjects and partially inhibited the response in the other two subjects. Doxepin, which possesses both anticholinergic and antihistaminic properties, had protective effects in three of five individuals. In a study on sheep, inhaled metabisulfite induced bronchoconstriction that could be prevented by pretreatment with either ipratropium bromide or nedocromil sodium, but not by chlorpheniramine (64). Sulfite-induced bronchoconstriction in these sheep was also associated with a nine-fold increase in immunoreactive kinins. Consequently, Mansour et al (64) concluded that sulfite-induced bronchoconstriction in sheep involves stimulation of bradykinin B_2-receptors with subsequent activation of cholinergic mechanisms. Studies in guinea pigs suggest that capsaicin-sensitive sensory nerves may play a role in sulfite-induced bronchoconstriction (60).

Possible IgE-Mediated Reactions

Adverse reactions to sulfites appear most commonly in atopic individuals, and studies have

attempted to identify an immunologic basis for these reactions. Several reports have demonstrated positive skin tests to solutions of sulfiting agents in some sensitive patients. The positive skin tests and other related evidence may point to the existence of an IgE-mediated mechanism in at least some sulfite-sensitive individuals.

Prenner and Stevens (21) reported a patient with a positive scratch skin test to an aqueous solution of sodium bisulfite at 10 mg/mL. This patient also exhibited a dramatic response to intradermal testing at the same concentration. Three non-sensitive control subjects had negative skin tests. The patient of Twarog and Leung (25) also showed a positive intradermal skin test response to an aqueous solution of bisulfite at 0.1 mg/mL whereas controls were negative with concentrations up to 1.0 mg/mL of the solution. Yang et al (24) also identified several asthmatic subjects with either positive prick or intradermal skin test to sulfites. Boxer et al (66) identified two additional cases with positive skin tests who also had positive oral challenges to sulfiting agents. Selner et al (67) reported positive intradermal and skin prick tests (SPTs) with 0.1 mg/mL and 10 mg/mL of potassium metabisulfite solutions, respectively, in an SSA subject. This patient also had a positive intradermal test with a 0.1 mg/mL solution of acetaldehyde hydroxysulfonate, a major bound form of sulfite in wine and other foods (67). Control subjects had negative skin tests.

Further evidence for an IgE mechanism can be found in positive passive transfer tests (PK transfer). Several investigators have successfully transferred skin test reactivity to non-sensitized subjects with sera from sulfite-sensitive individuals (21, 24, 68). The effect can be abolished by heating sera to 56°C for 30 minutes (68). These observations suggest the presence of a serum factor (IgE). However, specific IgE antibodies to sulfiting agents have not been demonstrated (66, 68).

Sulfiting agents can induce mediator release from human mast cells (MCs) and basophils obtained from some sensitive individuals. Histamine release has been demonstrated in mixed peripheral blood leukocyte studies in sulfite-sensitive individuals (23, 25). Similarly, Meggs et al (31) noted a significant rise in plasma histamine levels in two of seven subjects with systemic mastocytosis undergoing a sulfite challenge. No clinical response was observed in these patients, however. In a skin test-positive asthmatic, a tripling of plasma histamine level was observed during an asthmatic response to sulfite challenge. When challenged intranasally with 5 mg of potassium metabisulfite in distilled water, four subjects with asthma or rhinitis attributed to sulfite exposure demonstrated increased histamine levels in nasal lavage fluid 7.5 minutes after challenge (69). Similar results were obtained in chronic rhinitis control subjects, although the histamine levels generally fell below those found in patients with sulfite sensitivity (69). In contrast, other investigators have not been successful or noted inconsistent results in attempting to demonstrate histamine release from the MCs or basophils among sulfite-sensitive individuals (6, 27, 70, 71). Histamine, per se, may not play a significant role in sulfite-induced airflow obstruction since H_1 receptor antagonists fail to block the response (58).

Indirect evidence for the role of MC mediators in the production of bronchoconstriction due to sulfiting agents has also been found. Friedman (36) mentions that inhaled sodium cromolyn prevented the asthmatic response. In preliminary studies, Simon et al (65) found that inhaled cromolyn inhibited sulfite-induced asthma in four of six subjects and partially inhibited the response in two other subjects. Schwartz (72) reported that oral cromolyn at a dose of 200 mg blocked an asthmatic response to oral sulfite challenge in a single individual.

Sulfite Oxidase Deficiency

Other possible mechanisms for sulfite sensitivity have also been suggested. Simon (73) proposed that a deficiency in sulfite oxidase, an enzyme that metabolizes sulfite to sulfate, may promote the adverse reactions. The skin fibroblasts of six sulfite-sensitive subjects exhibited less sulfite oxidase activity than normal controls. However, the major source of sulfite oxidase activity in humans is the liver. Further investigation will be needed to determine the importance of this suggested mechanism.

Diagnosis

The diagnosis of sulfite sensitivity cannot be established by the patient's history alone. Our group (8) was able to correlate the presence of a positive sulfite challenge with the patient's history, and vice versa. The diagnosis of sulfite sensitivity should, therefore, be made only in individuals who demonstrate an objective response upon appropriate challenge.

Skin testing, by both prick and scratch methods, has identified some individuals with positive responses (24, 66). In contrast, some individuals who have equally severe bronchospasm or other reactions had negative skin tests.

Diagnostic Challenges

Because diagnostic challenges represent the only effective confirmatory technique, and because such challenges may pose significant risk to sensitive subjects, patients must be informed of the risks involved. Physicians instituting such provocation procedures should have available all equipment necessary for the treatment of a severe bronchospastic or anaphylactic reaction, including airway intubation and mechanical ventilation. The end point for objective assessment of reactivity should be ascertained before the challenge begins. Such measures might include changes in airway function in asthmatics or the appearance of urticaria in patients with this type of response. Patients may be challenged with capsules, neutral solutions, or acidic solutions of metabisulfite. Some protocols previously reported in the literature are shown in Tables 24–1 and 24–2 (74). Currently, a capsule challenge is the preferred option, because most sulfite exposure is likely to involve bound forms of sulfites in foods rather than solutions.

When conducting challenges in a single-blind fashion, positive results should be confirmed by a double-blind procedure. Moreover, if a placebo day and an active challenge day are conducted on two separate occasions, the possibility of order effects on the results must be considered. For example, if a patient receives placebo on the first day and experiences no response, he or she may experience a reaction on the subsequent challenge day regardless of whether placebo or active challenge with sulfite is administered, because of increased anxiety. To overcome this possibility, the order of administration of active and placebo challenges should be randomized and a third challenge day, either active or placebo, potentially instituted.

Table 24–1.
Capsule and Neutral-Solution Metabisulfite Challenge*

Adapted from (74). Used with permission.

cause individuals may vary in their sensitivity to sulfited foods, it may be necessary to perform challenges with foods containing sulfites to determine which ones the patient can tolerate.

Table 24–2.
Acid-Solution Metabisulfite Challenge*

Adapted from (74). Used with permission.

Treatment

Avoidance of Sulfited Foods and Drugs

Sulfite-sensitive individuals should avoid sulfite-treated foods (75, 76) and drugs (74, 77) that have been shown to trigger the response. Be-

Some bronchodilator solutions, subcutaneous lidocaine, intravenous corticosteroids, and intravenous metaclopramide may pose a risk for sensitive subjects. Many pharmaceutical companies are aware of this possibility, however, and are taking steps to eliminate sulfiting agents from their products. A partial list of sulfited medications appears in Table 24–3. Package inserts for suspect medications should be consulted for the latest information.

Use of Injectable Epinephrine

Although some forms of epinephrine contain sulfite used as a preservative, administration of this drug has not been shown to cause a reaction in sulfite-sensitive individuals. Apparently, epinephrine's action overcomes any adverse effects attributable to the preservative. Thus, patients who are inadvertently exposed to sulfites typically find self-administration of epinephrine useful. Self-injection with an automatic dispenser of epinephrine, which delivers 0.3 mL of a 1:1000 solution (0.3 mg) for adults, is available (Epi-Pen, Dey, Napa, CA). A similar device available for children delivers 0.15 mL of a 1:1000 solution of epinephrine.

Use of Blocking Agents

Limited studies have been conducted with a variety of agents that may block the responses to sulfite, including cromolyn sodium, atropine, doxepin, and vitamin B_{12} (65, 78). Although these treatments have demonstrated beneficial effects in limited numbers of patients, they remain investigational and cannot be recommended for standard use.

Table 24–3.
Some Anti-asthma Preparations That Contain Sulfites

Epinephrine	Adrenaline (Parke-Davis)
	Ana-Kit (Hollister-Stier)
	Epi-Pen (Dey)
	Micronefrin, aerosol (Bird)
	AsthmaNefrin, aerosol (Menley & James)
Isoetharine HCl	Isoetharine HCl (Roxane)
Isoproterenol	Isoproterenol parenteral solution, injectable (Abbott)
Hydrocortisone	Hydrocortone phosphate, injectable (Merck)
Dexamethasone	Decadron LA, injectable (Merck)
	Decadron phosphate, injectable (Merck)
	Dexone, injectable (Keene)

Use of Sulfite Test Strips

Chemically treated strips to test foods for sulfite content have been available in the past. Both false-positive and false-negative reactions have been encountered using these devices, so they are not reliable (79). Sulfite test strips are no longer commonly available.

A better understanding of the mechanisms involved in sulfite sensitivity would allow for more specific interventions to treat and perhaps prevent these reactions.

Food and Drug Uses

Food

Sulfites are added to many different types of foods for several distinct technical purposes (Table 24–4). The key technical attributes of sulfites in foods include a host of uses characterized as processing aids (1). Some uses of sulfites have now been restricted by federal regulatory actions in the US, as will be described later in this chapter. In many food products, sulfites serve multiple purposes. In white wines, for example, their primary function is to prevent bacterial growth and acetic acid formation; an important secondary effect is to prevent browning (1). Because of their important attributes, sulfites are utilized in an enormous number of specific applications in a wide variety of foods. Several reviews have appeared that provide more details on these applications (1, 80).

Table 24–4.
Technical Attributes of Sulfites in Foods

Technical Attribute	*Examples of Specific Food Applications*
Inhibition of enzymatic browning	Fresh fruits and vegetables*
	Guacamole*
	Pre-peeled raw potatoes
	Salads*
	Shrimp (black spot formation)
Inhibition of non-enzymatic browning	Dehydrated potatoes
	Dried fruits
	Other dehydrated vegetables
Antimicrobial actions	Corn wet milling to make cornstarch and corn syrup
	Wines
Dough conditioning	Frozen pie crust
	Frozen pizza crust
Antioxidant action	No major US applications
Bleaching effect	Hominy
	Maraschino cherries

*No longer allowed by US FDA.

Given the wide variety of applications for sulfites in foods, a broad range of use levels and residual sulfite concentrations can also be found in foods (Table 24–5). Residual sulfite concentrations in foods can range from undetectable (less than 10 ppm) to more than 2000 ppm (mg SO_2 equivalents per kg of food). Although SSAs vary in their degree of sensitivity to ingested sulfites, all such individuals can tolerate some sulfite. Certainly, the more highly sulfited foods pose the greatest hazard to SSAs.

Table 24–5.
Estimated Total SO_2 Level as Consumed for Some Sulfited Foods*

>100 ppm†
Fruit, dried (excluding dark raisins and prunes)
Grape juice (white, white sparkling, pink sparkling, red sparkling)
Lemon juice, nonfrozen
Lime juice, nonfrozen
Molasses
Pickled cocktail onions
Sauerkraut juice
Wine
50–99.9 ppm
Fruit topping
Gravies and sauces
Maraschino cherries
Potatoes, dried
Wine vinegar
10.1–49.9 ppm
Corn starch
Corn syrup
Hominy
Jams and jellies, imported
Maple syrup
Mushrooms, fresh
Pectin
Pickled peppers
Pickles/relishes
Potatoes, frozen
Sauerkraut
Shrimp, fresh
< 10 ppm
Beer
Coconut
Cod, dried
Cookies
Crackers
Fresh fruit salad
Gelatin
Grapes
High-fructose corn syrup
Instant tea
Jams and jellies, domestic
Malt vinegar
Pie dough
Pizza dough, frozen
Potatoes, canned
Soft drinks
Soup mix, dry
Sugar (esp. beet sugar)

†1 ppm = 1 μg/g
*Adapted from The re-examination of the GRAS status of sulfiting agents. Life Sciences Research Office, Federation of American Societies for Experimental Biology, January, 1985.

Inhibition of Enzymatic Browning and Other Enzymatic Reactions

Sulfites can inhibit numerous enzymatic reactions, including those involving polyphenoloxidase, ascorbate oxidase, lipoxygenase, peroxidase, and thiamine-dependent enzymes (1, 81). The inhibition of polyphenoloxidase helps to control enzymatic browning, which occurs to varying degrees and at variable rates on the surfaces of cut fruits (especially apples and pears) and vegetables (especially potatoes), at the edges of shredded lettuce, and in guacamole. In the presence of oxygen, polyphenoloxidase catalyzes the oxidation of mono- and ortho-diphenols in these fruits and vegetables to quinones. The quinones can cyclize, undergo further oxidation, and condense to form brown pigments. Black spot formation in shrimp is a similar type of reaction, in which tyrosinase (a type of polyphenoloxidase) catalyzes the oxidation of the amino acid tyrosine in the shrimp tissue.

The mechanism of action of sulfites in inhibition of enzymatic browning appears to be complex (1). Sulfites may directly inhibit the polyphenoloxidase (1). They may also react with intermediate products, especially the quinones, formed during the browning reaction, thereby preventing the ultimate formation of the brown pigments (81). Sulfites also act as reducing agents that promote the conversion of the quinones back to the original phenols.

The amount of sulfites necessary to prevent enzymatic browning varies according to the level of activity exhibited by the polyphenoloxidase, the nature and concentration of the substrate, the desired period of control, and the presence of other inhibitors or controlling factors. When only monophenols such as tyrosine are present, fairly low levels of sulfite can produce an effect (as in potatoes and shrimp). When diphenols are present (as in guacamole and cut fruit), much higher concentrations of sulfites may be necessary. The concentrations of these phenolic substrates in fruits and vegetables vary widely, with high levels found in guacamole and low levels present in lettuce. Sulfites do not irreversibly inhibit enzymatic browning, so the required concentrations of sulfites remain dependent on the length of time that the reaction must be inhibited.

The US FDA has prohibited many of the uses of sulfites for the control of enzymatic browning. Several alternatives exist for the control of enzymatic browning (1). Because polyphenoloxidase activity depends on oxygen, exclusion of oxygen through modified atmosphere packaging is a viable alternative. The use of acidulents (e.g., citric, acetic, or erythorbic acids) to slow the activity of the enzyme, and the addition of reducing agents (e.g., ascorbic acid) to convert the quinones back to phenols, are the most common alternatives; these techniques are often used in combination. Blanching of fruits or vegetables can also inactivate the enzyme—but then the products are no longer fresh. Freezing slows the activity of polyphenoloxidase markedly. For this reason, frozen potatoes do not require the addition of appreciable levels of sulfites, unlike fresh or refrigerated potatoes. This freezing effect also avoids the necessity of adding sulfites to other fruits and vegetables. Alternatives to sulfites for prepeeled raw potatoes and shrimp have proved difficult to develop due to the level of activity of the enzymes, the long period of inhibition desired, and especially the ability of sulfite to penetrate into subsurface tissue. 4-Hexylresorcinol has been identified as an effective alternative in shrimp and other foods (82, 83), although its application remains rather limited.

Inhibition of Nonenzymatic Browning

Nonenzymatic browning is a term used to describe a family of reactions that commonly involve the formation of carbonyl intermediates and, ultimately, brown polymeric pigments. The final pigments closely resemble those produced by enzymatic browning; the key difference is the lack of any enzyme to catalyze these reactions. Examples of nonenzymatic browning include the reaction between amino acids and reducing sugars, and the caramelization of sugar. Specific foods in which sulfites are used to control nonenzymatic browning include dehydrated potatoes, other dehydrated vegetables, dried fruits, white wines, white grape juice, nonfrozen lemon and lime juices, grated coconut, pectin, and some varieties of vinegar.

Sulfites control nonenzymatic browning by reacting with any of the carbonyl intermediates or substrates for this reaction (1, 84). Once bound to sulfites, the carbonyls can no longer condense to form the brown pigments. The stability of the carbonyl-sulfite reaction products varies from virtually irreversible to readily reversible. The sulfite level necessary to control nonenzymatic browning likewise varies with the nature of the carbonyls formed and the stability of the carbonyl-sulfite reaction products. Where unstable products result, more sulfite is needed.

No effective alternatives to sulfite for the inhibition of nonenzymatic browning have been identified despite intensive efforts (1). Removal of sugars by fermentation, application of glucose oxidase, changes in formulation, and leaching can produce the desired effect, but have obvious limitations because the resulting products are dramatically altered. Acids can slow, but not stop, nonenzymatic browning. Acidification has limitations as well because of the long shelf life of some of the affected products.

Antimicrobial Actions

Sulfites are not widely used in foods for their antimicrobial actions. In a few food processes, however, sulfites play a crucial role in the inhibition of undesirable bacteria (1, 85). In winemaking, sulfites allow the yeast to ferment sugar to ethanol while preventing the growth of undesirable bacteria that would lead to the formation of acetic acid. In corn wet-milling, the corn kernels are soaked in a sulfited steep liquor. The sulfite dissociates interactions between corn germ proteins and the starchy endosperm, thereby facilitating the removal of the starch. Another extraordinarily important function of the sulfites in corn wet-milling is the prevention of bacterial growth in the steep liquor. In addition, SO_2 is widely used during the transport and storage of table grapes to prevent mold growth (86). In general, sulfites work much more effectively against bacteria and molds than they do against yeasts.

The mechanism of antimicrobial action of sulfites is not well understood (1). Acidic pH enhances the antimicrobial activity of sulfites, suggesting that H_2SO_3 may be the active sulfite form in producing the antimicrobial effects. The amount of sulfites necessary to prevent undesirable microbial growth depends on the nature of the substrate, the length of time that growth inhibition is required, and the degree of binding between sulfites and other food components. Certain wine components (such as acetaldehyde and pyruvate) bind strongly to sulfites, and these bound forms of sulfite do not provide effective antimicrobial properties. Thus, sulfite must be added to wines in amounts sufficient to preserve enough free sulfite to prevent bacterial growth. In corn wet-milling,

the sulfite concentrations in the steep liquor are relatively high, but nearly all of the sulfite is removed following further purification of corn starch and corn syrup. With table grapes, distribution of the fruit is not allowed unless residual sulfites have dissipated to undetectable levels.

Although sulfites have few applications in foods for antimicrobial purposes, suitable alternatives have been difficult to find. The wide spectrum of antimicrobial activity is appealing in both corn wet-milling and winemaking. Other antimicrobial agents approved for use in foods have a narrower spectrum of activity and are ineffective replacements for sulfites. In table grapes, the gaseous nature of SO_2 is indispensable and replacement is, therefore, unlikely.

Dough Conditioning

Sulfites were widely used as dough conditioners in the baking industry, especially in frozen pizza doughs and pie crusts (1). Most of these sulfites uses have now been discontinued. Occasionally, they are used in crackers, cookies, biscuits, and tortilla shells. Sulfites act by breaking the cysteine disulfide bonds that are prevalent in the gluten fraction of the dough (87). The levels of sulfites required for dough conditioning are relatively low. Some dough formulations do not require sulfite, and such options are growing in favor.

Antioxidant Uses

Sulfites are not used in the US to prevent oxidative rancidity of fats because other additives are favored for this application. Sulfites are used for this purpose in other countries, especially in meat products. It is illegal to add sulfites to meats in the US. (The ability of sulfites to inhibit enzymatic and nonenzymatic browning might also be described as an antioxidative effect.) At one time, sulfites were routinely added to beer to prevent undesirable, oxidative flavor changes (85), but they are no longer used in US beers.

Bleaching Actions

Sulfites have major applications in the bleaching of cherries for the production of maraschino cherries and glace fruit, and the bleaching of hominy (1). Other minor uses include bleaching of pectins and the development of translucency in orange, lemon, grapefruit, and citron peel (1). In other products, their bleaching effects are considered detrimental to quality. Relatively high concentrations of sulfites are necessary to produce the bleaching effect, although further processing removes much of the sulfite from these products. Consequently, exposure to sulfites from these foods is minimal. No alternative bleaching agents have been identified.

Drugs

Sulfites are added to many pharmaceutical products (3, 4). Table 24–3 contains a list of drugs intended for asthmatics that may contain sulfites. With the increased concern over sulfite-induced asthma, these substances have been removed from some drugs in recent years, especially from drugs intended for asthmatics. Sulfites are used in drugs intended for oral, topical, respiratory, and internal use.

Sulfites have two primary functions as drug ingredients. First, they act as antioxidants, typically preventing the oxidation of one of more of the active drug ingredients. Second, they prevent nonenzymatic browning, which involves the reaction of reducing sugars with amino acids or amines. The addition of sulfites prevents those reactions, which can occur in enteral feeding solutions and dextrose solutions. The latter stages of the nonenzymatic browning reaction involve the condensation of quinones. Epinephrine can undergo a similar reaction that diminishes its potency. Consequently, sulfites are routinely added to epinephrine to prevent such condensation reactions.

The usage levels of sulfites in pharmaceutical products vary from 0.1% to 1.0%, although a few products may contain higher concentrations. Exposure to sulfites via drugs can be high but would be sporadic in most cases. The active ingredients of the drug may, in a few cases, counteract the effects of sulfite in sulfite-sensitive individuals. Until recently, sulfites were common additives in certain bronchodilators but, except in a few rare cases (39, 88), the bronchodilating effect of the active ingredient overwhelms the bronchoconstricting effect of sulfite. As noted earlier, epinephrine easily overwhelms the bronchoconstricting effects of sulfites. Thus, sulfite-containing epinephrine should never be denied to or avoided by an SSA, because it can act as a life-saving antidote (3, 89).

Many existing alternatives could replace sulfites as antioxidants in pharmaceutical products. These alternative formulations have been widely adopted in drugs commonly used by asthmatics.

On the other hand, alternatives for sulfite do not exist for the prevention of nonenzymatic browning. The development of effective alternatives for this purpose will be extremely difficult. Sulfite-sensitive individuals should stay alert to the possible presence of sulfites in medications and seek out alternative formulations. Epinephrine is the exception; epinephrine can be administered if necessary to sulfite-sensitive individuals.

Fate of Sulfites in Foods

SO_2 and its sulfite salts are extremely reactive in food systems. The wide range of technical attributes of sulfites in foods is a direct result of this reactivity. Thus, these substances often react with a variety of food components. A dynamic equilibrium exists between free sulfites and the many bound forms of sulfite (1). Thus, the fate of these food additives will vary widely, depending on the nature of each individual food.

SO_2 and the sulfite salts readily dissolve in water and, depending on the pH of the medium, can exist as sulfurous acid (H_2SO_3), bisulfite ion (HSO_3^-), or sulfite ion (SO_3^{2-}) (77). All of these forms react with a variety of food components with the extent and reversibility of these reactions relating to pH. At acidic pH (pH of < 4.0), SO_2 can be released as a gas from a sulfite-containing food or solution. Thus, sulfites can actually be lost from foods, albeit only under acidic conditions.

Sulfites react readily with food constituents including aldehydes, ketones, reducing sugars, proteins, amino acids, vitamins, nucleic acids, fatty acids, and pigments, to name a few (1). The extent of any reaction between sulfite and some food component depends on the pH, temperature, sulfite concentration, and reactive components present in the food matrix. An equilibrium always exists between free and bound sulfites, although the reversibility of the reactions varies over a wide range (1, 80). Some reactions, such as that between acetaldehyde and sulfite to form acetaldehyde hydroxysulfonate, are virtually irreversible. Other reactions, such as between the anthocyanin pigments of fruits and sulfite, reverse readily. The binding of sulfite by various food constituents diminishes the concentration of free sulfite in the food. While the dissociable, bound forms of sulfite can serve as reservoirs of free sulfite in the food, irreversible reactions tend to remove sulfite permanently from the pool of free sulfite. The desirable actions of sulfites in foods frequently depend on free sulfite, so the concentration of the pool of free sulfite is a critically important factor in technical effectiveness. Therefore, treatment levels for specific food applications aim to provide an active, residual level of free sulfite throughout the shelf life of the product.

In lettuce, high concentrations of sulfite (500–1000 ppm) have been recommended to prevent enzymatic browning. Because lettuce consists mostly of cellulose and water, the sulfite has few components with which to react. Consequently, most of the sulfite added to lettuce lingers in the form of free inorganic sulfite (90). Lettuce is unique in this regard, as most foods contain substances that readily react with sulfites. In most foods, therefore, the bound forms of sulfite would predominate.

A comprehensive discussion of the possible reactions between sulfites and food constituents lies beyond the scope of this chapter. An entire book has been written on the subject of the chemistry of sulfites in foods (80). Suffice it to say that the fate of sulfite in individual food products is dynamic, extraordinarily complex, and difficult to predict with any degree of precision.

Likelihood of Reactions to Sulfited Foods

Few trials have attempted to evaluate the sensitivity of SSAs to sulfited foods. Based on the suspected mechanisms of sulfite-induced asthma, one might predict that acidic foods and beverages capable of generating SO_2 gas would be more hazardous than other forms of sulfited foods. Clinical challenges with acidic solutions of sulfite in lemon juice or some other vehicle appear to support this conclusion (56, 89). In all foods, the fate of sulfite may be an important determinant of the degree of hazard faced by the sulfite-sensitive consumer. Little evidence currently exists, however, regarding the hazard levels posed by the various forms of food-borne sulfite. The overall concentration of residual sulfite in the food also represents an important determinant of the likelihood of a reaction.

Clinical challenges have documented several features of sulfite-induced asthma. First, all SSAs exhibit some tolerance for ingested sulfite. The threshold levels vary from one patient to another, ranging from approximately 0.6 mg of SO_2 equivalents (1 mg of $K_2S_2O_5$) to levels of 120 mg of SO_2 equivalents (200 mg of $K_2S_2O_5$). Second, clinical challenges have confirmed that free, inorganic sul-

fite presents a hazard to SSAs. Third, more asthmatics will respond to inhalation of SO_2 or ingestion of acidic sulfite solutions than to ingestion of sulfite in capsules.

From these facts, several predictions can be made about the likelihood of reactions to sulfited foods among SSAs. First, reactions will be more likely and probably more severe to highly sulfited foods such as lettuce, dried fruit, and wines. Certainly, no evidence exists to implicate foods with low levels of residual sulfite (< 10–50 ppm) in adverse reactions in sensitive individuals (91). Second, foods containing a higher proportion of free inorganic sulfite may offer greater risks than foods in which the bound forms of sulfite predominate. Sulfited lettuce is certainly the best example of a food with a high proportion of free inorganic sulfite (92). This prediction assumes, however, that the bound forms of sulfite are less hazardous than free inorganic sulfite—an assumption that has not been clinically established. Finally, one might predict that acidic foods or beverages containing sulfites would pose greater danger than other sulfited foods. Examples of these hazardous foods would include wines, white grape juice, nonfrozen lemon and lime juices, and perhaps lettuce treated with an acidic salad freshener solution. These predictions appear to match the practical experiences of SSAs.

Few experiments have been conducted to test these predictions. Halpern et al (91) tested 25 nonselected asthmatics with 4 oz of white wine that contained 160 mg of SO_2 equivalents per liter. Because patients were not prescreened for sulfite sensitivity, the results of this clinical trial are difficult to evaluate. Only one (4%) of the 25 patients exhibited reproducible symptoms with the wine challenge, however.

Howland and Simon (93) conclusively demonstrated that sulfited lettuce can trigger asthmatic reactions in confirmed SSAs. The five patients in this trial were exposed to 3 oz of lettuce containing 500 ppm of SO_2 equivalents. All of these patients had documented reactions to sulfite ingested in capsule form. Taylor et al (75) confirmed the reactivity of SSAs to ingestion of sulfited lettuce, including one subject who responded to only acidic solution challenges of sulfite.

In their study, Taylor et al (75) assessed the sensitivity of eight SSAs to a variety of sulfited foods, including lettuce, shrimp, dried apricots, white grape juice, dehydrated potatoes, and mushrooms. The eight SSAs were identified by double-blind, capsule-beverage challenges. Despite the positive double-blind challenges, four of these patients failed to respond to any of the sulfited foods or beverages. The other four patients experienced bronchoconstriction after ingesting sulfited lettuce, although this test was the only positive food challenge for the acidic beverage reactor. Curiously, this patient did not react adversely to a challenge with white grape juice, which is an acidic, sulfited beverage. Two of the remaining three patients also reacted to dried apricots and white grape juice; the third patient did not complete these challenges. Only one of the three patients reacted to challenges with dehydrated potatoes and mushrooms; in the case of dehydrated potatoes, however, her response to multiple double-blind challenges with dehydrated potatoes was not consistent. None of these patients responded to sulfited shrimp.

While these results were somewhat confusing, they illustrated that SSAs will not react equivalently to the ingestion of all sulfited foods. The likelihood of a response could not be predicted on the basis of the dose of residual SO_2 equivalents in the sulfited foods. The nature of the sulfite present in these foods varied widely. In lettuce, sulfite levels are high and free inorganic sulfite predominates (90). In white grape juice and especially dried apricots, sulfite levels are high, the foods are acidic, and sulfite may be bound to reducing sugars (1, 75). In dehydrated potatoes, sulfite levels are intermediate, the food is not acidic, and sulfite is typically bound to starch (1, 75). In mushrooms, sulfite levels are low and variable, but the form of sulfite remains unknown. In shrimp, sulfite levels are intermediate, the food is not acidic, and sulfite is probably bound to protein (1, 75). The likelihood of a reaction to a sulfited food depends on several factors: the nature of the food, the level of residual sulfite, the sensitivity of the patient, and (perhaps) the form of residual sulfite and the mechanism of sulfite-induced asthma (75).

Detection of Sulfited Foods

A comprehensive discussion of the methods for the detection of sulfite residues in foods lies beyond the scope of this chapter, although the subject has been extensively reviewed elsewhere (1, 80). The numerous procedures available include distillation-titration, ion chromatography, polarography, enzymatic oxidation with sulfite oxidase, gas chromatography, and enzyme-linked immunosorbent assay (ELISA) (1, 80). These pro-

cedures are highly specialized and should be undertaken only by skilled analytical chemists. One of these procedures, the Monier-Williams distillation-titration procedure, is probably the method of choice because it has been officially sanctioned by the Association of Official Analytical Chemists (94). None of the available methods alone can measure all forms of sulfite in foods, including free inorganic sulfite plus the many bound forms of sulfite (1). Most of these methods aim to detect free sulfite plus some of the reversibly bound forms of sulfite. Although they are termed methods for measuring total SO_2, they actually measure "subtotal SO_2." A few procedures intended to measure only free inorganic sulfite in foods are used on rare occasions (1, 90). Because clinicians do not know which forms of sulfites in foods pose hazards to SSAs, it is impossible to develop a procedure that detects only clinically relevant forms of sulfite. Instead, in the absence of information on hazardous forms of sulfite, the focus has been on measuring as much of the residual sulfite—both free and bound—as possible.

Avoidance Diets

As noted earlier, the most common treatment for individuals with sulfite-induced asthma is the avoidance of sulfite in the diet. Of course, asthmatics with a low threshold for sulfites must take greater care to avoid these substances than individuals with higher thresholds. Certainly, all SSAs should be instructed to avoid the more highly sulfited foods, which are defined as having in excess of 100 ppm of SO_2 equivalents (Table 24–5). Individuals with lower thresholds for sulfite might be advised to remove all sulfited foods from their diets, although adherence to such diets can be difficult. Packaged foods containing more than 10 ppm residual SO_2 equivalents must declare the presence of sulfites or one of the specific sulfiting agents on their labels. Thus, sulfite-sensitive consumers should be able to avoid significantly sulfited foods by careful perusal of labels. They must also be instructed that the terms sulfur dioxide, sodium or potassium bisulfite, sodium or potassium metabisulfite, and sodium sulfite indicate the presence of sulfites or sulfiting agents. Some sulfite-sensitive individuals may know that they can safely consume certain foods declaring sulfite on the labels because the amount of available sulfite in that particular food falls below their threshold doses. Such patients should be warned that the concentration of residual sulfite in any specific food is variable and that continued consumption might occasionally elicit an adverse reaction. No absolute evidence exists to suggest that sulfite-sensitive individuals need to avoid foods having less than 10 ppm residual SO_2 equivalents.

Whereas avoiding sulfited packaged foods is relatively straightforward, the same cannot be said for restaurant foods. The FDA has banned sulfite from fresh fruits and vegetables in restaurants, but other sulfited foods in restaurants remain unlabeled. With the banning of sulfites from salad bar items, many of the problems with sulfite-induced asthma in restaurants disappeared. The major continuing problem is sulfited potatoes. Sulfite-sensitive individuals should be instructed to avoid all potato products in restaurants except baked potatoes with the skins intact.

Regulatory Restrictions

The US FDA, Bureau of Alcohol, Tobacco and Firearms (BATF), and Environmental Protection Agency (EPA) have moved to regulate certain uses of sulfites following the discovery of sulfite-sensitive asthma. The FDA initially moved to require the declaration of sulfites on the label of foods when sulfite residues exceeded 10 ppm; BATF followed suit with wines. FDA then banned the use of sulfites from fresh fruits and vegetables other than potatoes. This ban affected lettuce, cut fruits, guacamole, mushrooms, and many other applications, especially the once-common practice of sulfiting fresh fruits and vegetables placed in salad bars. Potatoes remain the sole exception to the ban of sulfite use on fresh fruits and vegetables. In these actions, FDA has not distinguished between uses that result in low levels of residual sulfite and uses that create much higher levels. Under EPA restrictions, imported table grapes must be detained at their port of entry until sulfite residues can no longer be detected. FDA has also enacted a regulation specifying the allowable sulfite residue levels in shrimp.

The actions have helped to protect sulfite-sensitive individuals from the hazards associated with sulfited foods. Unfortunately, at this time, the FDA has taken no action to limit the use of sulfites in drugs. Certainly, any regulation is only as effective as its enforcement, so sulfite-sensitive individuals and their physicians should remain alert to avoid inadvertent exposures.

Conclusion

Sulfite sensitivity affects a relatively small subgroup of the asthmatic population. The symptoms of sulfite-induced asthma can, on occasion, prove quite severe and even life-threatening. Sulfite sensitivity should ideally be diagnosed with a double-blind bronchoprovocation protocol. Many unknowns remain regarding sulfite-induced asthma, including the mechanism of the illness and the likelihood of reactions to specific sulfited foods. Reactions to sulfited foods certainly derive in part from the concentration of residual sulfite in the food and the degree of sensitivity exhibited by the individual patient. In addition, the form of sulfite in the food and the mechanism of the sulfite-induced reaction may affect the likelihood of a response to a specific sulfited food.

SSAs should be instructed to avoid highly sulfited foods. The FDA and other US federal regulatory agencies have moved to protect SSAs from unlabeled uses of sulfites in foods. Nevertheless, sulfites continue to be used in many foods and drugs, and sensitive individuals must be cautious to avoid inadvertent exposures.

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25

Monosodium Glutamate

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In 1968, a physician by the name of Dr. Robert Ho Man Kwok wrote a letter to the editors of the New England Journal of Medicine describing "numbness at the back of the neck . . . radiating to both arms and the back, general weakness and palpitation" which he experienced only when dining in Chinese restaurants (1). His symptoms were transient, appearing 15 minutes after the start of the meal and lasting up to 2 hours. Dr. Kwok thought perhaps his symptoms might have been attributable to the alcohol in Chinese cooking wine, sodium in Chinese food, or the flavoring ingredient monosodium glutamate (MSG). Entitled "Chinese Restaurant Syndrome," Dr. Kwok's letter sparked great interest and perhaps even greater controversy among the general public and medical circles regarding the issue of MSG-associated adverse reactions. The complex of symptoms Dr. Kwok described came to be known in the medical literature and in the general media as the "Chinese Restaurant Syndrome," or the "MSG Symptom Complex." Suspicion about MSG's ability to cause adverse physical reactions continues: as of October 1998, 2621 adverse reactions had been reported to the FDA's Center for Food Safety and Applied Nutrition. In addition to the Chinese Restaurant Syndrome, MSG ingestion has been anecdotally associated with conditions as diverse as asthma, urticaria and angioedema, headache, shudder attacks in children, psychiatric disorders, and convulsions. Studies undertaken in the intervening decades since Dr. Kwok's letter have been unable to demonstrate a clear and consistent relationship between MSG ingestion and the development of these or any adverse reaction.

The Fifth Taste: L-Glutamate

Humans can detect four primary tastes: sweet, salty, bitter, and sour. There is also a fifth primary taste called umami. Umami describes the palatability or deliciousness of a food, and has been called a "brothy mouth-watering sensation" (2). For centuries, the umami taste has been a characteristic of Asian cuisine, particularly in dishes which have used kelp, fermented fish sauces, or soy sauce. In 1908, Ikeda discovered that the common amino acid L-glutamate in dried kelp ("kombu") was responsible for the savory taste of soup stock. He named this taste "umami," from the Japanese word for savory (3). The L-form of glutamate is unique in its ability to impart the umami taste, though it is not palatable by itself. Its isomer, D-glutamate, does not possess this characteristic taste. Because glutamate is an amino acid commonly found in food, the evolutionary purpose of the glutamate taste receptor may have been to indicate the presence of edible dietary protein. A molecular mechanism for umami taste transduction has been discovered: G-protein coupled receptors bind extracellular L-glutamate in a clamshell-shaped cleft present on taste buds (4). Certain nucleotides such as 5′-ribonucleotides synergize with L-glutamate to increase umami intensity. Such nucleotides are present in many edibles and include inosinate, found in meat, fish, and poultry, and guanylate, found in high concentration in shiitake mushrooms (5–7).

Glutamate is a nonessential amino acid that constitutes up to 20% of dietary protein. In food, it occurs naturally in two forms: non-protein-

bound and protein-bound. Some foods contain naturally-occurring high levels of free glutamate, such as tomatoes (0.34% MSG), Parmesan cheese (1.5% MSG), and soy sauce (1.3% MSG) (8). In the US, average dietary intake of naturally occurring free glutamate is about 1 g per day (9); an additional half gram per day comes from foods flavored with MSG (8). The sodium salt of glutamate, MSG is the most common form of free glutamate added to food (Fig. 25–1). It is a white powder resembling salt, and is made from fermented sugar beet or sugar cane, molasses or starch, or from the fermentation of bacterial strains capable of synthesizing glutamate in large quantities. Like MSG, other salts of free glutamate have the ability to impart an umami taste to food, such as monopotassium glutamate and monoammonium glutamate. Thus, glutamate salts are used widely in the food manufacturing and restaurant industries and often flavor foods such as crackers, potato chips, canned and dry soups, canned seafood, meats, frozen dinners, salad dressings, Chinese and other Asian food. Only free glutamates added in the food manufacturing process are required by the FDA to be specifically identified by name on the food label (Table 25–1).

Forms of protein-bound glutamate are also added to or exist naturally in food. Glutamate is a protein-bound component in partially hydrolyzed vegetable or plant proteins. It is also present in autolyzed yeast extract, additionally known as yeast nutrient or yeast food. The content of MSG in hydrolyzed vegetable protein (HVP), hydrolyzed plant protein (HPP), or hydrolyzed soy protein (HSP) can vary from 10%–30%. In these cases, glutamate is not required to be identified by name since it is a component of the food itself. Therefore, since 1986, the FDA has permitted glutamate to be indirectly identified on food labels as HVP, HPP or HSP (10). In 1988, the FDA also permitted the words "flavoring" or "seasoning" or the phrase "natural flavoring" to be used on food labels to refer to MSG present as a component of these specially processed proteins (10). Use of these various names means the consumer may be unaware that MSG is present in the food he or she is ingesting (Table 25–1).

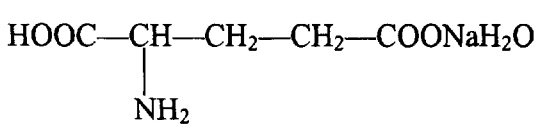

Figure 25–1. Chemical structure of monosodium glutamate.

Table 25–1.
Food Labeling of MSG

Free Glutamate:	*Bound Glutamate:*
MSG	Hydrolyzed vegetable protein (HVP)
Monopotassium glutamate	Hydrolyzed plant protein (HPP)
Monoammonium glutamate	Hydrolyzed soy protein
Glutamic acid	Natural flavoring
Glutamic acid hydrochloride	Flavor(s) or flavoring
Glutamate	Seasoning
	Kombu extract
	Autolyzed yeast extract

Glutamate is readily and rapidly metabolized by the human body. Intestinal epithelium transaminates glutamate, forming metabolites which can be used via the Krebs cycle (11). Other metabolites include gamma aminobutyric acid (GABA), glutamine, and glutathione (11). The readily available intestinal and hepatic metabolism of glutamate means that serum glutamate levels are only slightly elevated even when large doses (> 30 mg/kg body weight) are administered without food (12). Furthermore, dose-related increases in plasma levels are markedly attenuated in the presence of carbohydrate. Carbohydrates are a source of pyruvate for intestinal epithelia. Pyruvate facilitates transamination of glutamate and thereby reduces the release of glutamate to the peripheral circulation, blunting the rise of serum glutamate (13). Glutamate is rapidly metabolized: elevated plasma levels due to doses exceeding 5 g of MSG administered without food returned to basal levels in less than 2 hours (14). Fetal and neonatal exposure to glutamate is likely to be small: though glutamate can cross the placenta, fetal plasma concentration does not increase significantly even if maternal serum concentration is appreciable, and breast milk glutamate does not increase significantly even with ingestion of 100 mg/kg of MSG (15). Infants, including premature babies, can metabolize greater than 100 mg of MSG per kg body weight administered in infant formulas (16).

Glutamate and its chemical derivatives have long been added to foods to improve palatability. MSG has been used by the American food processing industry since the 1940s, and has been listed as Generally Recognized as Safe (GRAS) under section 201(s) of the Food, Drug and Cosmetic Act of 1959, alongside other food ingredients such as vinegar, sugar, and salt. In 1969, the FDA reevaluated the safety of all GRAS substances. In 1980, the Select Committee on GRAS Substances (SCOGS) convened by the Federation of American

Societies for Experimental Biology (FASEB) under contract by the FDA concluded that MSG and hydrolyzed proteins were safe for the general population at current consumption levels. In 1986, the FDA's Advisory Committee on Hypersensitivity to Food Constituents concluded that MSG posed no threat to the general public but that brief reactions might occur in some people. In 1987, the Joint Expert Committee on Food Additives (JECFA) of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) placed MSG and other glutamate salts among other food ingredients judged to be the safest for human consumption. To date, there is no evidence which suggests that MSG is carcinogenic or teratogenic (17–20). Its quantity in food is limited only by its palatability.

Monosodium Glutamate and Neurotoxicity

Whether MSG could cause brain pathology was questioned after experiments in the late 1960s demonstrated neurotoxicity in certain MSG-exposed neonatal laboratory animals. Olney et al (21) reported that neonatal rodents developed necrosis of the arcuate nucleus in the hypothalamus within hours of an intragastric bolus of approximately 500 mg of MSG/kg/body weight. This bolus of MSG was followed by permanent derangement of reproductive capacity and body weight regulation in these animals, possibly due to hypothalamic dysfunction. It was hypothesized that the hypothalamic median eminence might be particularly susceptible to circulating glutamate because of its lack of a blood-brain barrier. However, this lesion could not be reproduced when administered in identical manner to mature rats or administered in the diet of immature rodents (22, 23). Around the time of the original 1969 study, MSG was being added in large amounts to infant formula to increase palatability. Because of concern regarding possible MSG-induced neurotoxicity, JECFA recommended at that time that food additives in general (including MSG) should not be used in infant foods intended to be consumed prior to 12 weeks of age, and manufacturers of infant formulas voluntarily withdrew MSG from their products.

Because MSG affected the hypothalamus in the neonatal mouse, there was concern that perhaps MSG administration could influence pituitary function. Olney et al (24) found that 1 g/kg of MSG injected into adult rats increased luteinizing hormone and testosterone, presumably via stimulation of the hypothalamic hormone gonadotropin-releasing hormone, although gonadotropin-releasing hormone was not measured in this study. Until a definitive 1996 study by Fernstrom et al (25), there were little data regarding the neurohormonal effects of MSG in humans. In a double-blinded, placebo-controlled (DBPC) study, the authors demonstrated that high circulating glutamate levels were not likely to affect the pituitary. Prolactin was chosen as the primary outcome measure because both physiologic (ingestion of a high protein meal) and pharmacologic (thyrotropin releasing hormone) positive controls exist for stimulation of this hormone. Eight healthy men received 12.7 grams of MSG orally in a noncaloric beverage versus placebo after an overnight fast. Blood glutamate levels, prolactin, luteinizing hormone, follicle stimulating hormone, growth hormone, thyroid stimulating hormone, and cortisol were measured. The subjects also underwent physiologic and pharmacologic positive control testing for prolactin stimulation. Serum fasting glutamate levels measured 50 nmol/mL and increased 11-fold after subjects ingested MSG. There was no significant change in glutamate levels after ingestion of the placebo vehicle or protein meal. As expected, both positive controls increased serum prolactin levels in all subjects. MSG was not associated with significant changes in any of the hormones surveyed, including prolactin. Fernstrom et al (25) concluded that extremely high levels of circulating glutamate do not exert any appreciable effect on anterior pituitary function. The majority of studies done thus far indicate that glutamate does not cause neural damage or neurohormonal dysfunction.

Chinese Restaurant Syndrome (MSG Symptom Complex)

Due to continuing reports from the public of adverse reactions associated with MSG, the FDA contracted with the FASEB in 1992 to perform a scientific safety review of the effects of glutamate in foods. In a 1995 report (26), FASEB stated that a subgroup of the population ingesting MSG may experience an acute, temporary, and self-limiting syndrome that may include: 1) burning sensation of the neck, forearms, and chest; 2) facial pressure or tightness; 3) chest pain; 4) headache; 5) nausea; 6) upper body tingling and weakness; 7) palpitation; 8) numbness in the back of the neck, arms,

and back; 9) bronchospasm (in asthmatics only); and 10) drowsiness. FASEB also stated that these symptoms were more likely to occur in response to an oral bolus of MSG ≥ 3 g in the absence of food.

Stating that the phrase "Chinese Restaurant Syndrome" was pejorative and inaccurate, FASEB suggested substituting the phrase "MSG Symptom Complex" for "Chinese Restaurant Syndrome."

A restaurant syndrome is defined as "an adverse reaction to foods occurring within 2 hours of ingestion, frequently while patients are still dining in restaurants" (27). Dr. Kwok's letter in 1968 triggered many anecdotes implicating MSG for similar symptoms experienced while eating in Asian restaurants. In 1968, Schaumberg wrote, "With the enthusiastic cooperation of the Shanghai Café, one of us ate Chinese food for breakfast, lunch and dinner until the search had been narrowed to either hot and sour soup or wonton soup . . . Upon sampling of the individual ingredients, the dagger of suspicion pointed at monosodium glutamate" (28). In single- and double-blind experiments using himself as a subject, Schaumberg experienced a burning sensation over the back of his neck, forearms, and chest, facial pressure and tightness, and chest discomfort within 15 minutes after he ingested 5 g of MSG. These sensations lasted 45 minutes without sequelae. Ingestion of D-glutamic acid (the isomer of L-glutamic acid) did not cause this reaction, and pretreatment with diphenhydramine did not prevent or decrease the intensity of the reaction. Additional anecdotes of Chinese meal-induced symptoms prompted more formal evaluations to determine the cause of these reactions, and over the past 30-plus years many human challenge studies have been undertaken with this in mind.

One of the first formal studies of the effects of MSG in a human research group was conducted by Schaumberg et al (29) in 1969. These investigators noted that if taken into an empty stomach, MSG ingestion could result in three categories of symptoms: burning, facial pressure, and chest pain. Headache was also noted in a minority of subjects. In a single-blinded, uncontrolled study, they established an oral dose-response curve in 36 normal subjects who experienced some of these symptoms after ingesting up to 12 g of MSG. These investigators concluded that their results indicated that "the Chinese restaurant syndrome is the normal response to this agent" (29). Double-blind studies by Kenney (30) and Kenney and Tidball (31) identified individuals who experienced symptoms specifically associated with MSG only when MSG was ingested in amounts or concentrations far greater than normally encountered in a routine diet. In contrast, double-blind studies from Italy and the United Kingdom found no difference between symptoms experienced after MSG versus placebo ingestion (32–34).

Because of its characteristics, MSG poses a difficult challenge in particular in oral challenge studies. The distinct taste properties of MSG make blinding the subject to oral glutamate administration hard to achieve and consequently may skew experimental results. Furthermore, ingestion of MSG without accompanying carbohydrates does not mimic the real-life patterns of glutamate ingestion. As previously described, MSG metabolism is greatly enhanced by the presence of accompanying carbohydrates, which may mean that extrapolating food-free challenge data to everyday "in-use" situations may not be valid (35). Commonly, symptoms are not reproducible on MSG re-challenge, or are noted on both MSG and placebo administration. In a study by Kenney in 1986, six individuals believing themselves to be MSG-sensitive were tested with 6 g in a DBPC manner. Four of the six subjects did not react on testing, while the remaining two reacted to both placebo and MSG (36).

Large doses of MSG given without food may cause symptoms in people believing themselves to be MSG-sensitive. Geha et al (37) in 2000 conducted a DBPC, multiple-challenge crossover study in 130 self-identified MSG-sensitive people. Positive reactions were defined as experiencing two or more symptoms from a list of 10 symptoms reported to occur after ingestion of MSG-containing food. The study design involved three sequential challenge protocols using 5 g of MSG administered without food. Those subjects responding to MSG only and not to placebo were advanced to each subsequent challenge stage. Of the 130 subjects tested, only two subjects responded consistently to 5 g of MSG but not to placebo on each challenge, although their symptoms themselves were not reproducible. When tested with MSG accompanied by food, both of these subjects responded to only one out of three MSG challenges. Thus, among a small proportion of those who believe themselves to be MSG-sensitive, large doses of MSG given without food may elicit symptoms, but no persistent or serious effects of MSG ingestion are likely to occur.

Estimating the prevalence of adverse reaction to a particular food ingredient through question-

naires is potentially fraught with subjectivity and bias. This has complicated the estimation of the incidence of MSG Symptom Complex in the general population. Reif-Lehrer (38) reported that 25% of a population surveyed by questionnaire believed they had experienced MSG-related symptoms. This survey included several leading, closed-ended questions, which likely evoked many false-positive responses. In a 1979 study, Kerr et al used a National Consumer Panel to select a representative group to improve the accuracy of extrapolation to the US population at large, and found the prevalence of the MSG Symptom Complex to be 2% (39).

The pathophysiology of MSG Symptom Complex remains elusive. Many mechanisms have been proposed but none proven. It is clearly not the result of an IgE-mediated process. Since glutamate is an important excitatory neurotransmitter, neurologic mechanisms have been among those proposed. As an acetylcholine precursor, glutamate may possess parasympathomimetic effects: MSG-induced spasms of guinea pig ileum are blocked by atropine (40), and in 1971, Ghadimi et al (41) demonstrated an attenuation of symptoms in MSG reactors pretreated with atropine prior to MSG exposure. Glutamic stimulation of peripheral neuroreceptors has also been suggested, since use of an axillary cuff restricted a burning sensation to the arm where MSG had been administered intravenously which then spread to the chest and neck when the cuff was removed (29).

Non-neurologic mechanisms for MSG Symptom Complex have also been proposed. Smith et al (42) in 1982 investigated whether the high sodium levels associated with Asian cuisine may be a cause and found plasma sodium levels to be elevated after ingestion of a Chinese meal. Vitamin B_6 is involved in glutamate metabolism, which led Folkers et al (43) in 1981 to explore whether subjects with vitamin B_6 deficiency were more susceptible to the MSG Symptom Complex. In their blinded study, eight of nine positive reactors supplemented with this vitamin had negative repeat blinded challenges. Esophageal reflux was suggested by Kenney in 1986 (38). Chin et al (44) in 1989 suggested that histamine naturally present in ingested food might be responsible for the symptoms associated with MSG ingestion. Although they found that the amount of histamine per food portion was below the toxic level, the amount of histamine ingested in multiple food portions might result in the sensations involved in the MSG Symptom Complex. Scher and Scher (45) in 1992 proposed that nitric oxide production may play a role in the pathogenesis of the MSG Symptom Complex. Nguyen-Duong (46) in 2001 demonstrated that glutamate-induced vasorelaxation of porcine coronary arteries was potentiated by glycine and proposed that this vasodilatory action might be responsible for the flushing and palpitation associated with MSG Symptom Complex. Despite these assorted hypotheses and over 30 years of research, the causative mechanism remains unknown.

Asthma

Since the early 1980s, several reports have suggested that MSG can provoke bronchospasm in asthmatics. In a 1981 letter to the New England Journal of Medicine, Allen and Baker (47) reported two patients who described a history of asthma exacerbations several hours after dining in Chinese restaurants. In these patients, single-blind oral challenges with 2.5 mg of MSG were associated with severe bronchospasm about 12 hours post-challenge. Subsequently in 1987, Allen et al (48) performed in-hospital, single-blind, placebo-controlled MSG challenges on 32 asthmatics; 14 were suspected MSG reactors by history and 18 were unstable asthmatics with bronchospasm due to aspirin, benzoic acid, tartrazine, or sulfites. As described in detail by Stevenson in 2000 (49), several problems were associated with this study: theophylline was discontinued 1 day prior to placebo challenges, some patients received inhaled bronchodilator treatment within 3 hours prior to first challenge, bronchospasm was measured by effort-dependent peak expiratory flow rates (PEFR) rather than flow-volume loops, and baseline PEFR exhibited large variations consistent with unstable asthma. Although Allen et al (48) concluded that 14 patients developed asthma exacerbations 1–12 hours after ingesting 1.5–2.5 g MSG, these factors rendered these results difficult to interpret. What was interpreted by the study authors as MSG-related bronchospasm may merely have been peak flow variability indicative of underlying active asthma. Despite these significant study limitations, it was primarily on this data that FASEB based their decision regarding bronchospasm in asthmatics ingesting MSG. FASEB stated to the FDA in their 1995 review of MSG safety data that a small subgroup of asthmatics may have temporary worsening of their asthma after ingestion of 0.5–2.5 mg MSG (26). Because of the suboptimal design of the study performed by Allen et al (48), as well as lim-

ited data in general regarding MSG asthma exacerbation, the FDA stated in a 1996 edition of the Federal Register that a causal relationship had not been established between exposure to MSG and adverse asthmatic reactions (10).

Other blinded, placebo-controlled studies of asthmatics with and without histories suggestive of MSG sensitivity have not been able to demonstrate MSG-induced asthma. Schwarzstein et al (50) in 1987 studied 12 mild asthmatics, one of whom was a suspected MSG reactor. None developed bronchospasm within 12 hours following 1.5 g MSG ingestion. Also in 1987, Moneret-Vautrin (51) reported that two of 30 asthmatic patients developed bronchospasm after ingesting 2.5 g of MSG. However, the definition for bronchospasm in this study was a decrease in peak expiratory flow of 15%, which was less stringent than the criteria used by Allen et al (48), whose criterion for bronchospasm was a decrease in peak expiratory flow rate of 20%. If the Allen et al peak flow criteria were to be applied to the Moneret-Vautrin study, Moneret-Vautrin would have observed an MSG-related bronchospasm incidence of zero since all peak expiratory flow rates in that study declined by less than 20%. A 1998 DBPC study by Woods et al of 12 asthmatics with a suggestive history was negative for MSG-related bronchospasm following oral challenge with 5 g of MSG given after a standardized breakfast (52).

Although it had previously been suggested by Allen et al that those asthmatic patients with bronchoconstriction due to food additives or aspirin were more likely to experience MSG-related bronchospasm, a study by Woessner et al (53) in 1999 demonstrated this not to be the case. In this study, two groups were tested: 30 asthmatics who believed MSG ingestion exacerbated their asthma, and 70 aspirin-sensitive asthmatics. Asthma maintenance medications including inhaled and systemic corticosteroids and theophylline were continued, though inhaled β-agonists were not. Patients were enrolled in an inpatient-based DBPC challenge if their forced expiratory volume in 1 second (FEV_1) values were at least 70% predicted off of inhaled bronchodilators. The first day consisted of a single-blind placebo day to assess pulmonary baseline. If FEV_1 values varied by 10% or less on placebo day, the patients were challenged the following day with 2.5 g of MSG after a low-MSG breakfast. Adverse symptoms and FEV_1 values were recorded during the following 24 hours. Patients whose FEV_1 values decreased by at least 20% next underwent two additional MSG challenges in a blinded, placebo-controlled manner. On initial MSG challenge, only one patient of the 30 tested experienced an FEV_1 decline of 20%, though she remained asymptomatic. Her FEV_1 remained stable on the subsequent two MSG challenges. None of the aspirin-sensitive asthmatics reacted to MSG ingestion.

Because the number of studies performed thus far is limited, further rigorous investigation involving history-positive asthmatics should be undertaken before it can be decided with certainty whether ingestion of MSG can induce asthmatic bronchospasm. However, given the rarity of reports of MSG-related bronchospasm, as well as the absence of scientific documentation of MSG-induced bronchospasm in humans, MSG is unlikely to contribute to the worsening of asthmatic disease.

Urticaria and Angioedema

Urticaria and angioedema thought to be provoked by MSG ingestion have rarely been reported in the literature. Only one case report of angioedema associated with MSG ingestion exists. In a letter to the Lancet in 1987, Squire (54) described a 50-year-old man with angioedema of the face and extremities following ingestion of a soup made with an MSG-flavored soup base. A blinded, placebo-controlled challenge with the soup base resulted in angioedema 24 hours after ingestion; placebo-controlled ingestion of 250 mg of MSG was associated with angioedema seen at 16 hours post-ingestion. Avoidance of MSG-containing foods reportedly caused clinical remission of angioedema episodes.

Though not extensively evaluated, there have been several studies evaluating the role of MSG in urticaria. Genton et al (55) in 1985 evaluated 19 patients with chronic idiopathic urticaria (CIU) for sensitivity to 28 food additives, including MSG. In a single-blind protocol, four of the 19 subjects reacted, defined by an increase in urticaria within 18 hours following challenge. In 1986, Supramaniam and Warner (56) evaluated 36 children with asthma or urticaria. There were three reactors in this placebo-controlled study in which one placebo, eight food additives, and aspirin were administered at 4-hour increments. Whether the reactions were pulmonary or dermatologic was not specified. A 1988 study in Spain by Botey et al (57) detailed the evaluation of five children with angioedema or urticaria who presented for evalua-

tion of possible drug allergy. Particular attention was paid to the dietary history regarding additives, including MSG. Following a 2-day diet without known additives, these patients were administered 50 mg of MSG orally in a single-blinded fashion; if there was no reaction in 1 hour, an additional 100 mg was given. Three children had recurrence of urticaria at 1, 2, and 12 hours following ingestion; one developed pruritic erythema of the skin at 1 hour; and the fifth developed abdominal pain and diarrhea following ingestion of 50 mg of MSG. The authors of the study did not provide details regarding concomitant medication use. In 1995, Zuberbier (58) challenged a group of chronic urticaria patients with multiple food additives and found no reactors (defined as urticaria 24 hours post-challenge) following a dose of 200 mg of MSG. Because the majority of studies evaluating the role of MSG as an agent in angioedema or urticaria did not detail whether medications such as antihistamines had been discontinued prior to the challenge protocols, a "positive" reaction may simply have been due to a flare in baseline disease unmasked by discontinuation of disease-controlling drugs.

A recent study of MSG ingestion in patients with urticaria, however, did account for patterns of baseline disease and concomitant medication use. In 2000, Simon (59) evaluated 65 patients with chronic idiopathic urticaria/angioedema who had urticaria > 50% days in the preceding 6 weeks prior to study enrollment. Subjects continued to take antihistamines at their minimally effective doses. Twenty of the 65 subjects gave a history of sensitivity to food additives, including four to MSG. Subjects initially underwent a single-blind placebo-controlled screening challenge after a baseline skin score was obtained. Additive-filled opaque capsules containing multiple food additives (including 2.5 g MSG) were administered, with repeat skin scores determined every hour for 4 hours. A positive screening challenge was considered to be a 30% increase from baseline urticaria. Subjects with positive screening challenges then underwent double-blind additive challenges, including 2.5 g of MSG. Two of the 65 subjects exhibited a positive challenge on screening protocol but had negative challenges on double-blind challenge protocol.

Given the rarity of reports in the literature, it is unlikely that MSG-induced urticaria or angioedema occurs often or possibly at all, even in patients believing themselves to be MSG reactors. Further evaluation of angioedema or urticaria suspected to be related to MSG should be undertaken in a DBPC setting, with attention given to the baseline activity of disease so that false-positive results can be minimized or eliminated.

Headache

Though many people believe they have adverse reactions to foods and food additives, few people have confirmed sensitivity on objective examination. Not surprisingly, therefore, MSG has been associated anecdotally with a myriad of physical and psychiatric complaints. Of all adverse symptoms thought to be associated with MSG ingestion, headache was the symptom most often reported to the FDA's Adverse Reaction Monitoring System between 1980 and 1995 (60). In 1969, Schaumberg et al (29) performed one of the first formal studies of the symptoms potentially associated with MSG ingestion. That study suggested that the three main symptoms consisted of a burning sensation, facial pressure or tightness, and chest pain. Headache occurred in a minority of the subjects. Ratner et al in 1984 described four patients with MSG-related headaches (61). They were evaluated with double-blind testing consisting of sublingually administered soy sauce with and without 1.5–2.0 g of added MSG. These patients developed recurrent headaches within 15 minutes to 2 hours of sublingual administration of the MSG-added soy sauce but not to "placebo" soy sauce, and reportedly had relief of symptoms with MSG avoidance. No attempt was made to disguise the tastes of the two soy sauce formulations. Furthermore, since glutamate is likely to be present in any soy sauce, soy sauce could not be expected to serve as a true glutamate-free placebo. Scopp in 1991 described two chronic headache patients who decreased the frequency of their headaches through MSG avoidance. No objective testing was performed (62).

Theories regarding the etiology of MSG-induced headache are scarce. Merritt et al (63) in 1990 found that high concentrations of glutamate caused concentration-dependent contractions of excised rabbit aorta. These authors suggested that a similar vascular response might account for MSG-induced headache. No well-designed scientific studies confirming the existence of headaches due to MSG exist to date. Low-MSG diets should not be empirically recommended for the chronic headache patient since they are not based in clear scientific fact and are only likely be an unnecessary burden for these patients.

Conclusions

Overall, the available data on MSG reflect that it is safe for use as a food additive in the population at large. MSG toxicological data has demonstrated no serious nervous system effects, metabolic studies performed in infants and adults have shown ready and rapid utilization of excess glutamate, and serum glutamate levels have remained stable even when very large levels of MSG were ingested with carbohydrate. DBPC studies indicate that MSG ingestion is likely to be without adverse effect even in people suspecting themselves to be MSG reactors. MSG has not been clearly documented to cause bronchospasm, urticaria or angioedema, or headache. It is possible that large doses in excess of 3 g of MSG ingested into an empty stomach and unaccompanied by carbohydrate may be result in the adverse reactions known as the MSG Symptom Complex. This syndrome is likely to be infrequent, and resolves within 2 hours without treatment. In conclusion, there is no clear evidence in the current scientific literature documenting MSG as a cause of any serious acute or chronic medical problem in the general population.

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26

Tartrazine, Azo, and Non-Azo Dyes

Donald D. Stevenson

Ten coal tar derivatives are currently accepted under the Food, Drug and Cosmetic Act (FD & C) for use as dyes in food, drink, and color coding for capsules and tablets (1). All of these dyes contain aromatic rings, and some contain azo linkages (-N:N-). Azo dyes include tartrazine (FD & C Yellow No. 5) (Fig. 26–1), sunset yellow (FD & C Yellow No. 6), ponceau (FD & C Red No. 4), and carmoisine (EEC No. E. 122). By contrast, the non-azo dyes do not contain the -N:N- linkages. A few examples of non-azo dyes include brilliant blue (FD & C Blue No. 1) (Fig. 26–2), erythrosine (FD & C Red No. 3), and indigotin (FD & C Blue No. 2). Since all of these dyes are approved for use in humans, addition of any dyes to processed food and drink occurs routinely in the developed countries of the world. Therefore, the issue is not whether we are exposed to these chemicals but rather what harm they cause in humans. Some claim that all chemicals are harmful and should be banned from the diet. Others focus on certain subpopulations as being vulnerable to the adverse effects of dyes. The purpose of this chapter is to review the data that are relevant to the above issues. Tartrazine is selected for special emphasis because an extensive literature is already available to help us analyze this azo dye.

In 1984, Simon (2) reviewed the subject of generalized adverse effects of dietary dyes on the general population and unequivocally took the position that the evidence was speculative that azo and non-azo dyes were harmful to humans. Despite numerous claims by health food advocates and physicians who practice environmental medicine, no credible evidence supports global claims that these dyes cause injury, mental disease, or other known chronic disorders in the general population. Whether or not some individuals, who are perhaps genetically vulnerable, experience immune or pseudo-immune reactions to these dyes, is a different question. This chapter is organized into sections dealing with dye-induced urticaria and angioedema reactions, asthma, anaphylaxis, various cutaneous reactions, and hyperkinesis.

Urticaria/Angioedema Reactions Associated with Tartrazine and Other Dyes

In 1959, Lockey (3) described three patients who gave a history of developing a rash after ingesting yellow color-coded medications. The author conducted unblinded challenges with dilute solutions of tartrazine and concluded that the itching and other subjective complaints, which the patients experienced over the next few hours, were evidence of allergic reactions to tartrazine. In 1972, Juhlin et al (4) reported a prevalence of tartrazine-associated urticaria that ranged between 49% and 100% of subjects who ingested 1–18 mg of tartrazine. During the remainder of the 1970s, others reported tartrazine-induced urticarial reactions, but the prevalence of reactions was less (5, 6). In 1981, Juhlin (7) reported that 18 (10%) of 179 patients with chronic urticaria reacted to tartrazine during single-blind challenges. Challenge doses reflected the belief at that time that tiny concentrations of tartrazine (0.1 mg) in color-coded medications were capable of inducing urticarial reactions. During single-blind challenges in pa-

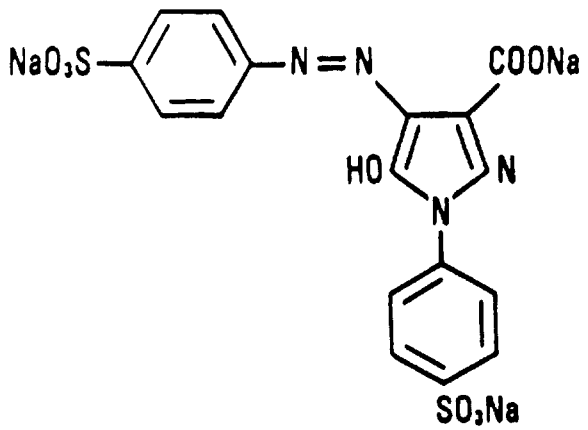

Figure 26–1. An example of an azo dye (tartrazine or FD & C Yellow No. 5). Note azo linkages.

tients with chronic urticaria, antihistamines were discontinued before the placebo challenges, which were always conducted first. By the time tartrazine was given, the therapeutic effects of antihistamine would have worn off and the appearance of hives could have been due to withdrawal of this essential treatment. The same author, however, reported a prevalence of tartrazine-induced urticaria of 100% in 1972, and 9 years later, in a second population of patients from the same country, a prevalence of 10%. Even taking into effect the potential (but difficult to carry out) reduction in tartrazine ingestion in the general population, a drop in the reaction rate of 90% seems astounding.

Doeglas (8), Thune and Granholt (9), and Gibson and Clancy (10) reported tartrazine-induced urticarial reactions in, respectively, 21%, 30%, and 34% of patient populations with chronic urticaria who underwent single-blind challenges with tartrazine. In the six studies reviewed above, all challenges were single-blinded with placebo challenges always given first. Antihistamines were withheld in two studies, and no information on the use of antihistamines was provided for the other four studies.

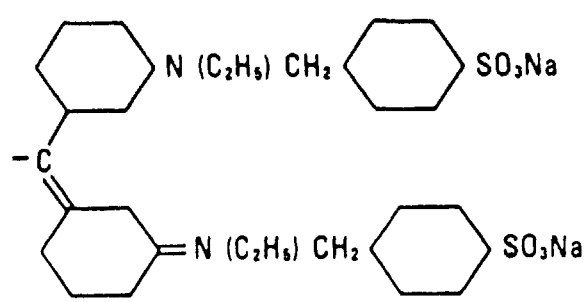

Figure 26–2. An example of a non-azo dye (brilliant blue or FD & C Blue No. 1).

Up through 1976, three studies were conducted that relied on double-blind, placebo controlled (DBPC) oral challenge techniques. Gibson and Clancy (10), reported that 26 (34%) of 76 patients with chronic idiopathic urticaria reacted to tartrazine during DBPC tartrazine challenges. Three of the 26 reactors were rechallenged with tartrazine 1 year later and no longer experienced an urticarial reaction to tartrazine. The authors interpreted this change in reactivity to be secondary to the institution of a tartrazine-exclusion diet, which these three patients believed they were following during the year. In 1975, Settipane and Pudupakkam (11) conducted DBPC challenges in two patients with chronic urticaria and 18 with aspirin-induced urticaria. In one of the two patients with chronic urticaria, ingestion of 0.22 mg of tartrazine correlated with an urticarial flare during a double-blind challenge. In the aspirin-induced urticarial patients, 2 (11%) of the 18 experienced a flare of urticaria during double-blind challenges with tartrazine. In a 1976 report, the same authors conducted double-blind tartrazine challenges in 38 patients with chronic urticaria (12). Of these 38 patients, 10 (26%) experienced flares of urticaria after ingesting aspirin. Using tartrazine doses of 0.22 mg during double-blind challenges, 3 (8%) of the 38 experienced a tartrazine-associated flare of acute urticaria.

Of the nine studies reviewed above (4–12), only the last three were DBPC. In seven studies (5–10, 12) the study population had chronic idiopathic urticaria, including the three double-blinded studies. In the remaining two studies (4, 11), the study population was never described. Antihistamine therapy was withheld in five studies (5–7, 10, 11), but the timing of withdrawal relative to the beginning of the challenges was not clearly stated. The remaining six studies (4, 6–9, 12) did not provide information about concomitant use of antihistamines during the challenges. The presence or absence of aspirin-induced urticaria in the study populations was not clarified in most studies, with the exception of the studies by Settipane (11, 12). Nevertheless, in three studies (6, 9, 10), tartrazine-induced urticaria occurred in at least some patients with chronic idiopathic urticaria whose urticaria was not flared by aspirin. Therefore, a tight linkage between aspirin-induced urticaria and tartrazine-induced urticaria was not established.

In 1986, Stevenson et al (13) reported the results of tartrazine challenges in 10 patients sus-

pected of having flares of urticaria that were believed to be caused by ingestion of tartrazine. None of these patients had chronic idiopathic urticaria, and all were classified as having acute intermittent urticaria. Most were not taking regular antihistamines because their urticaria was not chronic. For the challenge study protocol, antihistamines were not allowed during the study. A screening single-blinded, placebo-controlled tartrazine challenge was conducted first, using doses of 25 mg and 50 mg. If this challenge was negative, a double-blind challenge was not conducted. If the screening challenge with tartrazine was positive, the protocol called for a confirmatory double-blind challenge. At the beginning of the challenges all 10 patients were free of urticaria or angioedema. One patient developed hives 30 minutes after ingesting 25 mg of tartrazine during the single-blind challenge. She was rechallenged 5 days later using a DBPC method with 25 mg of tartrazine and two placebos. During this second challenge, her urticaria also flared 30 minutes after ingesting 25 mg of tartrazine. Later she underwent a single-blinded aspirin challenge with doses up to 650 mg; this challenge did not induce urticaria.

In a second group of nine patients with chronic idiopathic urticaria, antihistamines were continued during the challenges (13). None of these patients experienced an urticarial flare during single-blind challenges with 25 mg and 50 mg of tartrazine. Five (56%) of these patients developed flares of urticaria during challenges with aspirin. Finally, in a separate group of five aspirin-sensitive urticaria patients, challenges with tartrazine again failed to induce urticarial reactions on the day before aspirin challenges induced generalized urticarial reactions.

Murdoch and colleagues (14) studied 24 patients who were suspected of having dye-induced urticaria because their disease was in remission while consuming a diet free of dyes and additives. During multiple double-blind challenges with a variety of drugs and additives, the following results were recorded. Fifteen (63%) of the 24 did not react to any challenge substance. Four patients experienced urticarial reactions during aspirin challenges, two reacted to sodium benzoate, and three reacted to a panel of azo dyes (tartrazine, sunset yellow, amaranth [FD & C Red No. 2], and carmoisine). Thus, only four (17%) of the 24 reacted to the substances that they were avoiding with presumed therapeutic success. Furthermore, three of the four subjects were admitted to hospital for more extensive challenge studies. Two of the three experienced urticarial reactions to each of the four dyes during DBPC challenges. The third patient did not react during any of the in hospital challenges. However, plasma and urine histamine levels increased during the challenges in all three patients. Simultaneously, prostaglandins were measured in the urine during the challenges. It was fascinating to note that even though patient #3 did not have clinical reactions, his plasma and urine histamine rose and prostaglandins were found in the urine during the active dye exposures in his placebo-controlled challenge sequences. Shock organ responsiveness appeared to diminish in this patient, even though mediators were released during interactions with the dyes.

In conclusion, tartrazine and several other azo dyes can provoke urticarial reactions in a small number of patients. It seems unlikely that tartrazine and other azo dyes are the hidden "cause" of chronic urticaria in the vast majority of patients afflicted with this disorder. Even in the carefully controlled Murdoch study, 83% of patients who eliminated dyes and additives in their diet and experienced "improvement," nevertheless did not react to these compounds during double-blind challenges. Finally, there does not appear to be any cross-reactions between tartrazine and aspirin or the other nonsteroidal anti-inflammatory drugs (NSAIDs) with respect to tartrazine-induced urticaria.

Asthma Associated with Tartrazine and Other Dyes

In 1958, Speer (15) wrote in his book that "agents used in artificial coloring were the cause of asthma in sick children." Data supporting this claim were not presented in his book. In 1967, Chafee and Settipane (16) discovered a patient who believed that food dyes were worsening her asthma. Using a double-blinded protocol, they introduced a new dye or placebo each day for 6 days. On the day she ingested tartrazine, she experienced coughing. Objective measures of lung function were not collected and the challenge with tartrazine was not repeated at another time. The possibility of coincidental coughing cannot be excluded in this study.

Samter and Beers attempted to link tartrazine sensitivity to aspirin intolerance (17, 18). In their first report of 80 asthmatic patients, challenges with unknown doses of tartrazine, using unknown challenge protocols, produced three "reactions" to

tartrazine (17). In their second report, 14 (8%) of 182 asthmatic patients were said to have reacted to tartrazine (18). The report did not indicate how many subjects were aspirin-sensitive, by what criteria this fact was established, and how many of the 14 experienced urticaria or asthma during tartrazine challenges.

In 1975, Settipane and Pudupakkam (11) conducted DBPC tartrazine challenges in 20 asthmatic patients. Using small doses of tartrazine (0.44 mg), they reported that 3 (15%) of 20 experienced a 20% drop in forced expiratory volume in 1 second (FEV_1) values during challenges with tartrazine. Whether or not asthma medications were withheld during the challenges was not stated.

Stenius and Lemola (19) conducted oral challenge studies using small doses of tartrazine (0.1–10 mg). Following ingestion of tartrazine, 25 (22%) of 114 unselected asthmatics dropped their peak flow measurements by 20% from baseline values. In the same study, a separate population of 25 aspirin-sensitive asthmatics underwent tartrazine challenges and 12 (50%) reacted with a greater than 20% decline in peak flow values. It is generally agreed that peak flow measurements are less reproducible that timed flow/volume measurements (20, 21). Most investigators use wedge spirometers and obtain FEV_1 values during repetitive measurements of lung function. This subject was reviewed in detail by Stevenson (22). During placebo challenges in patients with irritable airways, FEV_1 values may decline by as much as 43%. Therefore, it is incumbent upon the investigator to treat the underlying asthma and demonstrate that the FEV_1 values do not vary by more than 10% during placebo challenges, before beginning single- or double-blind challenge studies. Most investigators use a $\geq$ 20% decline in FEV_1 as evidence of bronchospasm during challenge studies, assuming that the baseline challenge with placebo was stable (22). However, in a 1977 report by Freedman (23), a 14% decline in FEV_1 was used as an endpoint and was provided as proof that a tartrazine-induced bronchospastic reaction had occurred.

Specter and coworkers (24) conducted one of the largest studies investigating the prevalence of tartrazine-induced bronchospasm. In their studies, bronchodilators were withheld for 6–12 hours before beginning double-blind oral challenges, with one challenge substance or placebo each day during inpatient hospitalizations. A 20% decline in FEV_1, when compared to the placebo day, was considered to be evidence of a bronchospastic reaction. Tartrazine provoking doses ranged from 1 mg to 50 mg. The results of their study are summarized as follows. There were 277 asthmatic patients in their study. All were challenged with aspirin, and 44 (16%) experienced respiratory reactions. Of the remaining 233 patients (who were aspirin-tolerant), none experienced a 20% decline in FEV_1 values on the days they ingested tartrazine. By contrast, when the 44 aspirin-sensitive asthmatics were challenged with tartrazine, 11 (25%) experienced a 20% decline in FEV_1 values. Unfortunately, of the 11 tartrazine reactors, "five did not undergo placebo challenges" (i.e., did not have a placebo challenge baseline day with proven airway stability before challenges with tartrazine). The authors stopped antiasthma medications in a group of aspirin-sensitive asthmatics whose asthma was severe enough that they were hospitalized, failed to consistently perform baseline placebo challenges, and then noted a 20% decline in FEV_1 values during challenges with tartrazine. Thus, it is not possible to know whether these changes in lung function were due to discontinuing antiasthma medications, inherent hyperirritability of the airways, or tartrazine- and aspirin-induced asthma.

The most revealing study in this area of controversy was performed by Weber and associates (25). Using standard single blind oral aspirin challenges, they identified 13 of 44 asthmatic patients as having aspirin-sensitive asthma. After challenges with tartrazine in doses ranging from 2.5 mg to 25 mg, and withholding morning bronchodilators, 7 (16%) of the 44 patients experienced a 20% decline in FEV_1. Tartrazine challenges were repeated in the same seven patients 1 week later; this time they received their morning bronchodilator medications. During these follow-up challenges, which used the same provoking dose of tartrazine, FEV_1 values remained steady throughout the testing period. These patients were also challenged with six other azo dyes and did not experience any reactions. Furthermore, if one took the position that morning bronchodilator treatment prevented the tartrazine reactions, one is faced with the task of explaining why 13 (30%) of these patients experienced a $\geq$ 20% decline in their FEV_1 values during oral challenges with aspirin while taking the same bronchodilators.

In a study by Vedanthan and associates (26), 49 aspirin-tolerant children and five aspirin-sensitive asthmatic children underwent oral challenges with tartrazine. Standard asthma medications, including cromolyn, theophylline, and corticosteroids,

were continued during the challenges. None of the subjects reacted to tartrazine. The five aspirin-sensitive asthmatics underwent aspirin challenges and experienced a > 20% decline in FEV_1 values. Therefore, the end point criterion of a 20% decline in FEV_1 values as evidence of induced bronchospasm was sensitive enough to detect changes in bronchial airways during aspirin challenges. If tartrazine could actually provoke bronchospasm, one would have expected this to occur in some of the five aspirin-sensitive asthmatic children.

In a study of adult asthmatics by Tarlo and Broder (27), bronchodilators were continued. One of 26 aspirin-tolerant asthmatics experienced a wheezing reaction and a > 20% decline in FEV_1 values during a double-blind challenge with tartrazine. The first point of this paper is the dissociation between aspirin sensitivity and tartrazine-induced asthma. Secondly, the authors stated that elimination of tartrazine from the diet in this one patient did not have any effect on the course of her asthma. This paper is instructive, since the original premise of detecting tartrazine-induced asthma was to then advise the patient to avoid it in the future. Although, one patient provides only an anecdotal report, the hypothesis that dietary tartrazine causes asthma did not gain any support from this patient's clinical course (15, 18, 24).

In the largest series of aspirin-sensitive asthmatics undergoing tartrazine challenges, Stevenson and his associates (13) were unable to detect tartrazine-induced asthma in any of 150 consecutive aspirin-sensitive asthmatics. The protocol for this study was as follows. All patients were admitted to an inpatient clinical research facility. Regular antiasthma medications were continued. All patients underwent single-blind, placebo-controlled oral challenges with 25 mg and then 50 mg of tartrazine. If the baseline placebo challenge was stable and the FEV_1 values dropped by 20% during one of the tartrazine challenges, patients were re-scheduled for a double-blind tartrazine challenge at a later date. However, if the single-blind tartrazine challenge was negative, the patient was classified as not having tartrazine-induced asthma. After tartrazine challenges were completed, all 150 patients underwent single-blind oral aspirin challenges on the next day, and only those patients with a positive oral aspirin challenge were classified as having aspirin-sensitive asthma and were included in this study. Of the 150 patients, 6 (4%) experienced a ≥ 20% drop in FEV_1values, compared to placebo challenges during the single-blind screening challenges. These patients were rechallenged with the same provoking dose of tartrazine in a DBPC oral challenge protocol at a later date. None reacted to tartrazine during these double-blind challenges. At the time of rechallenge, none of the patients were participating in aspirin desensitization treatment and all were taking the same or less antiasthma medications as they were during the first challenge. These studies were extended when another 44 aspirin sensitive asthmatic patients underwent oral single-blind tartrazine challenges at the same institution (28). Again, none of the patients reacted to 25 and 50 mg of tartrazine.

A 1986 study from Poland (29) identified tartrazine sensitivity during oral challenges in 16 (31%) of 51 aspirin-sensitive asthmatic patients. The authors reported that five of the 16 aspirin-sensitive asthmatics who also experienced reactions (dyspnea) to tartrazine when desensitized to aspirin could then take tartrazine without adverse effects. Obviously, there was something radically different about the results of this study and those of Stevenson et al (13). If the study from Poland was accurate, with a tartrazine cross-challenge rate of 31% Stevenson et al (13, 28) should have identified 61 (31%) of 194 tartrazine-sensitive patients to equal the proportion reported in this first European study.

In a large multi-institutional study in Europe, 156 known aspirin-sensitive asthmatic patients underwent screening single-blind oral challenges with tartrazine (30). Four participants (2.6%) reacted to 25 mg of tartrazine with a 25% decline in FEV_1 values during single-blind challenges, and were then challenged with the same dose of tartrazine with double-blind challenges. Again, the four patients experienced a 25% decline in FEV_1 values during double-blind tartrazine challenges. A full day of placebo challenges may have been performed for each patient before starting tartrazine single-blind challenges but this was not reported in their paper. However, comparative placebo challenges obviously were conducted as part of the DBPC follow-up challenges. The authors of this study are well known investigators with extensive experience in conducting oral challenges. The extremely low prevalence of positive single- and double-blind challenge studies with tartrazine (2.6%) in the 1988 European study, contrasts sharply with the prevalence in the 1986 study from Poland (31.4%).

On the basis of scientific facts, what conclusions can be drawn from the literature on this subject? Certainly many of the early studies reporting

large numbers of asthmatics with tartrazine reactions were actually measuring spontaneous asthma in patients whose antiasthma medications had been discontinued before the challenges. Most of the high prevalence rates of positive respiratory reactions to tartrazine are simply not credible. Even the very large study by Spector et al (24) in which 11 (25%) of 44 aspirin-sensitive asthmatics were said to have tartrazine-induced asthma had serious methodological flaws in performance of the challenges.

Second, there probably are a few patients with tartrazine-related reactions, which include urticaria (13) and bronchospasm (27, 30). Whether or not these reactions are IgE-mediated is not known but such an explanation is more attractive than the idea that tartrazine participates in pseudoallergic cyclooxygenase-1 (COX-1)-inhibiting cross-reactions. Tartrazine does not inhibit cyclooxygenase (31).

Third, except for the Samter study in 1968 (18), the Specter study in 1979 (24), and the 1986 study in Poland (29), the link between aspirin-sensitive asthma and tartrazine sensitivity is not supported by any of the more recent and larger studies (22, 28, 30). Since all the NSAIDs that cross-react with aspirin inhibit COX-1, and tartrazine does not inhibit COX-1, there is no logical reason to suspect cross-reactivity in the first place. Furthermore, the study by Tarlo and Broder (27), in which tartrazine-induced asthma was found in a aspirin-tolerant asthmatic, and the two studies by Stevenson and colleagues (13, 28), in which tartrazine-induced asthma was not found in 194 aspirin-sensitive asthmatics, make it difficult to link aspirin sensitivity with tartrazine-induced asthma attacks. Even the European study of tartrazine-induced asthma found only 4 (2.6%) of 156 aspirin-sensitive asthmatics that also reacted to tartrazine. Their reported incidence of 2.6% is too low to qualify as a cross-reacting chemical. COX-1-inhibiting NSAIDs cross-react in aspirin sensitive asthmatics 100% of the time, and the weak inhibitors of COX-1, acetaminophen and salsalate, cross-react 34% and 20%, respectively (32–36).

Finally, with respect to recommendations to patients, it is logical to warn all aspirin sensitive asthmatics and chronic urticaria patients to avoid cross-reacting drugs (NSAIDs, acetaminophen, salsalate) (37, 38). However, to make the same recommendation for tartrazine does not make any sense. Arguably, in the entire medical literature only five cases of tartrazine-induced asthma have been reported in which double-blind challenges were conducted (27, 30). Only four of the five cases were in aspirin-sensitive asthmatics and an even larger study, conducted in the same manner, reported no cases of tartrazine sensitivity in 194 aspirin-sensitive asthmatics (13, 28). In the Tarlo and Broder report (27), even when a case of tartrazine-induced asthma was discovered, elimination of tartrazine from the diet of that individual did not change the course of her ongoing asthma. In an extensive online review of 90 articles on the subject of tartrazine challenges and avoiding tartrazine in the diet of asthmatics, only 18 articles were potentially relevant. None of these articles presented evidence in which either challenge with tartrazine or avoidance of tartrazine in the diet significantly altered asthma outcomes in the study subjects (39). Therefore, rather than making generalized recommendations regarding tartrazine avoidance, my recommendation would be to screen patients on the basis of history and conduct oral challenges with tartrazine in those who gave a positive history. In those patients who experienced bronchospasm during DBPC tartrazine challenges, one could consider recommending avoidance of tartrazine on a trial basis. Reporting the results of such a rare occurrence and potential dietary manipulation in a letter to the editor would be helpful and appropriate.

Anaphylaxis from Ingestion of Dyes

Caucino et al (40) reports a case of anaphylaxis after a patient ingested an estrogen tablet containing FD & C Red No. 40 and FD & C Yellow No. 27. A puncture prick test of the skin with a suspension of the ground up estrogen tablet, including the dyes and other excipients, induced a wheal and flare cutaneous response. Prick tests to the estrogen and other excipients was negative. Oral challenges with the two azo dyes were not performed.

Atopic Dermatitis Reactions

In a small study of 12 children, ages 1–6 years, with atopic dermatitis (AD), multiple double-blinded challenges with 50 mg of tartrazine were performed (41). The 12 children were selected for the study because they had severe and intractable AD and a parental history that tartrazine ingestion caused flares of their dermatitis. In one child, flares of dermatitis occurred during the three tartrazine challenges and not when placebos were

administered. Both the symptom scores and the physician observer scores were significantly and consistently increased only after tartrazine challenges. In a sample of 12 patients, the probability that three positive challenges with tartrazine and three negative challenges with placebo would occur by chance alone was 0.46. Balanced against the fact that tartrazine sensitivity was probably observed in one patient is the striking prevalence of non-reactions in 11 (92%) of 12 atopic children in whom the parents were convinced that tartrazine was a provoking agent. This is consistent with a desire on the part of parents to fix their child's chronic disease by eliminating something. Since dyes and preservatives are found in many foods, drinks, and color-coded pills, there is a strong chance that a flare of dermatitis will coincidentally occur at the same time as a remembered ingestion of a yellow dye. Advertising of this relationship in the lay press increases the chance that the parents will notice yellow dyes as the perceived "cause" of their child's AD.

Contact Dermatitis to Tartrazine and Azo Dyes

Azo dyes are skin sanitizers and can induced delayed hypersensitivity reactions of the skin in a small number of patients (42). Positive patch tests to tartrazine and other azo dyes has been documented in a few patients (43). Skin contact with azo dyes is most likely to occur in workers in the textile industry (44) or in people who wear clothes that have been colored with azo dyes.

Other Cutaneous Reactions to Tartrazine and Azo Dyes

Reports of purpura after ingestion of tartrazine have been reported (45–47). In addition, hypersensitivity vasculitis has been documented in patients who were ingesting tartrazine on a regular basis (48). Discontinuing tartrazine was associated with disappearance of vasculitis in some cases (49).

Hyperkinesis and Tartrazine

Hyperkinesis and learning disorders have been attributed to ingestion of tartrazine in children (50, 51). There is considerable controversy surrounding this subject and some authors do not believe that tartrazine has any effects on either hyperkinesis or learning disorders (52). In reviewing the literature on this subjects the results are inconclusive. This is largely because reports of tartrazine-induced effects on mental function and behavior are plagued by poorly designed studies, imprecise definitions of hyperactivity, and poor reliability of behavioral outcome measures. Furthermore, it has been difficult to define study populations and segregate them from the background noise of a heterogeneous population of children. Placebo effects, as detected by vigilant parents, have consistently reflected parental attitudes and bias in favor of tartrazine as a perceived cause of their child's problems. A number of articles, where poorly performed studies of tartrazine and hyperkinesis were reported, were not selected for mention in this review.

Despite this gloomy introduction, there are a few studies that address most of the investigative issues and present a reasonable case in support of some children with tartrazine-induced mental abnormalities. Swanson and Kinsbourne (53) conducted oral tartrazine challenges in 40 hyperactive children with up to 150 mg of tartrazine in one day. The performance of the hyperactive children was impaired on the days they received tartrazine but not on the days when they received placebos. The control children (without a diagnosis of hyperkinesis) did not experience any differences in behavior on any days, whether ingesting the dye or placebo.

In another study, Rowe examined 220 children referred because of suspected dye-induced behavior problems (54). After interviewing all 220 children, the author admitted 55 to the study as a core group of suspected tartrazine-induced behavioral disorders. Further screening was then employed by restricting the children's diets to avoid dyes and preservatives over a study period of 6 weeks. At the end of this screening period 40 (73%) of 55 were reported by parents as improved in behavior. Of these 40 children, 14 were said to strongly exhibit abnormal behavior when ingesting foods containing azo coloring. For eight of these highly selected children, the parents agreed to enroll them in a DBPC crossover challenge study. Each day over a study period of 2 weeks, the children received placebo, tartrazine, or carmoisine. When the codes were broken, only two (25%) of the eight showed any correlation with ingestion of the dyes and abnormal behavior. The remaining six subjects did experience behavioral changes but such changes occurred on placebo days as well as on days when the dyes were given.

In summary, of 220 subjects, whose parents thought that tartrazine induced behavioral changes; in only two subjects did challenges with the dyes correlate with behavior changes.

The authors extended their studies in a group of 24 patients selected during challenge studies, from a referral population of 800 children, suspected by their parents of having hyperkinesis secondary to ingestion of tartrazine (55). A dose response effect was discovered during DBPC challenge tests in these 24 study subjects. The minimum dose of tartrazine associated with hyperactivity in affected children was 10 mg per day. However, some children did become hyperactive until they received larger doses.

The first conclusion was that only a small proportion of children suspected of having dye-induced behavioral problems are actually affected. Second, a dose of at least 10 mg of tartrazine was required before any behavioral changes in the affected population of children were. This makes it difficult to implicated color-coded tablets and capsules, in which the total dose of dye is < 1 mg. Finally, although the evidence is rather persuasive that dye-induced behavioral changes can occur in some children who ingest moderate to large doses of dyes, the claim that most children with behavioral disorders are the victims of dye-induced reactions is not supported by the facts.

Conclusions

Although a few well-designed studies have been conducted, the literature is filled with studies that are not of high quality and yet report dye-induced events, sometimes in large numbers of patients. After sifting through the maze of claims against tartrazine and other azo dyes, the paucity of documented adverse events caused by these dyes is apparent. Except for rare patients who experience mild asthma or urticaria, anaphylaxis, cutaneous vasculitis, and contact dermatitis as a consequence of exposure to dyes, the vast majority of humans tolerate them without any problem. In fact, the overwhelming majority of claims against the dyes are of mistaken identity or association, or misdirected blame.

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27

Adverse Reactions to Butylated Hydroxytoluene and Butylated Hydroxyanisole (BHT and BHA)

Richard W. Weber

Foods containing vegetable or animal fat turn rancid through chemical changes induced by exposure to oxygen, heat, moisture, or the action of enzymes. The rapidity with which rancidity develops depends on the source and storage conditions of the fats or oils. Unsaturated fats have carbon-carbon double bonds in their structure, and these sites are susceptible to the chemical changes that cause rancidity. Saturated fats are more resistant. Vegetable oils have more unsaturated fats, but also contain naturally occurring protective antioxidants such as tocopherols. Animal fats are more saturated, but have lower amounts of natural antioxidants, and therefore are at greater risk for spoilage (1, 2). Similar factors cause the "browning effect": fruits and vegetables losing their freshness and turning color. Antioxidants block these events, and may even restore "freshness" in some cases.

The phenolic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used in a large number of foods that contain oil and fat. Other chemicals having antioxidant activity are frequently used in combination with BHA or BHT to enhance their activity; such agents include propyl gallate, citric acid, phosphoric acid, and ascorbic acid. Additionally, the naturally occurring antioxidant compounds called tocopherols have varying amounts of vitamin E action. About eight forms occur naturally in foods such as vegetable oils, cereals, nuts, and leafy vegetables, and are used commonly in baked goods, cereals, soups, and milk products.

BHA and BHT are synthetic compounds and do not occur in nature. BHT, also termed 2,6-di-*tert*-butyl-4-methylphenol or 2,6-di-*tert*-butyl-*p*-cresol, is manufactured from *p*-cresol and isobutylene (3). BHA is a mixture of two isomers, 85% 2-*tert*-butyl-4-methoxyphenol and 15% 3-*tert*-butyl-4-methoxyphenol (Fig. 27–1) (4). BHT was initially patented in 1947. These substances were developed as antioxidants for petroleum and rubber products, but were found to be effective antioxidants for animal fats.

In 1949, BHA appeared on the new Class IV preservative positive (allowed) list of the Health Protection Branch of Health and Welfare Canada. Usage was restricted to levels under 0.02% (5). Animal studies from the manufacturers were submitted to the US Food and Drug Administration (FDA) in 1954 and 1955, and permission was granted prior to the 1958 Food Additives Amendment. Therefore BHA and BHT were given "generally recognized as safe" (GRAS) status and no additional studies were required. However, a number of items on the GRAS list have come under further scrutiny, and BHA and BHT remain on the list with provisional status. The FDA limits their use in food, either alone or in combination with other antioxidants, to $\leq$ 0.02% of the total fat and oil content (1).

These compounds are commonly added to various foods, cosmetics, and pharmaceuticals to prevent oxidation of unsaturated fatty acids, and are considered more potent than other antioxidants. Also, they are less expensive than some other antioxidants, such as nordihydroguaiaretic acid (NDGA) (5). BHA is used more than BHT because it is more stable at higher temperatures.

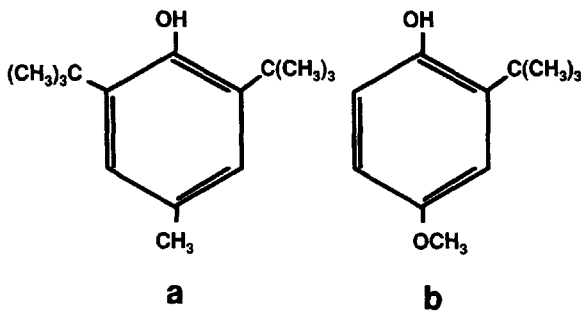

Figure 27–1. (a) Butylated hydroxytoluene (BHT, 2,6-di-*tert*-butyl-4-methylphenol). (b) Butylated hydroxyanisole (BHA, 2-*tert*-butyl-4-methoxyphenol). Commercial BHA also contains 15% 3-*tert*-butyl-4-methoxyphenol.

By 1970, the total amount of BHT used in foods was near 600,000 pounds, twice that used in 1960. The US average daily intake per person of BHT was estimated as 2 mg in 1970, while intake in the United Kingdom was estimated at half that rate (3). In 1974, the Joint Food and Agriculture Organization (FAO) of the United Nation/World Health Organization (WHO) Expert Committee on Food Additives had recommended 0.5 mg/kg as the acceptable daily intake (ADI) of BHA, BHT, or their sum, which has since been decreased (4). By 1976, the total annual production of BHT in the US was 19.81 million pounds, of which 8.86 million was for food use (3). With the greater present reliance of the North American diet on processed, packaged foods, more recent daily intakes of BHA and BHT are substantially larger. In 1986, the mean daily intakes for BHA was 0.13–0.39 mg/kg body weight per day. The intake for teenage males was 12.12 mg/person per day, with the average for both sexes of all ages at 7.40 mg/person per day (5). Daily dietary intake of BHA and BHT was estimated for the Netherlands in 1987–1988 (6). It appeared unlikely that the ADI for BHA (0–0.05 mg/kg body weight) was surpassed even in very high caloric intake (except for some extreme cases of 1–6 year olds). But the intake of BHT exceeded recommendations (FAO/WHO, 0–0.125 mg/kg; EEC, 0–0.05 mg/kg) in all age groups, and especially in 1–6 year old children. In Italy, theoretical maximum daily intake (TMDI) of the antioxidants BHA, BHT, erythorbic acid, and gallates were estimated using a hierarchical step-by-step approach (7). The likelihood of exceeding the ADIs was very low for all but BHT, which was above the current ADI. The three main food category sources for BHT (contributing over 74% of the TMDI) were 1) pastry, cake and biscuits, 2) chewing gums, and 3) vegetable oils and margarine. Recent estimates of the TMDIs of the phenolic antioxidants, BHA, BHT, and tertbutyl hydroquinone (TBHQ) in Brazil were 0.09–0.15, 0.05–0.10, and 0.07–0.12 mg/kg, respectively (8). These estimates based on household economic and packaged goods market surveys were supported by high-performance liquid chromatography/ultraviolet (HPLC/UV) detection in samples from selected food categories. The authors felt that it was unlikely that the average Brazilian consumer would exceed the recommendations for ADI. However, the ADIs given (BHA 0.05, BHT 0.3, TBHQ 0.7 mg/kg) were higher than the FAO/WHO guidelines.

BHA and BHT are used in breakfast cereals; chewing gum; snack foods; vegetable oils; shortening; potato flakes, granules, and chips; enriched rice; and candy (1). In addition to human food, BHT is added to animal feeds, such as fish meal in poultry feed. Passive food exposure to these antioxidants also occurs through their use in food-packaging materials like pressure-sensitive adhesives, paper and cardboard, lubricants, and sealing gaskets for food containers (1, 3). BHT was demonstrated to migrate from low-density polyethylene films into sunflower oil (used as a fatty food simulant) over 7 weeks at a greater rate than another antioxidant, α-tocopherol (9).

Toxicology

Despite the ease with which these antioxidants passed muster at the FDA, animal toxicology studies have revealed a variety of adverse events. These may or may not be related to their actions as antioxidants. BHA and BHT act as lipid soluble chain-breaking agents, delaying lipid peroxidation by scavenging intermediate radicals such as lipid peroxyls (10). In the process, the antioxidant has lost a hydrogen atom, thus becoming a radical. The antioxidant radical is generally less reactive than the peroxyl free radical, but under some circumstances can show pro-oxidant properties, frequently due to interactions with iron ions.

Single doses of BHT have been shown to induce interstitial pneumonitis and pulmonary fibrosis in mice, while BHA and other antioxidants did not appear to have this action (11). This BHT effect can be potentiated by oxygen given early but not late (12, 13). High-dose corticosteroids additionally may significantly worsen lung damage if given early, while late administration may allevi-

ate the injury (14, 15). Whether the lung injury is mediated through some unique property of BHT rather than through an antioxidant pathway is unclear; it does appear that the extent of damage depends on several factors interweaving both dose and timing. There are distinct mouse-strain differences in the chronic response to BHT, which may be due to cytochrome P450 2B isozyme conversion of BHT to the more pneumotoxic metabolite *tert*-butyl hydroxylated BHT (BHTOH) (16). CXB H mice became tolerant to the chronic administration of BHT, while BALB/cBy mice showed a chronic inflammatory process with activated alveolar macrophages and increased lung tumor multiplicity. Acute effects demonstrated 2- to 5-fold decreases in protein kinase Cα and calcium-dependent protease isozyme II (calpain II). BHT and BHTOH both induce apoptosis and are pneumotoxic, with BHTOH being more potent in both regards (17). Non-tumorigenic cells were more sensitive to BHTOH-induced apoptosis, suggesting a mechanism for BHT/BHTOH tumor promotion.

Yu and colleagues (18) studied BHA-induced toxicity of rat hepatocytes and found loss of mitochondrial transmembrane potential, with subsequent release of cytochrome c, activation of cysteine aspases (caspases)-3, -8, and -9, and ultimately, apoptosis. Cyclosporin A, which stabilizes mitochondrial permeability, inhibited this apoptotic cascade. BHT and metabolites can induce apoptosis in several cell lines, operating not only through cytochrome P450 mechanisms, but also via oxidative DNA damage hydrogen peroxide generation (19, 20).

Impact of dietary antioxidants on cancer prevention has received much scientific and media attention. BHA and BHT have been shown to both protect from and enhance tumor development in different systems. BHA, BHT, and NDGA have been shown to decrease skin tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate (TPA), benzoyl peroxide, and ultraviolet light. BHA achieves this result through decreased gene expression of ornithine decarboxylase, an indicator of skin tumor promotion and hyperprofileration (21). BHT, however, increased the incidence of liver tumors in male C3H mice (22). The same study showed increased colon cancer in BALB/c mice following one chemical carcinogen, dimethylhydrazine, but not another, methylnitrosourea. BHA, on the other hand, appeared to protect against the acute liver toxicity of a colon-specific carcinogen, methylazoxymethanol acetate (23). However, high-dose BHA was shown several years ago to produce cancers of the forestomach in rats (5). Since humans do not have forestomachs, and doses about 10,000 times higher than likely human consumption were used, it was felt by the FAO/WHO Joint Expert Committee on review of the data that the benefits of BHA outweighed the potential risks (5). The Netherlands Cohort Study begun in 1986 examined the association between dietary intake of BHA and BHT and stomach cancer risk (24). Complete data on BHA and BHT intake of 192 stomach cancer cases and 2035 subcohort members were available for case-cohort analysis. Mean intake of BHA or BHT were 105 and 351 μg/day, respectively. A statistically non-significant decrease in stomach cancer risk was seen with increasing BHA/BHT intake, therefore no risk was found with usual intake of low levels of the antioxidants.

Using human lymphocytes, Klein and Bruser (25) demonstrated BHT cytotoxicity with concentrations > 100 μg/mL. At 50 μg/mL, BHT inhibited the mixed lymphocyte reaction, but not PHA stimulation. A synergistic effect of PHA suppression was seen with co-incubation with either cortisol or prednisolone.

In mice studies, BHA inhibited several microsomal enzymes, but long-term administration also induced specific cytochrome P450 enzymes (26). In humans, BHA 0.5 mg/kg for 10 days had no appreciable effects on biotransformation capacity (27). Antipyrine and acetaminophen metabolism were unaffected. Urinary excretion of BHA metabolites was significantly increased on days 3 and 7 compared to day 1, suggesting either an inhibition of BHA metabolizing enzymes or bioaccumulation of BHA and/or its metabolites in the body.

Asthma/Rhinitis

Despite a wealth of animal toxicology literature on these antioxidants, there are only scattered reports of adverse reactions to BHA and BHT in humans. In 1973, Fisherman and Cohen (28) reported seven patients with asthma, vasomotor rhinitis, with or without nasal polyps, or the combination, who were suspected of intolerance to BHA and BHT. No clinical details were given, or explanations as to why BHA and BHT were suspected to cause difficulty. These patients were identified following open challenge with capsule ingestion of 125–250 mg of BHA/BHT and reproduction of symptoms of worsening vasomotor rhinitis, headache, flushing, asthma, conjunctival

suffusion, dull retrosternal pain radiating to the back, diaphoresis, or somnolence. No objective measures were noted. BHA/BHT intolerance was additionally documented by a doubling of a Duke earlobe bleeding time (termed the Sequential Vascular Response by the authors) in all cases. No rationale for this test was given, other than a proposed similarity to aspirin intolerance. In a follow-up paper the same year dealing with aspirin cross-reactivity, these authors had apparently found 21 patients with intolerance to BHA/BHT via the bleeding time, of whom 17 had clinical symptoms on challenge. No clinical details were given in this later paper (29).

The following year, in an unsuccessful attempt to duplicate these findings, Cloninger and Novey (30) performed a similar study using oral ingestion of 300–850 mg BHA in five asthmatics and two rhinitics. The baseline earlobe bleeding time was not reproducible in the degree suggested by the authors. None of the patients had clinical exacerbations, changes in peak flows, or more than a 50% change in the bleeding times; there was a non-dose related effect of drowsiness noted in four of seven patients. These authors therefore questioned the validity of clinical BHA intolerance as well as the validity and reproducibility of the sequential vascular response. Parenthetically, Goodman and colleagues (31), in a case of well-documented BHA/BHT-induced chronic urticaria (discussed below), could not demonstrate a positive effect of either BHA 250 mg or placebo on the earlobe bleeding time in either the patient or two controls.

Weber and colleagues found no asthmatic responses (defined as a > 25% drop in forced expiratory volume in 1 second, FEV_1) in 43 moderately severe perennial asthmatics undergoing single-blind capsule challenges with sequential doses of 125 mg and 250 mg of BHA and BHT (32). This was part of a larger study where single-blind challenges were validated by subsequent double-blind challenges. Aspirin sensitivity was documented in 44% of the patients, and reactivity to *p*-hydroxybenzoic acid, sodium benzoate, non-azo or azo dyes in 2%–5%. The author is aware of one unpublished case of a drop in pulmonary function following double-blind challenge with BHT 250 mg in a patient with food anaphylaxis and oral allergy syndrome, but this was not validated with additional blinded challenges. Therefore, at the present time, there are no reports of challenges with either BHA or BHT resulting in well-documented, reproducible asthmatic responses.

Urticaria

In 1975, Thune and Granholt (33) reported on 100 patients with recurrent urticaria evaluated with provocative food additive challenges. Sixty-two patients had positive challenges, with two thirds reacting to multiple substances. Positive responses with individual dyes, preservatives, or anti-inflammatory drugs ranged from 10%–30%. Most reactions occurred within 1–2 hours, with a number occurring between 12 to over 20 hours. Six (12.7%) of 47 tested to BHA reacted, and 6 (13.9%) of 43 reacted to BHT; it is unclear whether these were the same six patients. Test doses were given in 2–3 increments, with the total dose of BHA and BHT being 17 mg. The provocative challenges were not blinded, nor did the authors state criteria for a positive challenge.

In 1977, Fisherman and Cohen reported the results of provocative oral or intradermal challenges of a large number of suspected agents on sequential vascular response (SVR) in the assessment of 215 patients with chronic urticaria (34). Medications were withdrawn 12 hours prior to challenge, with the exception of hydroxyzine, which was held for 72 hours. Intolerance was found in 19 patients with challenges of 250–500 mg of BHA and BHT. Slight details of four reactors challenged with 250 mg each of BHA and BHT were included in a table: in addition to doubling of the earlobe bleeding time, two developed nasal congestion and three had urticaria, although it is not clear whether this was increased over baseline. These authors reported "single or partial etiologies" in 203 (94%) of the 215 patients, an astounding success rate in a clinical entity known for its resistance to defining a cause. The same criticism of lack of mechanism and non-reproducibility of the SVR in other hands also applies to these authors' urticaria evaluations as well as the asthma challenges.

In an urticaria review in 1977, Juhlin (35) reported provocative challenges with a mix of BHA and BHT in 130 urticaria patients. Incremental doses of 1 mg, 10 mg, and 50 mg each of BHA and BHT resulted in nine positive and five probably positive challenges (6.9%–11%). Details of the patients' symptoms, criteria for positive responses, or the blinding of the challenges were not reported. Four years later, Juhlin (36) published the results of a 15-day single-blind challenge battery of dyes, preservatives, and placebo in 330 patients with recurrent urticaria. Antihistamines were withheld for 4–5 days before the start of the challenge

sequence, and testing was accomplished when patients had "no or slight symptoms." Tests were judged positive if "clear signs of urticaria or angioedema" occurred within 24 hours. Of the 330 patients, 156 (47%) received a BHA/BHT challenge with cumulative doses of 1 mg, 10 mg, 50 mg, and 50 mg given (total dose 111 mg). Fifteen percent had positive reactions, and 12% had equivocal reactions. Lactose placebo was given in two doses on days 1, 3, 9, and 12, although modifications in the order did occur. Active substances were given in single to six divided doses at hourly intervals. Most patients did not undergo the entire challenge schedule; one third did not receive a placebo challenge.

Hannuksela and Lahti (37) published their results of an extensive double-blind challenge study in 1986. They evaluated 44 patients with chronic urticaria (> 2 months' duration), 91 atopic dermatitis (AD) patients, and 123 patients with resolved contact dermatitis. They used wheat starch as their placebo rather than lactose, because Juhlin had reported positive responses to lactose placebo. Patients were challenged to 9 mg of sodium metabisulfite, 200 mg of benzoic acid, a BHA and BHA mixture of 50 mg each, and a beta-carotene and beta-apo-carotenal mixture of 200 mg each. Positive reactions were repeated 4 days later to validate the response; challenges were rated as positive if the patient responded both times, and as equivocal if the repeat was negative. Of the 44 urticaria patients none had reproducible positive reactions to BHA/BHT, two responded to the first challenge but not the second 4 days later. The same response occurred with the AD patients; two had equivocal reactions to BHA/BHT. None of the contact dermatitis patients reacted to the antioxidants. One urticaria patient had reproducible responses to the wheat placebo, and another to benzoic acid, and one had an equivocal response to metabisulfite. One AD patient had positive reactions to carotenal/carotene, and another had an equivocal reaction to metabisulfite. One contact dermatitis patient had an equivocal reaction to the wheat placebo (second challenge not done). The authors contrasted their results to those of Juhlin (36), and cited challenge differences to explain their lack of responses. They also wondered whether a prolonged refractory period following the initial positive challenges could account for the negative follow-up trials, since they had waited only 4 days. In general, however, the authors felt that ordinary amounts of food additives do not provoke urticaria or influence AD (37).

In 1990, Goodman and colleagues (31) reported the first double-blind placebo controlled (DBPC) multiple challenge protocol documenting the link of BHA and BHT with chronic urticaria. Two patients with chronic urticaria and angioedema of 3–4 years' duration underwent oral challenges with several agents, which were performed 2–3 times for verification. The patients had improved on restricted diets, but had lost 20–30 pounds in the process. Both patients were admitted, placed on an elemental diet formula, and observed for 5–7 days to establish an estimate of baseline activity. The patients ranked pruritus severity, and skin lesions were ranked from 0–4+ based on intensity and body distribution. Only challenges inducing lesions within 12 hours of ingestion and involving an entire extremity or body area, or generalized, were considered positive. Those occurring 12–24 hours were considered equivocal. A mixture of 125 mg each of BHA and BHT was given. If no major reaction occurred, BHA/BHT 250 mg each was given 2–4 hours later. One patient was additionally challenged to BHA 250 mg alone. Placebo capsules were either dextrose or lactose. The patients were also challenged to sodium benzoate, *p*-hydroxybenzoic acid, and tartrazine and other azo dyes. Both patients reacted within 1–6 hours to BHA and BHT, at all times, and did not react to the other additives or placebo on numerous trials. There were no delayed reactions past 6 hours.

The oatmeal one patient had been routinely eating for breakfast contained BHA and BHT. Both patients were placed on diets specifically avoiding BHA and BHT, resulting in sustained diminution of frequency and severity of urticaria. At 7- and 1-year follow ups, respectively, both patients continued to do well on avoidance, with minor exacerbations after ingesting foods containing BHA and BHT. Each patient returned to his pre-illness weight and was able to resume his normal occupation.

After the initial challenges were completed on the first patient, and the code broken, the patient as well as two normal controls underwent an additional double-blind session, using BHA 250 mg and placebo. This was done to evaluate the predictive value of the sequential vascular response test (SVR) of Fisherman and Cohen. Serial earlobe bleeding times were all unchanged with both placebo and BHA in the patient and control subjects, despite the patient having a brisk urticarial response to the BHA. Skin prick tests with serial dilutions of BHA, BHT, sodium salicylate,

and para-hydroxy benzoic acid (OHBA) were also performed, and all were uniformly negative.

Serial plasma determinations throughout the challenge period for the first patient for CH_{50}, activated C_3 and factor B, and prostaglandin (PG)E2, $PGF_{2\alpha}$, and dihydroxy-keto $PGF_{2\alpha}$ were all unrewarding. CH_{50} decreased 30%–35% randomly on both placebo and active compound days. Activated C_3 and factor B were sporadically elevated on four occasions, twice with placebo and once during the prechallenge baseline period. The prostaglandin levels all decreased as the day progressed, regardless of whether placebo or active compound was given. Therefore, despite the extensive evaluation, the mechanism of action is unclear. An immunological process was not supported by the inconsistent changes in complement components, negative immediate skin tests, and lack of vasculitis on biopsy. The strict elimination diets did not totally ablate lesions in either patient. It may be that the antioxidants acted as potentiators of an underlying unrelated process, similar to the action of aspirin in chronic urticaria (38).

Osmundsen reported a case of contact urticaria due to BHT contained in plastic folders (39). Contact with the folders on unbroken skin resulted in a strong urticarial reaction within 20 minutes. The patient had positive wheal and flare responses to 1% BHA and BHT in ethanol.

The importance of these antioxidants causing or aggravating chronic urticaria is not clear. The true incidence of urticarial adverse reactions to BHA and BHT is unknown. Identification of provokers in a disease of waxing and waning nature may be difficult: are reactions truly causally related, or only spontaneous exacerbations of the process? This is especially an issue when a background of urticarial activity persists during challenges. A sharp definition of what constitutes a positive reaction is necessary. Additionally, the observer rating the severity of the reaction must be blinded as well as the subject, since he is just as susceptible to expectation bias. The studies of Thune and Granholt, and Juhlin fail on both counts (33, 35, 36). The 13%–15% incidence of BHA/BHT reactions reported in these studies is most likely an overestimate. Results of single-blind, placebo-controlled food additive panel challenges at Scripps Clinic have been unrewarding (40). In evaluating more than 20 chronic urticaria patients, a panel including tartrazine, potassium metabisulfite, monosodium glutamate, aspartame, sodium benzoate, methylparaben, BHA, BHT, and sunset yellow (FD & C Yellow No. 6) has revealed no responders. In an unpublished series from 1990 to the present, Weber, after the two patients reported above, found no further positive reactions to BHA or BHT in more than 30 chronic urticaria or angioedema patients undergoing blinded challenges. The importance of double-blinding in such studies has been pointed out by Weber and colleagues (32), and reinforced by Stevenson and associates (41). In the former study, of 15 patients who reacted to dyes or preservatives on open challenge, only three responded under repeat double-blind conditions.

Dermatitis

A variety of non-urticarial skin eruptions have been attributed to food additives. Contact dermatitis may occur to a large number of food additives, especially antioxidants, spices, gums, and waxes. Evidence for such responses can be objectively obtained through patch testing for delayed hypersensitivity.

Flyvholm and Menne (42) reported no positive reactions in 1336 consecutive eczema patients patch tested to BHT. However, a patient with contact dermatitis from TBHQ in hair dye showed cross-sensitization to BHA and BHT (43). In 1987, Tosti and colleagues (44) reported two cases of contact dermatitis due to BHA (0.1% and 0.2% concentrations) in topical agents for psoriasis and eczema, and cited 14 cases of BHA-induced contact dermatitis in the literature. Patch testing was positive for BHA but not BHT in both cases. Orton and Shaw (45) reported another two cases of contact dermatitis from a topical cream containing BHA (0.4%). Interestingly, patch testing was positive with pharmaceutical grade BHA 2% in petrolatum, but not analytical grade BHA 2% in petrolatum, raising the issue of choice of reagents in previous test results. Contact sensitivity to latex gloves may not always be due to the usual rubber allergens, but to antioxidants such as BHA (46). Acciai and coworkers (47) found one case of contact dermatitis from BHA in a pastry cook during an investigation of 72 caterers with eczema. An evaluation of contact sensitivity in 69 women with pruritus vulvae revealed patch test positivity of clinical significance in 40 (58%), one of whom demonstrated sensitivity to BHA (2% in petrolatum) (48). The importance of these instances of contact sensitivity to food considerations is that in some cases, once the hypersensitivity has been initiated through cutaneous exposure, dermatitis symptoms could be flared by ingestion of the

causative agent. Roed-Petersen and Hjorth (49) found four patients with eczematous dermatitis who had positive patch tests to BHA and BHT. Dietary avoidance of the antioxidants resulted in remissions in two of their patients. When challenged with ingestion of 10–40 mg of BHA or BHT both patients had exacerbations of the dermatitis.

Cutaneous vasculitis from food additives in chewing gum has been induced by ponceau (FD & C Red No. 4), and also by BHT (50, 51). A case of acute urticarial vasculitis due to BHT was reported in 1986 by Moneret-Vautrin and associates (51). Biopsy revealed a heavy perivascular lymphoid infiltrate of the upper dermis, with immunofluorescence revealing IgM, C1q, C3, C9, and fibrinogen. Lesions resolved with discontinuation of chewing gum. A series of single-blind challenges showed a reproduction of the lesions with ingestion of BHT and not other ingredients.

Mechanisms

The reports of Roed-Petersen and Hjorth (49), Osmundsen (39), and Moneret-Vautrin (51) suggest that certain adverse reactions to BHA and BHT may be mediated through immunological mechanisms in addition to those seen in typical contact delayed hypersensitivity. Murdoch and coworkers (52) described histamine release from leukocytes following challenges of aspirin, benzoate, BHA/BHT, and azo dyes. The authors studied 12 urticaria patients and 18 healthy subjects. BHA and BHT caused histamine release one time each in an urticaria patient, but four healthy subjects reacted to BHT and one to BHA, raising the question of clinical relevance in these in vitro tests. These studies suggest that immune effector cells are probably involved in at least some of these adverse effects, and that different mechanisms are operant. The majority of data to date, however, do not indicate that these are immunologically specific reactions.

Several authors have felt that adverse cutaneous reactions in humans to BHA or BHT were akin to skin lesions induced by aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), and represented alterations in the arachidonic acid-prostaglandin cascade. There is no data at present supporting such an action of the phenolic antioxidants. The evaluation of the single patient of Goodman and associates (31) did not reveal obvious perturbations of prostaglandin metabolites despite clinical exacerbation. It appears reasonable that BHA and BHT are acting in these circumstances in a pharmacological manner, but the mode continues to be unclear.

Unsubstantiated Effects

In addition to the purported adverse effects of BHA and BHT advanced by Fisherman and Cohen (28) based on the non-reproducible SVR, in the past these two antioxidants gained notoriety in the health food lay press as life prolongation agents. Claims for their benefit in increasing life span are apparently based on mice studies performed 25 years ago (3). Unfortunately, these studies had somewhat contradictory results, and it is unclear whether the improved life span in the mice could not also be achieved by optimum normal diet. Recommendations have been made for the ingestion of 2 g of BHT daily as a counteragent for disordered nutrition, age-related problems, and genital herpes (4). As pointed out by Llaurado (4), however, the dosing recommended by these health food advocates is only tenfold lower than the lethal concentration noted in certain rat toxicology studies! Obviously, such careless dosing is to be strongly discouraged.

Summary

BHA and BHT are ubiquitous additives found in a variety of foods, but to the greatest degree in foods that contain larger amounts of fats or oils that may become rancid. These phenolic antioxidants are also added to plastic or paper products that are in contact with food items, and to cosmetics and medicines that may be applied to the skin or mucosa. They continue to be widely used despite concerns over animal toxicity studies. Continued provisional status on the GRAS list reflects that the toxicology studies in animals were done with much larger doses than those utilized in the food industry. Nevertheless, consumption has increased over the past three decades.

Adverse reactions in humans to date are best substantiated in the skin. Delayed hypersensitivity contact dermatitis through a variety of occupational or medicinal exposures is well documented, but are not common. The true incidence of antioxidant sensitivity in chronic urticaria is presently unknown. High reaction rates of adverse reactions to food additives have not been substantiated by careful double-blind studies. Although

earlier European reports suggested up to a 15% incidence of BHA/BHT intolerance in chronic urticaria patients, study design was weak so firm conclusions cannot be made. Likelihood is strong that a number of reactions were due to random fluctuations of disease activity, and were not true positive reactions to the antioxidants or other food additives. To date, there are no convincing reports of human respiratory adverse responses. Therefore, the true prevalence of adverse reactions to BHA and BHT remains unclear.

Oral challenges, preferably double-blinded, remain the desired approach to verifying suspected adverse reactions to these antioxidants. The recommended schedule is a truncated incremental challenge. The doses used may be considered high, and certainly far exceed an average daily intake. However, such doses are more likely to provide a definitive reaction. Clinical relevance can then be ascertained by elimination of the incriminated agent from the diet. It must be noted that such doses, while appropriate for urticaria evaluations, could be dangerous if one were examining potential asthmatic responses.

Considering the lack of success in identifying causes in chronic urticaria, a search for additive sensitivity is probably warranted, even considering the anticipated low yield. Strict elimination diets or the use of elemental formulas are difficult and poorly tolerated by patients. Open or single-blind challenges could identify possible agents, which should then be further authenticated with double-blind testing. The diet restrictions could then be rationally addressed.

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28

Adverse Reactions to Benzoates and Parabens

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Benzoic acid and sodium benzoate (benzoates) are widely used as antimycotic agents and antibacterial preservatives in foods and beverages. The methyl, n-propyl, n-butyl, and n-heptyl esters of para-hydroxybenzoic acid (collectively referred to as parabens) are used as preservatives in a limited number of foods and beverages, and more extensively in pharmaceuticals and cosmetics. Benzoic acid, sodium benzoate, methylparaben, propylparaben, and heptylparaben are approved as direct food additives by the US Food and Drug Administration (FDA) and have "generally recognized as safe" (GRAS) status (1).

Benzoates and Parabens as Food and Beverage Additives

Benzoates have been used since the early 1900s as preservatives in foods and beverages. Annual consumption worldwide has been estimated at greater than 10 million pounds, making benzoates one of the most commonly used additives. The benzoates have a broad range of antimicrobial activity, exhibit little or no toxicity in the concentrations used for food applications, and are relatively inexpensive to produce.

The chemical structures of benzoic acid and sodium benzoate are shown in Figure 28–1. Benzoic acid is a white crystalline solid with an acidic pH and limited water solubility (2). Sodium benzoate is a white crystalline powder with alkaline pH that readily dissolves in water (3). When sodium benzoate is dissolved in acidic solutions, it is partially converted to the free acid. Benzoates appear to be most effective as antimicrobial agents at acidic pH.

Benzoates are widely distributed in nature in the form of the free acid or as simple salts, esters, and amides. They occur naturally in prunes, cinnamon, cloves, tea, anise, and many berries. Raspberries and cranberries contain up to 0.05% by weight (2, 4). Benzoates as preservatives are found in alcoholic beverages, fruit juices, soft drinks, baked goods, cheeses, gum, condiments, frozen dairy products, relishes, and sugar substitutes to name a few. Orally administered benzoates are rapidly absorbed through the intestine and transported to the liver. Benzoate is converted to a thioester with coenzyme A to form benzoyl-CoA. Benzoyl-CoA then reacts with glycine to form hippuric acid, which is excreted in the urine.

The use of parabens as antimicrobial agents in pharmaceuticals, cosmetics, and food began in Europe in the 1920s and spread to the US in the 1930s. Although used primarily as preservatives in pharmaceuticals and cosmetics, parabens are also approved for use in foods by the US FDA, the European Community, the Joint FAO/WHO Expert Committee on Food Additives, and regulatory agencies of several other countries. Methylparaben and propylparaben are the forms most commonly used as food additives. Parabens are contained in coffee extracts, fruit juices, pickles, sauces, soft drinks, processed vegetables, baked goods, fats and oils, seasonings, sugar substitutes, and frozen dairy products. Concentrations vary between 450 ppm and 2000 ppm. The use limit for parabens as chemical preservatives in foods is 0.1%. The chemical structures of the parabens are

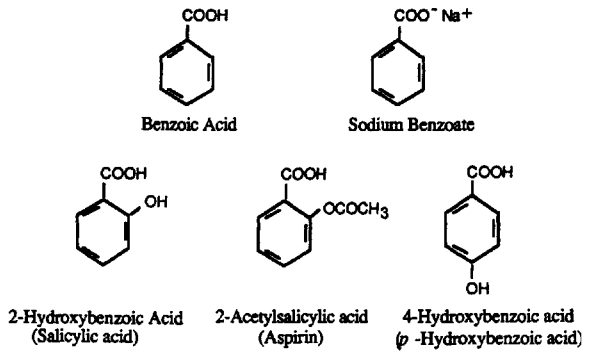

Figure 28–1. Benzoates and closely related congeners.

shown in Figure 28–2. They are white crystalline or powder solids that have essentially no odor or taste. Bactericidal activity is present over a wide pH range in contrast to the benzoates (5). Methylparaben has antimicrobial properties against cold-tolerant bacteria and is common as a preservative in prepared chilled foods (6).

Parabens are not described as occurring naturally in nature. Oral administration normally results in rapid absorption and subsequent hydrolysis to p-hydroxybenzoic acid. Glycine, glucuronic acid, and sulfuric acid conjugates are then formed and all are eliminated in the urine (7). Toxicity studies have demonstrated little or no adverse effects either acutely or chronically at doses far exceeding the current acceptable daily intake (ADI) of 55 mg/kg/day (8).

Benzoates, Parabens, and Associations with Chronic Urticaria-Angioedema

The prevalence of reactions to food additives in the setting of chronic urticaria has been studied frequently. Unfortunately, due to design problems

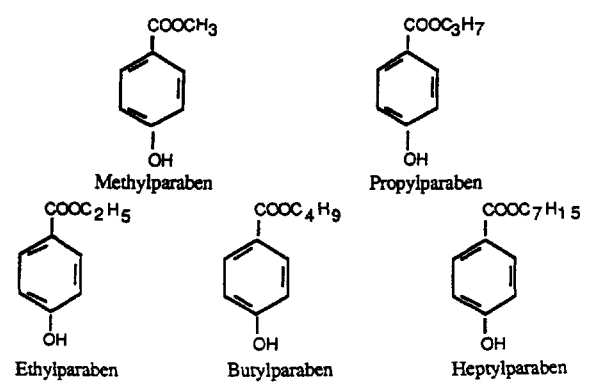

Figure 28–2. The paraben family of food additives.

with oral challenge studies in this patient population, variable study design, and lack of adequate controls in many studies, the prevalence of such reactions has not been definitively elucidated. Design considerations in food additive challenge studies are of critical importance. Selection of patients may include, for example, all patients with a history of chronic idiopathic urticaria, only those with histories suggestive of food additive reactions, or only those patients who appeared to improve on an additive-free diet. Depending on the selection criteria, different percentages of positive reactors have been reported. These variables have not been explicitly stated in many reports and add confusion to the already difficult task of comparing studies.

The relative activity or inactivity of the urticaria at the time of challenge appears to be a key factor. In a study by Lumry et al (9), only 1 (7%) of 15 patients whose urticaria was in remission experienced a reaction to aspirin (ASA). However, 7 (70%) of 10 patients whose urticaria was active at the time of challenge reacted to ASA. These challenges were performed using a semi-quantitative reaction criteria. Reactions were judged in comparison to a baseline observation period in each individual patient.

In most reported studies, a period of baseline observation for comparison with reaction data was never made. Further, most challenge studies report loosely defined criteria for identifying urticarial responses. Other potential confounding factors include discontinuation of medications (particularly antihistamines), timing and number of placebo challenges and additive doses. Finally, the importance of the double-blind challenge cannot be overemphasized. A more detailed description of design considerations for oral challenge protocols in chronic urticaria-angioedema can be found in another chapter.

One of the earliest open additive challenge studies in chronic urticaria patients was reported in 1975 by Doeglas (10). He observed that "four or five" of 23 patients reacted to sodium benzoate. Placebo-controlled challenges were not performed. Patients with physical urticarias were included. Also in 1975, Thune and Granholt (11) reported that two (6%) of 32 patients reacted to parabens and four (10%) of 41 patients reacted to benzoates after oral challenge. Overall, 62 (62%) of 100 patients reacted to at least one of 22 different additives used in the challenges. Again, placebo controls were not used, making any firm conclusions difficult to support.

A study performed by Juhlin (12) involved single-blind challenges using multiple additives. Benzoate hypersensitivity was reported in 19 (11%) of 172 participants. Overall, one or more positive reactions were observed in 53 (31%) of patients. This study utilized only a single administration of placebo, which was always given first, followed by multiple additive challenges. Reaction criteria were subjective and were determined to be "uncertain" in 57 (33%) patients. Previous studies by the same group reported a prevalence range of 44%–60% for benzoate hypersensitivity in chronic urticaria patients (13, 14). Because of study design limitations, firm conclusions are again difficult to support.

Supramaniam and Warner (15) reported that four (15%) of 27 children with urticaria reacted to sodium benzoate. Overall, 24 (56%) of 43 children reacted to one or more additives. The study did utilize a double-blind challenge design. However, only one placebo was interspersed with nine different additives, and a baseline observation period to determine the relative activity of the chronic urticaria was not utilized. Whether antihistamines were withheld or continued was not mentioned. Genton et al (16) also reported a significant reaction rate to benzoates in single-blind additive challenges on 17 patients with chronic urticaria and/or angioedema. Among these patients, 5 (30%) of 17 reacted to successive doses of sodium benzoate (10 mg, 50 mg, 250 mg, and 500 mg). Urticaria developed in 15 (88%) of the 17 patients with at least one of the six additives used. If urticaria or angioedema were "noticed by a physician during the 18-hour period after the test," the challenge was considered positive. All patients considered for the study were observed to have had "sufficient improvement" in their disease while on a 2-week elimination diet (free of additives). Explicit baseline disease activity was not reported.

Ortolani et al (17) studied 396 patients with chronic urticaria and angioedema. On the basis of history, 179 patients were considered for treatment with an elimination diet and 135 elected to proceed. Eighty-seven (64%) of the 135 patients had an 80% or greater reduction in urticaria symptom scores during the 2-week elimination diet compared to the 2-week baseline observation period. Only eight (9%) of the 87 patients who had improved on the elimination diet had a positive double-blind challenge to foods. Of the 79 patients who did not react to foods, 72 underwent double-blind, placebo-controlled (DBPC) oral food additive challenges. Three (4%) of the 72 had urticarial reactions with sodium benzoate (60 mg, 410 mg, and 410 mg). Twelve (16%) of the 72 reacted to one or more additives, including tartrazine and sodium metabisulfite. Parabens were not tested.

Hannuksela and Lahti (18) reported that one (2%) of 44 patients reacted to benzoic acid in a DBPC challenge study. One (2%) of the 44 patients also reacted to placebo. Several other food additives were tested in this study but no other reactions were observed. In a study with similar design, Kellett et al (19) reported that 4 (10%) of 44 chronic idiopathic urticaria patients reacted to benzoates and/or tartrazine. Ten percent also reacted to placebo.

Simon (20) studied 65 patients with active chronic idiopathic urticaria who continued antihistamines at the minimum effective dosage. Twenty of the participants reported a history of adverse reactions to additives. A baseline urticaria skin score was obtained in each patient using a semiquantitative method. Initially, participants were challenged with multiple additives (including benzoates and parabens) or placebo in a single-blind fashion. Two (3%) of the participants had positive additive reactions. These two individuals were then re-challenged utilizing a DBPC design at least 2 weeks later. Neither of them had a positive reaction. The author concluded with 95% confidence limits that the prevalence of additive sensitivity in patients with active chronic idiopathic urticaria is somewhere between 0% and 3%.

Several studies have utilized an elimination diet approach in their evaluation of food additive contributions to chronic urticaria. Unfortunately, no blind or placebo-controlled studies of this type have been reported. The Ros study (14) reported an additive-free diet to be "completely helpful" in 24% of patients with chronic urticaria. Another 57% of patients were "much improved," 19% were "slightly better" or had experienced "no change." Rudzki (21) observed clinical response to a diet free of salicylates, benzoates, and azo dyes in 50 (32%) of 158 patients. These studies did not investigate which particular additive was potentially inducing or exacerbating the urticaria.

Gibson and Clancy (22) reported the use of an elimination diet in 69 patients with chronic idiopathic urticaria (symptoms present for greater than 3 months; physical urticarias excluded). They found that 54 (92%) of the patients experienced complete remission within 2 to 4 weeks of beginning the diet. Challenge studies using multiple additives revealed that 18 (34%) of the 54 reacted

to benzoates. An initial placebo tablet was utilized in the challenges; blinding was not mentioned. Twelve patients agreed to re-challenge after remaining in complete remission for 1 year on the elimination diet. Three of the four in this group who had initially reacted to benzoates remained positive to benzoate challenge at 1 year. None of the three patients who had reacted initially to tartrazine remained positive at 1 year.

Ehlers et al (23) evaluated the response to an elimination diet in 16 children with chronic urticaria (of at least 3 months' duration). Nine (56%) of the 16 children were free of symptoms within 10 days of beginning the diet. An additional three patients "improved considerably." Six of the patients who responded to the diet were challenged in a DBPC fashion. Details of the challenge protocol and criteria for positive reactions were not discussed. Five (83%) of the six patients reacted to at least one of the additives. Four of them reacted to multiple additives (three or more). Parabens elicited reactions in three (50%) of the six. Benzoic acid caused a reaction in one (16%) of the six. The authors suggested that additives appear to play a significant role in pediatric chronic urticaria, a relatively uncommon condition.

Malanin and Kalimo (24) evaluated the utility of skin testing with additives in chronic urticaria patients. Ninety-one subjects were skin-tested with 18 food additives. Twenty-four subjects (26%) had at least one histamine equivalent positive food additive skin test. Ten of the 24 participants with a positive skin test underwent oral food additive challenges with the suspected additive(s). Only one (10%) of the 10 challenged reacted (benzoic acid). Overall, significantly more patients with positive skin tests responded to an elimination diet (16 [89%] of 18 with positive skin tests vs 17 [40%] of 42 with negative skin tests). The authors proposed non-IgE-mediated skin hyperreactivity as the mechanism for skin test positive reactions. The pathogenesis of additive reactions is presently unknown.

In summary, oral challenge studies with food additives in the setting of chronic urticaria-angioedema present many design problems. Meticulously designed studies that utilize DBPC challenges, such as the Ortalani study (17) and the Simon study (20) suggest that benzoates and parabens are uncommon provoking or exacerbating factors. In selected patients, a trial of an additive-free diet may be warranted followed by systematic reintroduction of additive-containing foods if significant clinical improvement was observed. DBPC additive challenges could then be utilized to diagnose the particular additive sensitivity if clinically appropriate.

Benzoates, Parabens, and Associations with Asthma

The prevalence of asthmatic reactions to food additives in the general population or select groups such as atopic asthmatics has not been definitively defined. Nevertheless, several studies suggest that such reactions are unusual. Weber and colleagues evaluated aspirin and additive sensitivity in a group of 43 moderate to severe persistent asthmatics (25). In the initial single-blind challenges, two (5%) showed a positive response (decrease in forced expiratory volume in 1 second [FEV_1] of 25% or more from baseline) to benzoates and parabens. Only one (2%) of the patients remained positive during double-blind testing. The prevalence of tartrazine sensitivity in this study was 16% during initial open challenges (7 of 43). This fell to 0% during subsequent double-blind challenges. Of note, bronchodilator medication was not withheld in the majority of patients because a number of apparent false-positive reactions had been obtained earlier in the study when these medications were withheld. This study emphasizes the importance of the double-blind challenge and observing a relatively stable baseline FEV_1 prior to the initiation of challenges in the asthmatic population.

Tarlo and Broder found only one patient with sodium benzoate hypersensitivity (FEV_1 fall of more than 20% from baseline) among 28 patients (4%) with persistent asthma (26). The protocol utilized a DBPC design and medications were not withheld. Of note, clinical improvement of this patient's asthma was not observed when benzoates were removed from the diet. Osterhalle et al (27) performed initial open multiple additive challenges in 46 children with persistent asthma. Eleven (24%) showed positive reactions (FEV_1 decrease > 20% of baseline). Confirmatory DBPC challenges gave only three positive responders.

Genton and associates (16) found 1 (6%) of 17 asthmatic patients who reacted to sodium benzoate in a single-blind, randomized placebo-controlled study. Garcia et al (28) reported no reactions to sodium benzoate among 62 patients with steroid-dependent asthma. Not surprisingly, other less rigorously controlled studies have reported more widely varying rates of asthmatic reactions to food additives (29–31).

Similar to chronic urticaria, some authors have suggested an additive-free diet is useful in selected patients with persistent asthma (32). This approach has not been evaluated in published controlled trials.

Benzoates, Parabens, and Anaphylaxis

Relatively few reports of possible anaphylactic/anaphylactoid reactions have appeared in the medical literature. Given the widespread consumption of these preservatives, one can conclude that such reactions are exceedingly rare. In 1944, Kinsey and Wright (33) reported an "anaphylactoid type" reaction in an individual four hours after he had received a 6-g oral dose of sodium benzoate to evaluate liver function. The following day identical symptoms of "shock" developed within 4 hours of another 6-g sodium benzoate dose. Michels et al (34) reported the case of a young woman who developed "flush, angioedema and severe hypotension (systolic blood pressure under 50 mm Hg)" 30 minutes after eating a meal containing sodium benzoate as a food preservative. One week earlier she had experienced "generalized itching" after eating cheese, which also contained benzoates. A placebo-controlled challenge with 20 mg of oral sodium benzoate produced urticaria confined to her arms and generalized pruritus. A second challenge, apparently several days later after treatment of a sinus infection, resulted in only "mild localized itching" after ingestion of 160 mg of sodium benzoate. Neither of the above cases provides conclusive evidence of systemic anaphylaxis related to ingested benzoates.

Orally ingested parabens have not been reported to cause systemic anaphylaxis. Nagel et al (35) did report a case of bronchospasm and generalized pruritus associated with administration of intravenous steroids containing parabens in an asthmatic child. Intravenous steroids without paraben preservatives did not induce any symptoms. Skin testing to individual parabens as well as passive transfer tests were positive. Skin testing with the steroid preparations with and without parabens provided further evidence of a hypersensitivity reaction induced by the paraben preservatives. Carr (36) reported two cases of hypotension and diffuse macular rash potentially associated with paraben preservatives contained in a topical lidocaine preparation used for intraurethral anesthesia prior to cystoscopy. One of the patients tolerated a preservative-free topical lidocaine preparation 2 months after his initial reaction. No mention was made concerning whether or not the second patient was able to tolerate a preservative-free preparation. Other reports have also associated paraben preservatives in local anesthetics as the likely etiology of IgE-type reactions (37, 38). Again, oral ingestion of parabens has not been linked to anaphylaxis.

Benzoates, Parabens, and Dermatitis

The development of contact dermatitis associated with topical parabens used in cosmetics and other skin care products has been reported extensively in the literature dating back to 1940 (7). A loosely controlled study by Veien et al (39) evaluated the possibility of oral paraben ingestion as an exacerbating factor in patients with chronic "dermatitis" and contact paraben sensitivity diagnosed by patch testing. Two (14%) of 14 patients reported flares of their "usual dermatitis" within 24 hours of ingesting parabens, and placebo challenges were negative. These two patients were subsequently followed on an elimination diet for 1–2 months. Neither the patients nor the physicians noted any significant improvement. Perioral contact urticaria has been reported in association with sodium benzoate in a toothpaste (40). Overall, reported contact reactions to benzoates are rare in comparison to the parabens.

The potential role of ingested benzoates or parabens in atopic dermatitis (AD) has received limited attention in the medical literature. Van Bever et al (41) investigated the role of food and food additives in 25 children with severe AD. All of the children were hospitalized and received an elemental diet by nasogastric tube. Topical therapy was continued in the hospital setting. All children were reported to be "almost free of active eczema" after 1–2 weeks of the elemental diet and topical therapy. Selective DBPC food and food additive challenges were performed after 1 week of the observed clinical improvement. Six of the children were challenged with sodium benzoate and three "reacted." The reactions consisted of "pruritus and redness of the skin," which had apparently resolved within the 4-hour period of observation post challenge, as no "late reactions" were seen. Exacerbations of underlying AD related to challenges were not reported. Any skin findings lasting more than 4 hours were not observed after any food or food additive challenge. Nevertheless, the authors reported that 24 (96%)

of the 25 children "reacted" to one or more foods and all children who were challenged with additives "reacted" to at least one. Clearly, reaction criteria were among the major flaws of the study. This study has also been criticized because no placebo reactions occurred after 132 placebo challenges. Hannuksela and Lahti (18) had observed equivalent reaction rates between placebo and active substances in patients with chronic urticaria and AD in an earlier study.

A carefully designed study by Worm et al (42) provides evidence of a link between food additives and AD. Fifty patients with AD were monitored while eating their usual diet for 4 weeks. Baseline AD skin status scores using a variation of the Costa Method were obtained. In phase two, 41 of the patients followed an elimination diet for 6 weeks. At the end of the dietary intervention phase, 26 of the 41 patients (63%) showed an improvement in their skin status scores of greater than 35%. Open oral provocation tests with an additive-rich diet given over a period of 2 days were performed in 24 of the 26 responders (two refused). The open diet challenge resulted in positive reactions (worsening of Costa Score above 10 points within 48 hours) in 19 of the 24 patients (79%). The authors reported no immediate reactions but rather solely late-phase reactions typically occurring between 24 and 48 hours. Ten patients who had not responded to the dietary intervention also underwent the open challenge as a control group. None of the control patients reacted. In 15 of the 19 patients reacting to the open challenge, DBPC oral food additive challenges were performed. The additives given together in a single capsule included sodium benzoate, p-hydroxybenzoate, azo-dyes, BHA/BHT and others. Six (40%) of 15 patients reacted to the additive challenge given as a single dose followed by a 48-hour observation period. One patient reacted to placebo. Additives were not tested individually. To date, no controlled study has implicated benzoates or parabens individually as pathogenic factors in AD.

Miscellaneous Reactions

Isolated case reports appear in the literature suggesting symptoms ranging from depression to rhinitis may be related to benzoate ingestion in certain individuals (43). Cutaneous vasculitis has been reported occasionally in association with sodium benzoate ingestion (44–46). In two of these reports the patients also had microhematuria. Challenge tests were reported to be associated with cutaneous vasculitic lesions and the patients improved with dietary intervention. A study by Lunardi et al evaluated the effects of an elimination diet in 5 patients with biopsy proven leukocytoclastic cutaneous vasculitis (46). Evidence for an associated autoimmune disorder, infection or neoplastic disease was not found. All patients improved on the elimination diet; four showed complete resolution of their skin lesions. All patients "reacted" to at least one food or food additive. One of the patients reacted to benzoates. The authors reported that with elimination of the offending foods and/or food additives, no relapses were seen in 2 years of follow-up.

The Melkersson-Rosenthal syndrome is a rare disorder characterized by recurrent or persistent orofacial edema, which typically involves the lips, variable facial paralysis and lingua plicata (fissuring of the tongue) (47). The syndrome's etiology is unknown but is reported to be more common in atopic individuals. A few reports have suggested that food additives, including benzoates, may play a role (47–51). However, another investigation using DBPC challenges in six patients found no evidence of food or food additive sensitivity (52).

Summary and Conclusions

Benzoates and parabens are used extensively as chemical preservatives in foods and beverages in the United States and throughout much of the developed world. These compounds have essentially no toxicity at approved concentrations and considering their widespread consumption, are extremely well tolerated. Benzoates and parabens have been investigated frequently in association with chronic urticaria-angioedema. Many studies with less stringent design criteria have implicated these agents, particularly the benzoates, as relatively frequent exacerbating factors. On the other hand, more rigorously designed protocols suggest that these chemicals are unusual provoking or exacerbating agents among urticaria patients.

Asthmatic reactions have also been reported and investigated in association with food additives including benzoates and parabens. Well-designed trials have not provided a conclusive link between persistent asthma and benzoates or parabens.

The association of AD with food additives has received relatively limited attention in the med-

ical literature. No well-designed study has implicated benzoates or parabens individually as pathogenic factors. A recent study using multiple food additives including benzoates provides evidence that at least some of these substances may be provoking factors in a minority of patients (42).

Rarely, anaphylactic-type reactions have been reported with ingested benzoates but definitive evidence of systemic anaphylaxis is lacking. Oral parabens have not been reported as potential causes of anaphylaxis. However, parabens have been implicated in systemic reactions related to their use in pharmaceutical agents, particularly local anesthetic preparations. Other miscellaneous reports have appeared suggesting benzoates as occasional inciting agents in cutaneous vasculitis.

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29

Food Colorings and Flavors

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Many foods contain added colors and flavors derived from either synthetic or biogenic sources. The vast majority of these food additives pose little to no risk of adverse events; however, some added colors and flavors have been implicated in adverse reactions to different foods. This chapter focuses on those added food colors and flavors implicated in adverse food reactions and discusses, where known, the mechanisms involved and prevention and treatment strategies.

Food Colorings

Food colorants are defined as any dye, pigment, or substance that can impart color when added or applied to food. Two broad categories of food colorants are dyes and lakes. A dye is a water-soluble form of color, and a lake is a water-insoluble form. Food colors are further classified by their derivation, as either synthetic or biogenic.

Historically, food colors were among the first classes of food additives to receive attention because of adverse reactions. One of the first cases on record of an adverse reaction to a food colorant occurred in 1848 when 21 individuals were poisoned at a public dinner in Nottingham by a blancmange (a dessert) colored green with copper arsenite (1).

Why Are Color Additives Used In Foods?

Color is an important property of food that influences its acceptance and enjoyment. Food color can be affected by the season, processing techniques, and storage. Manufacturers add color to certain foods to meet consumer expectations. Primary reasons for adding colors to foods include: 1) to offset color loss due to exposure to light, air, extremes of temperature, moisture, and storage conditions; 2) to correct natural variations in color; 3) to enhance colors that occur naturally but at levels weaker than those usually associated with a given food; 4) to provide a colorful identity to foods that would otherwise be virtually colorless; 5) to provide a colorful appearance to certain "fun foods"; 6) to protect flavors and vitamins that may be affected by sunlight during storage; and 7) to provide an appealing variety of wholesome and nutritious foods that meet consumer demands (2).

Legislation

The first legislation specifically governing food color additives, the Color Additive Amendment of 1960 (2, 3), requires coloring agents used in foods to be approved by the US Food and Drug Administration (FDA) before they are marketed.

Previously, the food additive amendment of 1958 exempted food additives safely in use before 1958 from obtaining FDA approval (2). Unlike other additives however, colors in use before this legislation were not allowed to be in continued use unless they underwent further testing to confirm their safety. Of the 200 provisionally approved colors, only 90 were listed as safe, and the reminder were removed by FDA or the industry at that time (2).

The Delaney clause to both the food additive amendment and color additive amendment includes a provision prohibiting approval of a substance found to cause cancer in humans or animals (2). Also, good manufacturing practice

regulations limit the amount of food color additive used in foods.

Regulation

Color additives in foods are regulated in the US by the FDA. Title 21 of the Code of Federal Regulations (CFR) parts 73 and 74 defines the regulations governing food color additives (4). For food color additives, two regulatory classes are identified by the US FDA: color additives exempt from batch certification (Title 21, part 73, subpart A) (3, 4) and color additives subject to batch certification (Title 21, part 74, subpart A) (3, 4).

Certified Colors

Certified colors are synthetic, and carry the Food, Drug and Cosmetic (FD & C) or Drug and Cosmetic (D & C) color label. Each batch is tested by the manufacturer and the FDA to ensure that it meets purity specifications (2, 4). If added in any quantity to any food in the US, the FDA requires that each certified color be individually specified by name on the ingredient statement for the product.

Structurally these colors are classified as either azo or non-azo dyes. Together the five azo and four non-azo dyes account for the nine FD & C certified colors that can be used in human food. Azo dyes include FD & C Yellow No. 5 (tartrazine, dye and lake), FD & C Yellow No. 6 (sunset yellow, dye and lake), FD & C Red No. 40 (Allura Red AC, dye and lake), Citrus Red No. 2 (skin of oranges, not intended or used for processing, not to exceed [NTE] 2.0 ppm by weight, where ppm is 1 μg/g), Orange B (casings or surfaces of frankfurters and sausages, NTE 150 ppm by weight). The non-azo dyes are FD & C Blue No. 1 (brilliant blue FCF, dye and lake), FD & C Blue No. 2 (indigotin, dye and lake), FD & C Green No. 3 (fast green FCF, dye and lake), and FD & C Red No. 3 (erythrosine, dye) (4, 5).

Synthetic coloring agents and their adverse effects are discussed in detail in Chapter 26. Therefore, the remainder of the color portion of this chapter focuses on color additives exempt from certification.

Colors Exempt from Certification

Colors exempt from certification are pigments derived from natural or biogenic sources (animal, vegetable, mineral). They can be manufactured and marketed without FDA batch certification, but they are subject to legal safety and purity standards similar to certified color (2).

Labeling

The Nutrition Labeling and Education Act of 1990 required that certified food color additives be specifically declared by individual name on the food label, but the requirements for the food colors exempt from certification were not changed (3). Under CFR Title 21, section 101.22, colors exempt from certification need not be specified by name on labels of foods to which they are added. They may be simply acknowledged using the terms "color added," "artificial color," or "artificial color added." Alternatively, such components may be declared as "colored with _____" or "_____ color." One of the above descriptive designations must appear on the label if one of these colors is added to the food. A listing of colors exempt from certification can be found in Table 29–1 (4).

Under current FDA regulations, there is no provision for listing these color additives as "natural." In fact the term "natural color" is prohibited because it may lead the consumer to believe that the coloring is derived from the food itself (3). Hence, there is no such entity designation as a "natural color additive" in the US regulatory system (3). Paprika, turmeric, and saffron or other colors that are also spices, shall be declared as "spice and coloring" unless declared by their common or usual name.

Reactions to Color Additives Exempt From Certification

Adverse reactions to color additives can be immunologic (food allergy/food hypersensitivity) or non-immunologic (food intolerance). The coloring principles of color additives exempt from certification are usually low molecular weight nonprotein chemicals, and hence would not be expected to elicit true food allergies. Hypotheses that these components may act as haptens are unproven. It is known, however, that some of these colors, being extracts from biological materials, can contain other components, including proteins retained during the manufacturing process. Type I IgE-mediated allergic reactions attributed to these colors are believed to be due to allergy to protein residues. This phenomenon has been described with annatto and carmine/cochineal col-

Table 29–1.
Color Additives Approved for Use in Human Food (Title 21 of CFR, Part 73, Subpart A: Color Additives Exempt from Batch Certification)

Title 21 CFR Section	*Straight Color*	*EEC#*	*Year Approved*	*Uses and Restrictions*
73.30	Annatto extract	E160b	1963	Foods generally
73.40	Dehydrated beets (beet powder)	E162	1967	Foods generally
73.75	Canthaxanthin	E161g	1969	Foods generally, NTE 30 mg/lb of solid or semisolid food or per pint of liquid food; may also be used in broiler chicken feed
73.85	Caramel	E150a–d	1963	Foods generally.
73.90	β-Apo-8′-carotenal	E160e	1963	Foods generally, NTE 15 mg/lb solid, 15 mg/pt liquid
73.95	β-Carotene	E160a	1964	Foods generally
73.100	Cochineal extract	E120	1969	Foods generally
	Carmine		1967	
73.140	Toasted partially defatted cooked cottonseed flour	—	1964	Foods generally
73.160	Ferrous gluconate	—	1967	Ripe olives
73.165	Ferrous lactate	—	1996	Ripe olives
73.169	Grape color extract	E163?	1981	Nonbeverage food
73.170	Grape skin extract (enocianina)	E163?	1966	Still and carbonated drinks and ades; beverage bases; alcoholic beverages (restrict. 27 CFR Parts 4 & 5)
73.200	Synthetic iron oxide	E172	1994	Sausage casings NTE 0.1 percent (by wt)
73.250	Fruit juice	—	1966	Foods generally
			1995	Dried color additive
73.260	Vegetable juice	—	1966	Foods generally
			1995	Dried color additive, water infusion
73.300	Carrot oil	—	1967	Foods generally
73.340	Paprika	E160c	1966	Foods generally
73.345	Paprika oleoresin	E160c	1966	Foods generally
73.450	Riboflavin	E101	1967	Foods generally
73.500	Saffron	E164	1966	Foods generally
73.575	Titanium dioxide	E171	1966	Foods generally NTE 1 percent (by wt)
73.600	Turmeric	E100	1966	Foods generally
73.615	Turmeric oleoresin	E100	1966	Foods generally

NTE, not to exceed.

orings (5–19). The level of protein residues in these color extracts may vary depending on the processing technique (20). Thus far, no cases have been reported that suggest cell-mediated allergic or food intolerance reactions to colors exempt from certification (20).

Although reactions to color additives are rare, the FDA requests they be reported. The agency operates the Adverse Reaction Monitoring System (ARMS) to collect and act on complaints concerning all food ingredients, including food additives (21). Consumers can register complaints two ways: 1) by contacting their FDA district office (see local phone directory), or 2) by sending written reports of adverse reactions to:

ARMS
HFS-636
Food and Drug Administration
200 C St, NW
Washington, DC 20204

Carmine

For centuries, the magenta or red colored pigment carmine, extracted from the female cochineal insect, has been used as an important natural dye. Carmine as a food color additive is obtained by aqueous extraction of the dried gravid female cochineal insects *Coccus cacti* or *Dactylopius coccus costa.* These scale insects belong to the superfamily *Coccoidea,* and reside as parasites on the prickly pear cactus (*Noplae coccinelliferna*) (22). Cochineal insects are harvested mainly in Peru, Central America, and the Canary Islands. Cochineal extract is the concentrated solution remaining after alcohol is removed from an aqueous-alcohol extract of cochineal insects. Carmine is the aluminum or calcium-aluminum lake on an aluminum hydroxide substrate of carminic acid. The active coloring principle of carmine/cochineal is thought to be carminic acid (Fig. 29–1).

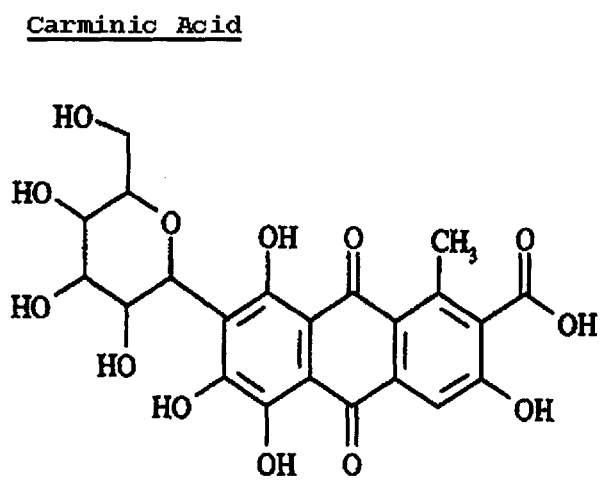

Figure 29–1. Carminic acid: $C_{22}H_{20}O_{13}$ (MW 492.39). The coloring principle of carmine, carminic acid is a hydroxyanthraquinone linked to a glucose unit. Carminic acid comprises approximately 10% of cochineal and 2%–4% of its extract.

Several studies exploring the safety of carmine in rats suggest no genotoxic, teratogenic, or carcinogenic properties (23–25). Most commercial preparations of carmine contain 20%–50% carminic acid, although it is usually sold diluted to contain 2.2%–3.5% carminic acid. Commercial cochineal extract contains 1.8% carminic acid. The quantity and stability of allergenic proteins in carmine may be affected by the extraction and food processing techniques (20).

Regulation and Labeling

As noted above, under Title 21 CFR (section 73.100), carmine can be added to foods without carmine or cochineal extract appearing on the label. The presence of carmine or cochineal extract needs to be acknowledged by one of the following descriptive terminologies: "colored with carmine"; "carmine color"; "cochineal extract"; "color added"; "artificial color"; or "artificial color added." Carmine may be labeled E120 in the European Union (EU), or sometimes as "Natural Red No. 4" or CI (Color Index) 75470.

Carmine Usage

In the US, carmine is widely used in foods, beverages, drugs, and cosmetics. By law it cannot be added to meat products. Under current US regulations it is the only organic pigment permitted for use as a cosmetic around eyes. Some common food items in which carmine can be an ingredient are listed in Table 29–2. The World Health Organization has set a daily consumption limit of 5 mg/kg/day for carmine. The FDA has no such recommendation limiting consumption (22).

Reported Adverse Reactions to Carmine

Carmine gained importance in the 1980s as a possible replacement for FD & C Red No. 3 lake, which was removed from use at that time. Despite widespread consumption, carmine has rarely been implicated in adverse reactions experienced by consumers. Although generally considered safe for human consumption, there is now a growing awareness of hypersensitivity reactions in the medical literature related to the ingestion of carmine.

The incidence of adverse reactions or hypersensitivity to carmine is unknown but clinically a variety of reactions have been reported. Worldwide at least two dozen cases of carmine "allergy" have been reported in various forms and they include occupational asthma (7, 8), extrinsic allergic alveolitis (9, 10), cheilitis (11), and food allergy manifesting as anaphylaxis, angioedema, and urticaria (7, 12–16).

Mechanisms Involved in Carmine Allergy

Although the coloring principle in carmine is thought to be carminic acid, the antigenic components of the colorant remain undefined. Evidence supporting an IgE-mediated mechanism in carmine-associated adverse reactions has come from studies that have demonstrated positive skin prick test (SPT) (12, 13, 15), positive Prausnitz-Küstner (PK) test (15), positive basophil histamine

Table 29–2.
Carmine Usage

Water-Insoluble ***Carmine Colors***	*Water-Soluble* ***Carmine Colors***	*Water-Soluble* ***Cochineal***
Cosmetics	Yogurt	Beverages
Pharmaceuticals	Ice cream	Yogurt
Dairy products	Fruit-based drinks	Ice cream
Baked goods	Beverages	Fruit fillings
Confections	Fruit fillings	Puddings
	Puddings	Confections
	Bakery mixes	
	Confections	
	Cosmetics	
	Pharmaceuticals	

release assay (13), positive radioallergosorbent test (RAST) IgE (12), and positive sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and IgE Immunoblot to 50 kilodalton (kDa) and 28 kDa proteins (6).

Sensitization to Carmine

The route of sensitization is not known. It has been hypothesized that sensitization to carmine via ingestion of carmine-containing foods is unlikely because of the low levels of carmine and carmine-associated protein residues present in foods and beverages. Sensitization is more likely to occur in a setting where a high level of exposure is possible. This includes occupational exposure or sensitization through a dermatologic route with cosmetics. Indeed, the vast majority of cases of anaphylaxis associated with the consumption of carmine-containing foods have occurred in carmine processing workers and in women. Once sensitization has occurred, low levels of exposure to residual proteins present in foods may be sufficient to cause allergic reactions.

Management

Carmine allergy is uncommon, but it is important to be aware of this possibility in patients who present with unexplained episodic cutaneous and systemic events such as urticaria, angioedema, asthma, or anaphylaxis after suggestive exposures. The value of diagnostic tests such as SPT with carmine, RAST IgE tests, and challenge testing, although all used by various investigators, has not been established.

At present, avoidance is the only therapy available for subjects with carmine hypersensitivity. Avoidance implementation remains problematic under current FDA labeling requirements for food (Title 21 of CFR, section 101) (26). Although cosmetics containing carmine are required to specify the ingredient by name, labeling often contains confusing terms such as "may contain" followed by an exhaustive list of colorants. Although such a "declaration" may meet legal requirements, it does nothing but further complicate and delay the diagnostic process. Until more clear and uniform labeling regulations requiring declaration of known allergenic substances such as carmine is mandated, avoidance of these substances by allergic individuals and accurate diagnosis of the allergen will remain problematic (26).

Annatto

Annatto dye is an orange-yellow food coloring extracted from seeds of the tree *Bixa orellana*. The annatto tree is a large, fast-growing shrub cultivated in the tropics. Capsular fruits from this tree are brown or crimson colored and contain seeds coated with a thin, highly resinous material, which is the raw material for the colorant annatto. The chief coloring principles in the annatto extracts are the carotenoid bixin in the oil-soluble extracts, and norbixin in the alkaline aqueous extracts (27).

Regulation and Labeling

Annatto as a food coloring is exempt from certification, under Title 21 CFR 73.30 for foods. It has the same labeling requirements as carmine and other color additives exempt from certification. Outside the US annatto can be referred to as CI Natural Orange No. 4, E160b, bija, rocou, orlean, or achiote.

Annatto Usage

Annatto is commonly used as a food coloring additive in a variety of foods including cheeses, snack foods, cereals, ice creams, margarines, oils, and beverages. It is often used to simulate butter or cheese color and may be added to butter or cheese flavoring to help produce the desired color.

Reported Adverse Reactions to Annatto

Despite extensive use in foods adverse reactions to annatto are rare. Although the actual prevalence of reactions to annatto in the general population is unknown, the prevalence has been estimated to be between 0.01 (lower limit) and 0.07 (upper limit) with a 95% confidence interval (28).

Annatto has been reported to cause urticaria (17, 18, 28), angioedema (17, 18), anaphylaxis (19), asthma (20), and atopic dermatitis (AD) (29).

The first and most convincing case of annatto anaphylaxis occurred in a 62-year-old male after ingestion of Fiber One cereal, which contained annatto extract. He developed anaphylaxis within minutes. The SPT to annatto was found to be positive at 1:1000 dilution. SDS-PAGE immunoblot using the patient's serum implicated a 50 kDa annatto protein. (19).

A randomized double-blind placebo-controlled (DBPC) oral food additive challenge was conducted in 101 patients with eczema. Five capsules containing different food additives were given to the subjects following a standard elimination diet. One of the capsules contained a 90 mg mixture of food colorings including annatto. (The mixture of food colorings included 10 mg annatto extract, 10 mg erythrosine, 10 mg ponceau 4R, 10 mg tartrazine, 10 mg patent blue V, 10 mg sunset yellow, 10 mg betanine, 10 mg curcumin, and 10 mg quinoline.) Although 25 patients showed reactions to the mixture of food colorings, statistical significance was not realized because 16 patients reacted to the placebo. On a second challenge the reaction could not be reproduced in nearly one third of the subjects. A reaction in this study was defined as a self determined flare of dermatitis that occurred within 3 days of the challenge (30).

In another study (18) by Juhlin in 1981, 112 patients with history of angioedema and recurrent urticaria were administered 5 mg and 10 mg of annatto in open oral challenges as a part of provocation testing to a variety of substances. No placebo controls were used. Ten (9%) had positive reactions defined as definite urticaria or angioedema occurring within 24 hours. Fourteen (12.5%) of the patients had "uncertain reactions" with doubt about the presence of urticaria or angioedema. The purported provoking dose(s) in those with "definite reactions" is not specified. The use of patients with chronic urticaria and angioedema leads to problems in interpreting the results. The withdrawal of medications used for symptom control prior to the study could also have contributed to false positive reactions. Hence this study was inconclusive.

In a study by Mikkelsen (17) in 56 patients with chronic urticaria and angioedema, 25 μL of annatto extract was given orally in a gelatin capsule. Fifteen (27%) patients had urticarial and angioedema symptoms within 1–10 hours, with a mean of 4 hours. The study lacked double blinding and placebo controls. Withdrawal of medications used for symptom control prior to challenge could have resulted in breakthrough symptoms. Additionally, some of the subjects in the study were not completely free of urticaria and angioedema symptoms. The drawbacks in the study create uncertainty in the interpretation of the results.

Fuglsang et al (29, 31) have conducted two double-blind placebo-controlled food challenges (DBPCFCs) using a mixture of food colorings. In the first study from 1993 (31), 271 children (98 controls and 173 with atopic symptoms) underwent open challenge with food additives prepared in a lemonade solution. Seventeen (6.3%) patients had reactions with the open challenge, and 12 of these went on for DBPCFC with a mixture of natural colorings. No positive reactions were noted. The mixture of natural colorings included, per 100 mL, 2.5 mg turmeric, 1.6 mg annatto, 6.0 mg β-carotene, 1.0 mg canthaxanthin, and 5.5 mg beet coloring.

A follow-up study in 1994 by the same authors (29) with a similar protocol, which included a DBPC oral challenge with the same mixture of natural colorings, demonstrated two positive reactions. The first patient experienced a flare of AD, and the second urticaria. The symptoms were noted within 24 hours of the challenge. As a mixture of colorants were used it could not be determined whether annatto or any other colors used in the challenge was responsible for the reactions.

Van Assendelft (32) reported a possible asthmatic reaction to an annatto-containing pharmaceutical product, but no skin tests or challenge tests were done in this subject to determine allergy to annatto.

Mechanisms Involved in Adverse Reactions to Annatto

The antigenic principle(s) in annatto that cause adverse reactions has not yet been identified. A 50-kDa protein was implicated in one case (19). Methods used that suggest an IgE-mediated mechanism include positive SPT and positive specific IgE-binding band on SDS-PAGE and immunoblot. Oral challenge data, as noted above, are difficult to interpret.

Management

Adverse reactions to annatto are uncommon. In evaluating patients with unexplained adverse reactions such as anaphylaxis, urticaria, and angioedema after ingestion of annatto containing foods, annatto should be considered in the differential. SPT, RAST IgE testing, and oral challenge to annatto may be diagnostically helpful, but their predictive value and safety have yet to be determined. Avoidance remains the cornerstone of therapy but problematic under current labeling regulations.

Turmeric

Turmeric has been used as a color for hundreds of years. It has been used in cosmetic formulations,

as a textile dye, as a color for foods, and as a medicine for many ailments and conditions in Asia for centuries (33). It is also known as Indian saffron.

Curcumin is the orange-yellow coloring principle in turmeric. It is extracted from the rhizome, or tuber, of the plant *Curcurma longa,* which is grown mainly in southeast Asia. Turmeric oleoresin is prepared by extraction from turmeric using one of several approved solvents.

Regulation and Labeling

Turmeric is permanently labeled as acceptable for use in foods in the US and is exempt from certification under Title 21 CFR 73.600, and Title 21 CFR 73.615. It is identified as E100 in the EU; other international codes include CI #75300 and CAS# 458-37-7.

Turmeric Usage

Turmeric has been used as a food additive in the East for centuries to enhance storage, taste, and presentation of curries. In the US, turmeric is used in baked goods, sugar-panned candy, chewing gum, breads, biscuits, crackers, popcorn products, salad dressings, and compressed vitamin tablets. Turmeric oleoresin is used in chicken-flavored soups, and in pickles mainly for the flavor.

Turmeric contains a complex mix of bioactive compounds called curcuminoids, which have free radical activity and antioxidant benefits. Consequently, turmeric is being studied for its effects on arthritis, cancer, and several other medical conditions (33).

Reported Adverse Reactions to Turmeric

Adverse reactions to turmeric are uncommon in the published literature despite widespread and heavy use in the South Asian and Indian subcontinents.

The studies by Fuglsang et al (29, 31) discussed in the section on adverse reactions to annatto involved DBPC challenge with a mixture of natural colors that included turmeric. However, because a mixture of colorants were used, it was not determined whether turmeric was responsible for the reactions.

A similar study involving oral challenge with a mixture of colorants that included curcumin showed reactions which were not significantly different from placebo, and were not reproducible in two thirds of the patients on rechallenge. The details of this study can be found in the section on adverse reactions with annatto (30).

Thus, no definitive evidence exists for allergic reactions to turmeric or curcumin. Of course, avoidance is prudent if turmeric is suspected of causing adverse reactions.

Carotenoids

Carotenoids are orange-yellow pigments derived from biogenic or synthetic sources. Carotenoids are precursor molecules to fat-soluble vitamin A, and are also known as provitamin A carotenoids. Vitamin A has many forms, of which retinol is the most active and usable form and is found in animal foods such as liver and eggs. Retinol can be converted to retinal and retinoic acid, other active forms of the vitamin A family, thus retinol is called preformed vitamin A.

β and α-carotenes are isomers of carotene, the naturally occurring carotenoid. Both synthetic and naturally occurring carotene are used as coloring agents. Canthaxanthin, isolated first from the mushroom *Cantharellus cinnabarinus,* and from other sources since then, is the other carotenoid pigment used as a food additive and also available both in synthetic and biogenic forms.

Regulation and Labeling

Carotenoids are food color additives exempt from certification under Title 21 CFR, section 73.75 for canthaxanthin (E161g), section 73.90 for β-apo-8′-carotenal (E160e), and section 73.95 for β-carotene (E160a).

Carotenoid Usage

Carotenoids are used to color foods yellow-orange. Examples include butter, cheese, cereal grain, and others (27).

Reported Adverse Reactions to Carotenoids

Two studies by Fuglsang et al (29, 31) involved DBPCFCs using a mixture of food colorings that included β-carotene and canthaxanthin. The details of these studies were already discussed in the section on adverse reactions to annatto. Because a mixture of colorants was used, it was not

determined whether carotenoids were responsible for the reactions noted in the second study (29).

A study by Juhlin (18) involved provocation testing to carotenoids (112 subjects underwent oral challenge with β-carotene 50 mg, 100 mg, 150 mg) and canthaxanthin (42 subjects with 10 mg, 200 mg, 200 mg) in patients with chronic urticaria and angioedema. In the β-carotene group 9% had positive reaction described as flaring of urticaria and angioedema within 24 hours of challenge, another 12.5% had doubtful reactions. In the canthaxanthin group 14% had positive reactions and 24% doubtful reactions. Details of this study have been discussed in the section on annatto reactions.

One case report of a possible reaction to carotenoid colors is found in literature (34). A 9-month-old male infant developed AD, vomiting, colic, and restlessness while consuming various foods that contain carotenoids. Sensitivity to the vitamin A drops was determined by the authors with two double-blind challenges; skin tests, however, were negative. The authors suggested that the source of the reaction was vitamin A and possibly the carotenoids. However, the suspected causative role for the carotenoids, and especially the vitamin A, in this case was not conclusively established and the mechanism is unknown.

Management

Adverse reactions to carotenoids are probably very rare and are yet to be conclusively established. Avoidance is prudent if reactions are noted with use of carotenoids. In suspicious cases DBPCFCs may be helpful.

Saffron

Saffron is derived from the bulb plant *Crocus sativa* L., and is the most expensive of all spices (35). The saffron spice consists of the dried stigmas and styles of the crocus flower (36). Each blossom of the crocus plant contains one pistil, consisting of three stigmas, a style, and an ovary (36). Saffron has a dark-yellow-orange to dark red color (35). Several coloring pigments and coloring components have been identified in saffron, including carthamine, saffron yellow A and B (safflor yellow), saffloamine A, ethereal oils (safranal, pinen, cineol), glycosides (picrocrocin), and pectins (35).

Regulation and Labeling

Saffron is exempt from certification under CFR Title 21 section 73.500, and has the same labeling requirements as other food colors exempt from certification. Saffron is identified as E164 in the EU.

Saffron Usage

Although used as a spice saffron is also often used as a coloring in soups, sauces, rice dishes, cakes, cheese, and liqueurs such as chartreuse liqueur.

Reported Adverse Reactions to Saffron

In 1997, Wuthrich et al (35) described a case of anaphylaxis in a 21-year-old atopic farmer who had asthma, AD, and oral allergy syndrome to apple, nuts, and spinach. This subject, after eating a meal of saffron rice and mushrooms, developed abdominal cramps, laryngeal edema, and generalized urticaria within a few minutes. He was transferred to the emergency room cyanotic and pulseless, and required resuscitation. SPTs using rice, saffron, mushrooms, garlic, and onion were performed, and all were negative except saffron, which showed a strongly positive result. IgE RAST to saffron extract using the patient's serum was positive. SDS-PAGE followed by immunoblotting revealed specific IgE antibodies for proteins with molecular weights between 40 kDa and 90 kDa.

This patient had a strong skin test and RAST IgE reactivity to celery and cooked celeriac as well. The anaphylaxis episode was attributed to saffron; however, the authors did not rule out celery salt which could have been a component of the dish in question (35).

Management

In patients with type I hypersensitivity or other significant reactions to saffron, avoidance remains the only viable therapy.

Grape Anthocyanins

The word anthocyanin is derived from two Greek words, *anthos* (flower) and *kyanos* (blue) (33). Anthocyanins are widely distributed in the plant kingdom, where they occur as different gly-

coside combinations that produce red, blue, or purple coloration in various fruits and vegetables (4). Most of the rich red, pink, blue, and violet ornaments of the plant kingdom owe their color to one of the anthocyanins (33).

Grape color extract and grape skin extract are anthocyanin-containing color additives that are approved for use in foods in the US. Grape color extract is available as both an aqueous solution and a water-soluble powder that is derived by dehydrating the aqueous solution. The aqueous solution is obtained from Concord grapes and contains anthocyanins, tartrates, malates, sugars, and minerals. 3-mono- and 3,5-di-glucosides of malvidin, delphinidin, and cyanidin and other acetylated derivatives are the water soluble pigments responsible for the purple color of the grape color extract (27).

Grape skin extract is a purplish-red liquid prepared by the aqueous extraction of the fresh deseeded marc remaining after grapes have been pressed to produce grape juice and wine (27).

Because these coloring compounds are distributed only in trace quantities in most of the flowers, fruits, and vegetables (780–5000 ppm) (33), they are difficult and expensive to extract. With careful techniques, however, large amounts can be obtained from grapes and red cabbage, the main commercial sources.

Regulation and Labeling

Labeling requirements for grape anthocyanins is similar to other color food additives exempt from certification. Four sections of the CFR regulate anthocyanins on the basis of source. They are: fruit juice, Title 21 CFR 73.250; vegetable juice, Title 21 CFR 73.260; grape skin extract, Title 21 CFR 73.170; and grape color extract, Title 21 CFR 73.169.

Internationally anthocyanins are identified as E163, cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin (33).

Anthocyanin Usage

Anthocyanins are consumed naturally in fruits and vegetables such as grapes, strawberries, raspberries, blueberries, apples, radishes, and red cabbage. Less familiar sources of anthocyanins include purple corn, black carrot, and passion fruit and a host of other exotic fruits. Grape color extract is used in nonbeverage food; grape skin extract (enocianina) is used in still and carbonated drinks and ades, beverage bases, and alcoholic beverages. Other examples of anthocyanin use include cherries in yogurt and ice cream, fruit fillings, candy, and confections (33).

Reported Adverse Reactions to Grape Anthocyanins

Numerous adverse reactions, sensitivities, and confirmed allergic reactions have been reported following ingestion of grapes or grape products (37–52), including rhinoconjunctivitis, urticaria, angioedema, and anaphylaxis. No reactions specifically implicating grape skin extract or grape color extract when used as a coloring agent have been reported so far (20). The reported allergic reactions to grapes are likely from exposure to protein in the grapes that would not be present in either grape skin extract or grape color extract in appreciable amounts (20).

Management

Avoidance of grape or grape products is recommended in cases where severe allergic reactions have occurred with their consumption. Overall it appears that consumption of anthocyanins from natural fruits and vegetables would greatly exceed the consumption of anthocyanins used as color additives, and thus additive reactions to anthocyanins without reactions to the parent foods would be unlikely (3).

Other Food Color Additives Exempt From Certification

There have been no reported allergic adverse reactions to any of the other food colors that are exempt from certification when used as a food color additive.

Titanium Dioxide

Titanium dioxide (Title 21 CFR section 73.575) is a reaction product of the element titanium found in earth. It is a white crystalline powder that is one of the few exempt colors whose use is restricted to 1% by weight in foods. Certain confections, cheeses, icings, medications, and cosmetics are colored by titanium dioxide (3).

Caramel

Caramel (Title 21 CFR sections 73.85, 73.1085, 73.2085) is a reddish-brown to brown black liquid or solid. It is the end product of controlled heat treatment of a variety of carbohydrates, and goes through several different manufacturing processes. It is used in colored baked goods, desserts, gravy, sauce products, prepared meats, beverages, and confections.

Paprika

Paprika (Title 21 CFR sections 73.340 and 73.345) is a deep red, sweet, pungent powder produced from the ground dried pod of the mild pepper *Capsicum annuum* (3). It is used as a coloring agent as well as a spice in canned goods, vegetable oils, processed meats, salad dressings, snack food coatings, popcorn oil, cheeses, and confections. Although reactions have been reported with paprika, no reactions have been reported to paprika when used as a food color specifically.

Beet Powder and Carrot Oil

Beet powder (Title 21 CFR section 73.40) and carrot oil (Title 21 CFR section 73.300) are consumed in large quantities when the parent vegetables are eaten. Examples of beet juice base color use in foods includes fruit preparations, condiments, dairy products, sauces, fillings, and candies. Carrot oil, which contains both α- and β-carotene, is used in sauces, salad dressings, meat seasoning products, pasta, margarine and other food products.

Toasted Partially Defatted Cooked Cottonseed Flour

The color additive "toasted partially defatted cooked cottonseed flour" (Title 21 CFR section 73.140) is derived by processing food quality cotton seed. The end product is light to dark brown in color. IgE-mediated reactions to cotton seed have been reported (53–55) and include urticaria, angioedema, and anaphylaxis. However, the food color additive derived from cotton seed has not been implicated in reactions specifically so far.

Iron oxide (Title 21 CFR section 73.200), ferrous gluconate (Title 21 CFR section 73.160, ferrous lactate (Title 21 CFR section 73.165), and riboflavin (Title 21 CFR section 73.450) are in limited use as food colors.

Food Flavorings

Food flavorings are a heterogeneous group of supplements added in small quantities to commercially and privately prepared foods to enhance flavor or quality. These substances are not used as primary ingredients, and as such are present in relatively minute quantities when compared to main ingredients. Flavorings may be naturally occurring compounds, as is the case with spices, or may be derived from natural sources after undergoing biochemical manipulation. In general, the allergenicity of these substances is minimal due to their low protein content resulting from the separation of protein from the flavorful molecules during the manufacturing process. However, some of the parent compounds on which natural flavorings are based are derived from proteins known to be allergens (56). Artificial flavors are synthesized compounds such as aromatic alcohols and terpenes, used singularly or in combination to mimic natural flavors (57). Although allergic reactions to food flavorings are rare, several substances have been identified as causing both type I and type IV allergic reactions.

Taste and Flavor

Taste is defined as the ability of the tongue to sense the classically described flavors sweet, salty, bitter, and sour and the more recently described sensation umami. The definition of flavor incorporates taste as well as olfactory input and other sensory information from the trigeminal nerve (58). The sensation of taste is generated from the interaction of volatile components of food and receptors in the olfactory and gustatory epithelium. Tastebuds, localized to specific areas of the tongue, contain specialized neurons capable of sending neurologic signals in response to contact with soluble flavor molecules. This sensory system has been conserved through evolution and is important for nutrient acquisition and the avoidance of noxious or toxic substances. The specialized neurons that recognize the four basic flavors appear to be conserved across species.

On the molecular level, receptors that mediate taste send neural signals when tastant molecules contact specific adsorption sites, selective ion channels, or structure-specific receptors con-

tained in the tastebud neurons. Intracellular signaling occurs though calcium and G protein-mediated pathways (59). Transduced signals from receptors are conducted through the facial, glossopharyngeal, and vagal nerves to various nuclei in the brainstem. These nuclei compare signaling rates between the different sensory nerves, and the brain perceives these signals as flavors (60).

Regulation and Legislation

As with other food additives, in the US food flavorings are monitored by the FDA. According to provisions in the Food, Drug and Cosmetic Act (section 403), ingredients used in commercially produced food must be listed on package labels. Ingredients are labeled such that the most plentiful ingredient is listed first, and others follow in decreasing order of amount. In contradistinction to food colors, many ingredients in the food flavoring category are not required to be listed individually and may be listed as "artificial and natural flavors." Currently CFR section 101.22 provides for the definition and labeling of these substances. The original intent of such nebulous labeling was likely to preserve a competitive marketplace among food manufacturers. Complete disclosure of the character and relative amounts of flavorings used in particular foods would be contrary to the maintenance of company or trade secrets. Except for certain ingredients known to cause reactions (e.g., sulfites), several potentially allergenic substances are not required to be labeled. For consumers with food allergies, this oversight is at best annoying and at worst life threatening.

Flavor Characteristics

Compounds that impart flavors to foods are generally chemicals of a volatile nature. These substances are of low molecular weight and are thus unlikely to be allergens. Many occur in nature and others are created from naturally occurring chemicals by various synthetic processes. Artificial flavors are synthesized chemicals with structures similar to naturally occurring flavors but are not directly derived from natural products. Overall flavorings are a minor component of the total content of foods (56). A simple example includes the heating process while grilling meat. Thermal energy causes chemical changes in amino acids and sugars, which gives grilled meat its characteristic flavor. More complex processes include fermentation and enzymatic reactions responsible for the flavor of dairy and cheese products.

Techniques such as distillation, solvent extraction, concentration, and microencapsulation may be used to produce flavorings. Once these compounds have been produced, they are converted to different physical forms including powders, crystals, essential oils, soluble essences, concentrated juices, or emulsions. These various forms provide a wide array of options for adding flavorings to foods without changing the overall consistency of the finished food product.

Factors Affecting Flavoring Antigenicity

Several steps in the flavor manufacturing process may alter the allergenicity of a particular flavoring. Parent compounds derived from known allergens increase the likelihood that the daughter compound will be allergenic. Many processing techniques involve mechanisms that reduce the protein content of food flavorings. For example, distillation separates the flavorful volatile portions of a substance, leaving the proteinaceous material behind. Several steps in flavor processing may involve heating. Although many allergenic proteins are heat labile, some are resistant to degradation and can remain allergenic after the heating process (56).

Although the low molecular weight of most food flavorings renders them unlikely to be allergenic, they may participate in allergic reactions by acting as haptens. This phenomenon is more likely to be important in contact sensitivity (type IV) reactions. Given the short period of time that most foods are in contact with any particular portion of the gastrointestinal (GI) tract, contact sensitization is unlikely. However contact sensitivity has been described for substances which maintain prolonged contact with the oral mucosa such as hard candies, toothpastes, or tobacco flavorings. Allergic reactions have also been described in those with occupational exposure to various flavorings. These subjects often are exposed to high concentrations on a continual basis during the manufacturing process.

Allergenic Agents and Their Reactions

Flavors in the cinnamon family are known contact sensitizers (61). Balsam of Peru, a flavoring compound that contains cinnamic aldehyde, cinnamic alcohol, cinnamic acid, methyl cinna-

mate, eugenol (clove oil), and vanillin, has been implicated in allergic reactions ranging from contact and generalized urticaria to contact dermatitis (62–64). Products that may contain balsam of Peru include toothpaste, hard candies, products with citrus peel (marmalade, juices, baked goods), ice cream, cinnamon, clove, or vanilla (56). Hand eczema was described as a manifestation of contact sensitivity in a chef who frequently worked with cinnamon (65). The recalcitrant hand eczema cleared upon avoiding contact or ingestion of cinnamon in the workplace or the small amounts contained in vermouth. Cinnamic aldehyde in toothpaste has been shown to cause cheilitis and eczematoid lesions of lips proven with mucosal biopsies and patch testing (66). A similar reaction ("Thin Mint dermatitis") was reported in a cookie factory employee who developed hand eczema after becoming sensitized to balsam of Peru and vanilla when she began working on a production line for mint-flavored cookies (67). Generalized urticaria has been reported that was related to the ingestion of cinnamon or balsam of Peru (65).

Several other flavoring agents have been implicated in contact dermatitis: anise, carvone, fennel oil, d-limonene, menthol, peppermint oil, phenyl salicylate, and spearmint oil (56). Typically, cases involve these substances when used as flavorings in hard candies, toothpastes, or denture cements. Sensitive individuals developed symptoms including cheilitis and lip eczema (56). Systemic allergic type reactions have also been described with some of the above flavoring agents. Natural vanilla and artificial vanillin have been described as exacerbating AD. A DBPCFC with these agents resulted in flares of eczema in young children (68).

Flavoring oils used in toothpaste have been linked to asthma exacerbations. A 21-year-old woman with a history of aspirin sensitivity, nasal polyps, and asthma experienced flares of her asthma after brushing her teeth. DBPCFC with spearmint, peppermint, and menthol all resulted in a 36% decrease in this patient's forced expiratory volume in 1 second (FEV_1). The authors of this report were unable to identify an immunologic mechanism for this patient's reaction, however the rapid onset of her symptoms suggested allergy as a factor (69).

Hidden Allergens

Several documented IgE-mediated allergic reactions, including rhinorrhea, wheezing, urticaria, and angioedema have been caused by flavorings that contained or were derived from proteins known to be allergens (70). These reactions typically occurred in subjects previously known to be sensitive to other allergens, particularly milk. One report documents four patients who had reactions after eating foods that had been labeled as milk-free. Investigators concluded that hydrolyzed sodium caseinate was responsible for these reactions. This compound was not explicitly labeled and was accounted for on the product labeling as "natural flavoring" (70). Because of changes in labeling requirements, sodium caseinate now must be disclosed. Systemic allergic reactions have also been attributed to milk proteins contained in a dill pickle seasoning used to flavor potato chips. Investigation determined that lactose used in a spice mix to flavor the potato chips was contaminated with milk proteins (56).

Although the vast majority of reactions as described above have occurred with milk based proteins, other allergenic foods, such as peanut, egg, soy, and seafoods could also potentially be involved in similar reactions. It is important that patients with known food allergies continue to read food labels, even on familiar products. Ingredients often change and flavorings may be reconstituted. We had a patient react to a food that he had previously tolerated. Upon investigation, it was discovered that the "mini" version of the cookie was manufactured on a different line than the "jumbo" version to which the patient reacted. The "jumbo" cookie line was also used to manufacture products that contained nuts (MS McMorris, written communication, April, 2001). Concerns over this cross contamination from manufacturing equipment has led to the trend of adding a phrase such as "may contain peanut" to product labels. The appearance of "natural flavorings" listed on the label of a food to which a reaction has been attributed should raise suspicions of a common allergen hidden in the mixture (71).

Spices

Spices are aromatic compounds derived from plants. As with other flavorings, they are present in small amounts compared to other ingredients in prepared foods. Spices may be listed as individual ingredients on food labeling or may be labeled as "natural flavorings" or "seasonings." Table 29–3 contains a list of spices that have been implicated as causing allergic reactions. Despite the preva-

Table 29–3.
Spices Implicated in Allergic Reactions

Anise
Caraway
Cardamom
Celery seed
Clove
Coriander
Cumin
Curry
Dill
Fennel
Fenugreek
Garlic
Ginger
Mace
Mustard
Nutmeg
Paprika
Parsley
Pepper (cayenne, red, black, and white)

Adapted from (56).

lence of spices in foods few IgE-mediated reactions to this class of flavoring have been documented. This lack of IgE-mediated reactions may be due to the small amounts of spices used compared to other ingredients, or they may represent an unidentified cause of anaphylaxis. Spices may not be considered in the search for a cause of anaphylaxis because they are not usually specifically identified on product labeling.

Occupational exposure-related allergic reactions to garlic have been reported. The CFR considers garlic to be a food, but because it is used as a flavorant, rather than for nutritive reasons, we will consider it as a spice. Falleroni et al (72) reported a 49-year-old male who worked in a spice packaging factory and was frequently exposed to clouds of garlic dust. Allergy to garlic was confirmed with SPT to crude garlic extract with four volunteers as negative controls. Polystyrene tube solid phase radioimmunoassay (PTRIA) confirmed the presence of garlic-specific IgE in the patient's serum. This subject developed rhinitis and wheezing with a nearly 40% decrease in his FEV_1 upon bronchial challenge with garlic.

Another case report identified a 40-year-old male who developed rhinoconjunctivitis after exposure to a commercially produced meat flavor. This patient was skin tested to the main ingredients of the spice mixture. Fresh ginger and ginger extract elicited positive skin test reactions in the patient, and resulted in no reactions in three negative control patients. Ginger-specific IgE was identified with Pharmacia's CAP-RAST. SDS-PAGE with immunoblotting demonstrated a 17-kDa IgE binding protein in the patient's serum (73).

Although the majority of allergic reactions to spices occur in those with frequent high dose exposure, reactions through exposure in food and medicinal products have been reported (74). Coriander, a spice common in Asian and Middle Eastern cuisine that cross reacts with mugwort (75) was reported to cause anaphylaxis in an adolescent. Sensitivity was confirmed based on a positive SPT with fresh coriander. A DBPCFC resulted in rhinorrhea, mucosal edema, pruritis, and chest tightness at the third dose (76). Fenugreek, a spice in the legume family closely related to the chickpea, caused anaphylactic shock following inhalation during food preparation. A 36-year-old female with a history of mild asthma inhaled the spice to identify it and rapidly developed cough, wheeze, syncope, and hypotension. Fenugreek is also used for medicinal purposes, and a sensitized individual developed anaphylaxis and angioedema following topical administration to cure dandruff (74). Both patients were found to be skin test positive. SDS-PAGE with immunoblotting with serum from both patients confirmed the presence of an IgE binding protein, and DBPCFC confirmed clinical reactivity in both patients with significant drops in peak expiratory flow rate (PEFR) (74).

Several other spices have also been implicated in IgE-mediated reactions. Patients with specific IgE to mace, white pepper, capsicum peppers, black pepper, celery seed, and mustard have been described. In vitro release of histamine from leukocytes isolated from sensitized patients upon specific spice exposure has also been demonstrated for the above spices (75). Allspice (77), anise (78), caraway (79), curry (78), and paprika (80) have been demonstrated by RAST and immunoblotting to generate specific IgE in sensitized patients. Cardamom, cumin, paprika, and parsley have also been reported to cause IgE-mediated reactions (56).

Fruit Concentrates and Essences

A final category of food flavorings includes fruit concentrates and essences. Concentrated fruit juices are used as flavoring in various products and can be responsible for immediate hypersensitivity reactions. Fruit essences are flavorings based on isolating the aromatic portions of fruits from the fruit proteins. They have been implicated in IgE-mediated reactions. Essence of banana used in a pediatric formulation of oral penicillin was found to cause urticaria in a patient with hay fever and oral allergy syndrome. This patient was posi-

tive on skin testing to fresh banana but was negative to penicillin. In addition, IgE to banana but not penicillin was demonstrated with CAP-RAST (81).

Evaluation and Diagnosis of Food Flavoring Reactions

The evaluation of a patient with a suspected allergy to a flavoring proceeds much like the evaluation for other food allergies. A complete history and physical exam, as well as a detailed history of exposures prior to the reaction are necessary. The evaluation of patients with known sensitivities to common food allergens such as milk or peanut should be directed toward flavorings that may have been derived from or contain traces of those proteins. Contacting the producer of flavorings can reveal ingredients that are not explicitly labeled.

Patients without known prior food sensitivities present more of a challenge. If at all possible, narrowing down the ingredients of the foods or medications containing flavors ingested around the reaction time can help determine potentially culpable allergen sources. It bears noting that up to 90% of commercially available cough and cold medications and antipyretics for children contain natural and artificial flavors (57). Complex ingredients (e.g., teriyaki sauce) may potentially be used for skin testing. Once a skin test reaction has been confirmed, directed skin testing using the ingredients of the proprietary food flavoring can be used. It should be noted, however, that many of the naturally occurring spices can be irritating to the skin, which may lead to misinterpretation and false positive reactions. Negative control subjects should be tested as well to identify nonspecific irritant reactions from true allergic reactions. Negative skin testing may then be confirmed with a DBPCFC.

For patients with contact sensitivities, patch testing may provide diagnostic information. This should be done using purified compounds of known contact sensitizers (e.g., balsam of Peru).

Summary

Food colors and flavorings are a heterogeneous group of compounds often derived from potentially allergenic proteins. The manufacturing process usually renders these additives free of appreciable protein, minimizing allergenic potential. However, allergic reactions of both IgE-mediated and contact sensitivity types have been reported with food colors and flavors. These are often among subjects involved in the manufacturing of these compounds who are exposed to levels significantly higher than those found finished food products. Overall, sporadic reactions occur rarely in consumers and may be due to both immunologic and non-immunologic mechanisms. Because of labeling regulations, avoidance and diagnosis of particular allergens can be a challenging medical problem.

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Part 4

Contemporary Topics in Adverse Reactions to Foods

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30

Pharmacologic Food Reactions

Stephen B. Fritz
James L. Baldwin

Many foods contain a variety of either naturally occurring or added components that have pharmacologic or drug-like activity (1). When consumed in moderation, however, only a small number of substances have been identified that account for the majority of clinically apparent adverse pharmacologic reactions to foods. This chapter focuses on the most common endogenous substances implicated in pharmacologic reactions to foods and discusses their mechanisms and strategies for prevention and treatment.

Definitions and Characteristics

Pharmacologic food reactions have been defined as adverse reactions to foods or food additives that result from naturally derived or added chemicals that produce drug-like or pharmacologic effects in the host (2). Unlike type I allergic food reactions, which affect only a selected group of atopic patients, pharmacologic food reactions can potentially be elicited in a wider, more diverse group of individuals. The dose or quantity of food necessary to elicit a clinically apparent reaction typically varies among individuals and even in the same individual over time. Pharmacologic food reactions depend on metabolic differences, concurrent medication usage, food freshness, and food preparation.

Pharmacologic substances in foods can mediate their effects directly or indirectly. In the direct route, the food substance interacts with host tissue to exert an effect. In the indirect route, the food substance activates one or more of the host's endogenous mediator systems, which in turn exerts the effect on the host tissue. Differences in host tissue and/or host mediator system susceptibility at the time of ingestion are two factors that can contribute to the variability of these reactions.

Endogenous Substances Responsible for Pharmacologic Food Reactions

The different classes of endogenous substances responsible for pharmacologic reactions to foods are shown in Figure 30–1. Vasoactive amines constitute the largest class of substances responsible for pharmacologic reactions to foods. Methylxanthines make up a second class of food components having pharmacologic activity. Finally, there is well-documented evidence for pharmacologic activity of several unrelated food components including capsaicin, ethanol, myristicin, psoralen, solanine, and glycyrrhetinic acid.

Vasoactive Amines

The vasoactive amines include dopamine, histamine, norepinephrine, phenylethylamine, serotonin, tryptamine, and tyramine. Of these substances, dopamine, histamine, phenylethylamine, serotonin, and tyramine may be present in appreciable amounts in foods, thereby producing clinically apparent pharmacologic effects.

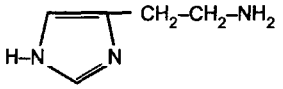
Histamine

The diamine histamine is perhaps the best known of the vasoactive amines present in, and responsible for, pharmacologic reactions to foods. Because of histamine's significant contribution to

Endogenous substances responsible for pharmacologic food reactions. (Vasoactive Amines and Others)

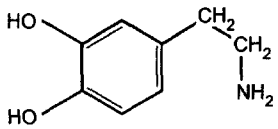
Vasoactive Amines:

Diamines: Histamine



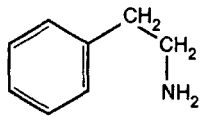
Monoamines:

Dopamine



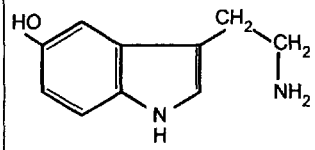
Fava Beans

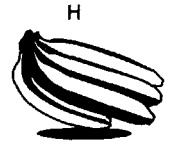
Phenylethylamine



Red Wine

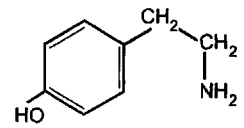
Serotonin





Bananas

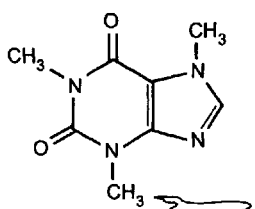
Tyramine



Cheddar Cheese

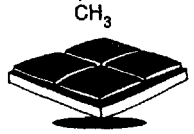
Methylxanthines:

Caffeine



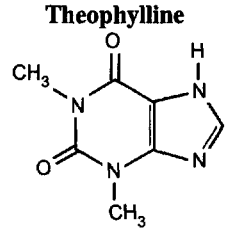
Coffee

Theobromine



Chocolate

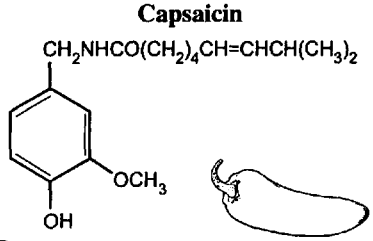
Theophylline



Tea

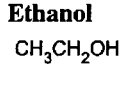
Other:

Capsaicin



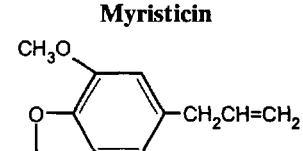
Chili Pepper

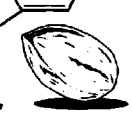
Ethanol



Martini

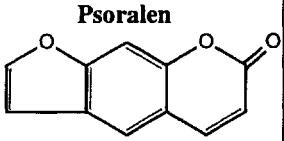
Myristicin

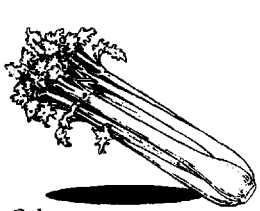




Nutmeg

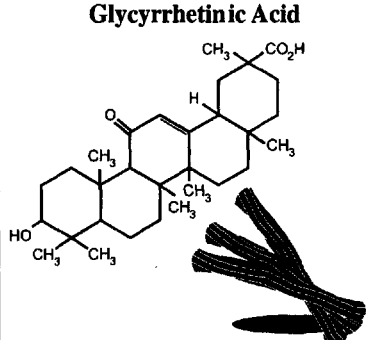
Psoralen





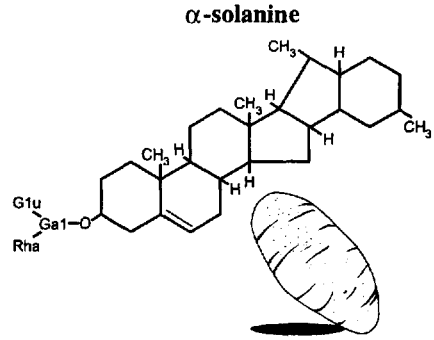
Celery

Glycyrrhetinic Acid



Licorice

α-solanine



Potato

Figure 30–1. Endogenous substances responsible for pharmacologic food reactions (vasoactive amines and others).

the pathophysiology of atopic disease, histamine-induced pharmacologic food reactions are frequently confused with food allergic reactions.

Synthesis: Histamine is synthesized in nature by the decarboxylation of its amino acid precursor histidine. This synthesis is catalyzed by the enzyme histidine decarboxylase and other enzymes that are widely distributed in nature. Canine intestinal bacteria may be capable of decarboxylation of dietary histidine to form histamine (3). Likewise, marine bacteria contaminating inappropriately refrigerated scombroid fish may convert the histidine present to histamine (4, 5). The wide distribution of enzymes capable of decarboxylating histidine to histamine partially accounts for the presence of histamine in many foods.

Physiologic Effects: Histamine mediates its effects on tissues through H1 receptors, H2 receptors, or both. The subsequent tissue responses to histamine, summarized in Table 30–1, can present following any type I hypersensitivity reaction in which histamine is released from mast cells (MCs) and/or basophils through an IgE-dependent mechanism. A clinically similar physiologic response can be noted in non-IgE-dependent pharmacologic food reactions in which histamine is either present in the food ingested or released from tissue stores due to some intrinsic histamine-releasing ability of the food ingested. The IgE- and non-IgE-dependent histamine-mediated events both occur within minutes of ingestion of the culpable food, and are clinically indistinguishable.

The physiologic effects of an oral ingestion of histamine depend on a number of factors, including individual susceptibility (6), metabolism, and dose ingested. Certain subjects are known to exhibit particular sensitivity to elevation of plasma histamine. For example, in response to elevated plasma histamine, acute bronchospasm can occur in asthmatics, and coronary artery spasm can develop in patients with variant angina pectoris (7, 8). Histamine-induced migraine can also be inhibited by H2 receptor blockade (9). Elevations in plasma and urinary histamine have been described following ingestion of a high-histamine-content food. Consequently, susceptible patients might be at particularly high risk for developing adverse events following ingestion of a high-histamine-content food.

Adverse responses to histamine, including abdominal cramping, flushing, headache, palpitations, and hypotension, appear to be roughly dose-dependent. Ingestion of 25–50 mg of histamine may precipitate headache, whereas 100–150 mg may induce flushing (10). These values are only rough estimates, however, and scombroid toxicity has been described with ingestion of as little as 2.5 mg of histamine (11). Although sensitivity and specificity of different histamine assays may account for some of the discrepancies, it is clear that individual susceptibility and factors affecting metabolism play prominent roles in clinical responses as well.

Metabolism: The duration of histamine's effect depends on its metabolism. In normal physiology, conversion of histamine to its major inactive metabolites by either histamine methyltransferase or diamine oxidase (DAO) generally occurs rapidly (12, 13). Figure 30–2 shows the two routes of histamine metabolism. Prolonged binding of histamine from normal dietary sources to H1 and H2 receptors is uncommon, and symptoms rarely occur with such incidental ingestions. When large ingestions of histamine occur (e.g., scombroid poisoning), however, the metabolic capacity is temporarily exceeded and a multitude of histamine-mediated effects are observed. Experimental administration of large oral quantities of histamine yields similar clinical responses (14).

Although methylation appears to be the primary route for metabolism of histamine administered by both the oral and intravenous routes, DAO is important as well. DAO is present in the intestinal mucosa in almost all mammalian species examined (15). Ingestion of a histamine-containing meal along with ingestion of drugs that inhibit DAO can produce histamine-induced symptoms.

Table 30–1.
Physiologic Responses Elicited by Histamine

Responses Mediated by H1 Receptors
Smooth muscle contraction
Increased vascular permeability
Mucous gland secretion
Responses Mediated by H2 Receptors
Gastric acid secretion
Inhibition of basophil histamine release
Inhibition of lymphokine release
Responses Mediated by H1 and H2 Receptors
Vasodilatation
Hypotension
Flush
Headache
Tachycardia

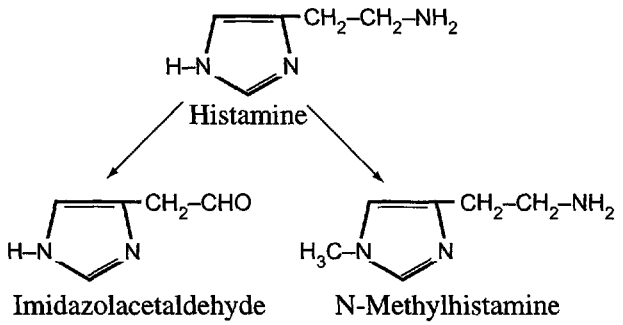


Figure 30–2. Histamine metabolism.

Pigs pretreated with the potent DAO inhibitor aminoguanidine and fed a high-histamine-content meal experienced severe clinical histamine-induced signs including, in some cases, shock and death. Conversely, pigs fed the same meal without pretreatment were generally asymptomatic (16). Isoniazid is a potent DAO inhibitor and, when combined with a histamine-containing meal, has resulted in severe histamine-induced symptoms (17, 18). In vitro experiments have shown a number of drugs (e.g., chloroquine, pentamidine, clavulanic acid, dobutamine, pancuronium, imipenem, and others) to be potent human intestinal mucosal DAO inhibitors. The in vivo clinical relevance of these findings remains uncertain (16).

Histamine-Containing Foods: Accurate measurement of the histamine content of foods has been difficult because of low specificity and sensitivity of the bioassays and chemical assays used. Certain foods are generally accepted as having higher histamine content than others (19, 20). Three cheeses (Parmesan, blue, and Roquefort), two vegetables (spinach and eggplant), two red wines (Chianti and Burgundy), yeast extract, and scombroid fish have histamine content adequate to raise postprandial 24-hour urinary histamine levels (19). For this reason, dietary histamine restrictions are recommended for patients undergoing 24-hour urinary histamine determinations.

The histamine content in red wines is commonly cited as one of the possible causes of wine intolerance. The symptoms most often reported by susceptible individuals include flushing of the face, headache, nasal congestion, and/or respiratory distress. A recent French study, however, found no significant difference in the occurrence of adverse reactions in wine-intolerant individuals who underwent two double-blind provocation tests, one with a wine poor in histamine (0.4 mg/L) and one with a wine rich in histamine (13.8 mg/L) (21). The histamine-rich wine also contained higher levels of other biogenic amines including tyramine, ethylamine, putrescine, and phenylethylamine (21). This suggests that the histamine content of wine may not be directly linked to adverse reactions to wines. It is also interesting to note that fermented cheeses contain amounts of histamine that are much greater than those found in wines, yet signs typical of intolerance to histamine have rarely been reported after ingestion of cheeses (22). Although the reason for this is not known, it suggests the role of a biogenic amine other than histamine, or the presence of a histamine-releasing substance, in wine that is not available in other foods such as cheese.

Several symptoms generally attributed to monosodium glutamate (MSG) resemble those associated with histamine toxicity. Using a radioenzymatic assay technique, the histamine content of several common Asian dishes, condiments, and basic ingredients was measured. Although the amount of histamine in individual food portions was determined to fall below the level generally thought necessary to induce symptoms, consumption of multiple portions could result in ingestion of enough histamine to produce symptoms (23).

Scombroid Poisoning: Histamine poisoning from ingestion of foods with high histamine content is well documented. The prototype for this kind of histamine toxicity is scombroid poisoning. Marine bacteria decarboxylate histidine present in improperly refrigerated scombroid fish (e.g., tuna, mackerel, skipjack, and bonito) and nonscombroid fish (e.g., mahi mahi, bluefish, amberjack, herring, sardines, marlin, and anchovies), thereby increasing the histamine content of these fish. Ingestion of such fish can result in symptoms of scombroid poisoning, which include flushing, sweating, nausea, vomiting, abdominal cramps, diarrhea, headache, palpitations, urticaria, dizziness, a metallic, sharp, or peppery taste, and, in severe cases, hypotension and bronchospasm (11, 24). These symptoms, which usually begin within an hour of ingestion of such fish and last for several hours, have been definitively linked to histamine in spoiled fish (11). The US Food and Drug Administration (FDA) has established a hazard concentration for histamine poisoning of greater than 450 μg per 100 g of tuna (25). Levels from 2.5

to 250 mg of histamine per 100 g of fish have been reported in most cases of scombroid poisoning. Treatment is supportive and includes H1 and H2 receptor blockade. Improper warming between the time that the fish is caught and when it is prepared can lead to histamine production sufficient to cause poisoning. Scombroid poisoning can be prevented only by proper handling and refrigeration of fish (24, 26).

Histamine-Releasing Foods: Some foods without significant histamine content may contain substances capable of triggering degranulation of tissue MCs, with resultant histamine release. Substances thought to be responsible for this histamine-releasing activity include enzymes in foods, such as trypsin, and other agents from both animal and vegetable sources, such as peptone. Foods with this unproven intrinsic histamine-releasing capacity include egg whites, crustaceans, chocolate, strawberries, ethanol, tomatoes, and citrus fruits (27).

Monoamines

Monoamines of dietary significance include dopamine, phenylethylamine, serotonin, and tyramine. Of these substances, phenylethylamine and tyramine account for the majority of pharmacologic reactions, although adverse effects of both dopamine- and serotonin-containing foods have been reported as well. These vasoactive monoamines are found in the greatest amounts in fermented foods.

Synthesis: Naturally occurring amino acids are converted into the vasoactive monoamines by a number of microorganisms that possess the amino acid decarboxylases necessary for this conversion. For example, tyrosine is the precursor for both dopamine and tyramine, phenylalanine is the precursor for phenylethylamine, and tryptophan is the precursor for serotonin. Amine production by these microorganisms varies depending on a variety of different conditions, including pH, temperature, and sodium chloride content (28).

Metabolism: The vasoactive monoamines are metabolized by the enzyme monoamine oxidase (MAO), which includes two subtypes: MAO-A and MAO-B. The genes for both MAO-A and MAO-B have been mapped to the short arm of the X chromosome (Xp11.23) (29), and appear to be derived from a duplication of a common ancestral gene (30). MAO is found in a variety of tissues, where it is localized to the outer membrane of mitochondria. It catalyzes the oxidative deamination of a variety of neurotransmitters as well as the monoamines of dietary significance. Dopamine and tyramine can be metabolized by both MAO-A and MAO-B. The polar amines (serotonin, epinephrine, and norepinephrine) are metabolized primarily by MAO-A, whereas the nonpolar amine phenylethylamine is metabolized primarily by MAO-B (31).

Patients with rare deletions in their MAO-A gene have increased levels of serotonin, epinephrine, and norepinephrine detectable in their urine, whereas MAO-B deficient subjects have increased urinary phenylethylamine levels (32). Although no studies have examined pharmacologic food reactions in these individuals, it is interesting to note that the MAO-A deficient individuals clinically have problems with impaired impulse control, including a propensity towards stress-induced aggression. MAO-B deficient individuals do not seem to have clinically apparent disturbances in their behavior (32). Although the reasons for these clinical differences are not known, it may be that raised serotonin levels in MAO-A deficient individuals have a disruptive effect on the developing brain (32).

Specific Monoamines

Tyramine: Many fermented foods contain tyramine derived from the bacterial decarboxylation of tyrosine. Foods with particularly high levels of tyramine include Camembert and cheddar cheeses, yeast extract, wine (especially Chianti), pickled herring, fermented bean curd, fermented soya bean, soy sauces and miso soup, and chicken liver. Smaller but still detectable amounts are present in avocados, bananas, figs, red plums, eggplant, and tomato (33–35).

Although tyramine exerts an indirect sympathomimetic effect by releasing endogenous norepinephrine (36), dietary tyramine usually does not cause detectable clinical effects. However, it is thought to be responsible for adverse clinical effects involving migraine headache and the hypertensive crisis experienced by patients receiving concurrent treatment with MAO inhibitors.

Foods and beverages containing tyramine have been linked to headache in some patients with food-induced migraine. In one study employing

double-blind, placebo-controlled (DBPC) challenges in 45 patients with food-induced migraine, 75 (80%) of 94 tyramine (125 mg) challenges evoked a migraine, whereas only five (8%) of 60 placebo challenges were followed by migraine (37). Several other studies, however, have failed to demonstrate a relationship between migraines and tyramine (38, 39). Two trials have examined the effect of a low-tyramine diet on the frequency of migraine headaches in pediatric and adult populations. Neither study was able to find a difference in headache indices between high tyramine and regular diets (40).

Although dietary tyramine has not been proven to cause migraines, it is possible that there is a subgroup of migraine patients that are susceptible to the effects of dietary tyramine. In these patients tyramine could induce headaches through the release of norepinephrine and its agonist effect on α-adrenergic receptors. This theory is supported by a study which found that pretreatment with indoramin, a selective α-adrenergic blocking agent, significantly reduced the likelihood of developing headaches induced by intravenous tyramine (40).

As noted earlier, ingestion of foods and beverages containing large quantities of tyramine can lead to headache and hypertensive crisis in patients being treated with MAO inhibitors (34). Normally, monoamine oxidase found in the gastrointestinal (GI) tract readily metabolizes dietary monoamines. When MAO inhibitors block MAO function, however, exogenous dietary monoamines are absorbed and release endogenous norepinephrine. The resulting pressor effect is linked to palpitations, severe headache, and hypertensive crisis. These episodes can be averted by avoiding foods rich in tyramine and other monoamines. Treatment involves slow intravenous administration of the α-adrenergic antagonist phentolamine, which is given until blood pressure stabilizes.

Dopamine: Dopamine exerts both an indirect sympathomimetic effect, by releasing endogenous norepinephrine, and a direct sympathomimetic effect, by interacting with α- and β-1 adrenergic receptors. Although tyramine in foods and beverages accounts for the majority of MAO inhibitor-associated hypertensive crises, dopamine present in fava beans or broad beans can also precipitate such a crisis. Avoidance of those foods is recommended for patients taking MAO inhibitors (34).

Phenylethylamine: Like the other monoamines, phenylethylamine may be found in several fermented foods and beverages, especially Gouda and Stilton cheeses and red wine. Unlike the other monoamines, however, phenylethylamine is also found in chocolate (33, 41).

Several mechanisms have been implicated in producing phenylethylamine's action (42, 43). It appears likely that phenylethylamine, like tyramine, exerts primarily an indirect sympathomimetic effect by releasing endogenous norepinephrine. Consequently, phenylethylamine has been implicated in both food-induced migraine (44) and MAO inhibitor-associated hypertensive crisis (34).

Serotonin (5-Hydroxytryptamine): Serotonin is found in highest concentrations (> 3.0 μg/g) in certain fruits, vegetables, and nuts, including banana, kiwi, pineapple, plantain, plum, tomato, walnuts, and hickory nuts (33, 45). Serotonin is present in moderate amounts (0.1 to 3.0 μg/g) in avocados, dates, grapefruit, cantaloupe, honeydew melon, black olives, broccoli, eggplant, figs, spinach, and cauliflower (45). The only non-plant foods with significant amounts of serotonin are certain mollusks, especially octopus (33).

Serotonin acts on at least two distinct receptors and a variety of cell types. Its actions are complex and exhibit wide species and receptor variability. Two major effects attributed to serotonin are skeletal muscle vasodilatation with flushing and both intracranial and extracranial vasoconstriction. Although these effects are often seen with endogenous serotonin production from carcinoid tumors, dietary serotonin does not appear to produce any immediate clinical symptoms, even in patients concurrently taking MAO inhibitors. In fact, oral feeding of serotonin equivalent to as many as 30 bananas failed to elicit clinical symptoms (46). The urinary excretion of the major metabolite of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), increases following ingestion of large amounts of serotonin. In this circumstance, a false diagnosis of carcinoid tumor may be entertained. Consequently, patients collecting 24-hour urine for 5-HIAA measurement should avoid serotonin-containing foods.

Although not proven, dietary serotonin has been implicated in a form of endomyocardial fibrosis seen in Uganda, similar to the endomyocardial fibrosis noted in patients with carcinoid syndrome (47). It appears that this entity is no longer a major health problem (45).

Methylxanthines

The three dietary methylxanthines are caffeine, theophylline, and theobromine. All are methylated derivatives of xanthine, which is a dioxypurine. Theobromine is extremely weak physiologically compared to theophylline and caffeine. Whereas all methylxanthine-containing beverages and foods contain caffeine, theophylline is present in only very small amounts in these foods and beverages, and theobromine is present in significant amounts in only cocoa and chocolate products. Consequently, caffeine accounts for most of the adverse responses from dietary methylxanthine consumption. This section will, therefore, focus on dietary caffeine and its effects.

Physiologic Effects

By far the most common physiologic effect of the methylxanthines involves stimulation of the central nervous system (CNS). The methylxanthines also exert effects on the cardiovascular, respiratory, GI, renal, and musculoskeletal systems (48). These effects are outlined in Table 30–2.

Mechanism of Action

The mechanism of action of the methylxanthines has been studied in various systems (48), and at least three have been suggested. Initial investigations focused on the ability of these agents to inhibit the enzyme phosphodiesterase. In many systems, however, it appears that under physiologic conditions this mechanism plays a minor role at best. In the CNS, the methylxanthines appear to act as adenosine antagonists, producing excitation by blocking adenosine's inhibitory effects. In addition, caffeine has been shown to compete for binding at the benzodiazepine site of central chloride channels, causing excitation by limiting activation of these channels (49).

Table 30–2.
Some Physiologic Effects of the Methylxanthines

Central Nervous System:
Psychostimulation (anxiety, insomnia)
Cardiovascular:
Increased contractility, blood pressure, pulse; increased cerebrovascular resistance
Respiratory:
Relaxation of respiratory smooth muscle; increased diaphragm contractility
Renal:
Diuretic effect
Gastrointestinal:
Decreased lower esophageal sphincter pressure; increased gastric secretion, nausea
Skeletal Muscle:
Increased contractility

Absorption, Distribution, and Metabolism

The three dietary methylxanthines are readily absorbed from the GI tract and distributed throughout body water. They are extensively metabolized in the liver, primarily to uric acid derivatives that are, in turn, excreted in the urine. Females taking oral contraceptives have significantly slower rates of catabolism of caffeine than females not taking oral contraceptives and males (48). In addition, fluoroquinolones impair caffeine and theophylline metabolism, resulting in increased serum concentrations (50).

Methylxanthine-Containing Foods

The methylxanthine content of foods and beverages has been widely studied via high-performance liquid chromatography (HPLC) (48, 51). Rough estimates of the quantities of the methylxanthines are given in Table 30–3. These values may fluctuate widely, depending on the variety of foods and their preparation. For example, Robusta coffee blends yield higher caffeine content in general than Arabica blends (48). Furthermore, brewing times and methods can alter the caffeine content by 100% in certain teas and coffees (48).

Adverse Effects of Caffeine

As noted, caffeine exerts pharmacologic effects on a variety of organ systems. Consequently, adverse pharmacologic reactions to caffeine-containing foods and beverages are manifested in many ways.

Large quantities of coffee and tea are known to produce clinical symptoms that mimic anxiety and panic disorders (52). In a blinded, placebo-controlled trial of caffeine consumption in patients diagnosed as having panic disorder or agoraphobia with panic attacks and in normal controls, caffeine produced significantly greater increases in subject-related anxiety, nervousness, fear, nausea, palpitations, restlessness, and tremors in patients compared with controls (53). Furthermore, these effects were correlated with plasma caffeine levels and

Table 30–3.
Methylxanthine Content of Foods and Beverages

	Theophylline (mg)	*Caffeine (mg)*	*Theobromine (mg)*
Coffee (1 cup)	100–150	80–175	NA
Cola (12 oz)	40	36–100	NA
Tea (1 cup)	30–40	20–90	1
Cocoa (1 cup)	4	6–11	13
Milk chocolate (1 oz)	6	6–10	44–60
Baking chocolate (1 oz)	35	25–47	390–450

Source: USDA nutrient database for standard reference, release 14 (2001).

were reported to resemble those experienced during panic attacks. The only somatic effect that differed significantly from baseline in the normal controls was an increase in tremors (53). In addition, caffeine abstention has been reported to reduce the frequency of panic attacks in this patient population (54). A central adenosine receptor dysfunction in patients with panic attacks has been proposed as an explanation for their increased sensitivity to caffeine (55).

Two cases of caffeine-induced urticaria reported in the literature were diagnosed by DBPC challenge (56, 57). Although the mechanism remains obscure, both cases were inhibited by pretreatment with terfenadine, suggesting mediator release and H1 receptor stimulation in the pathogenesis of the reactions.

Withdrawal from caffeine often produces migraine headache in susceptible individuals. Other common effects described in normal healthy individuals and attributed to dietary caffeine include insomnia, palpitations, nausea, and diuresis.

Capsaicin

The genus *Capsicum* encompasses many species, including chili peppers, red peppers, paprika, Tabasco pepper, and Louisiana long pepper. *Capsicum* peppers have been used for centuries by cultures around the world to enhance the flavor of relatively bland foodstuffs, as well as for its medicinal and irritant properties. Although more than 100 volatile compounds are present in capsicum oleoresin, capsaicin is the most important biologically active compound and is used most frequently for its pharmacotherapeutic benefits (58). About 70% of the irritant effect of these foods that accounts for their "hot" sensation derives from their capsaicin content (59).

Capsaicin's initial irritant action is mediated by release of the neurosecretory compound substance P from nocioceptive nerve fibers. Substance P depolarizes neurons to produce vascular dilation, smooth muscle stimulation, and pain. Repeated exposure to capsaicin results in blockage of substance P synthesis, diminishing the neurons' ability to transmit pain. This process is the basis on which capsaicin creams are used for painful conditions such as rheumatoid arthritis, osteoarthritis, diabetic neuropathy, postherpetic neuralgia, postmastectomy pain syndrome, and reflex sympathetic dystrophy (58).

The most common adverse effect associated with capsaicin is the "burning" oral sensation associated with its ingestion. In this instance, capsaicin binds strongly through its lipophilic side chain to the lipoproteins of oral mucosal receptors. To hinder this strong interaction and "cool the burn," a lipophilic phosphoprotein such as casein (present in milk, nuts, chocolate, and some beans) is more effective than cold water (60). A case of plasma cell gingivitis has also been attributed to oral exposure to capsaicin (61).

Adverse pharmacologic effects associated with capsaicin have also been reported in several tissues following exposure by different routes. Gastric installation has been shown to cause significant increases in gastric acid and pepsin secretion, as well as mucosal microbleeding and exfoliation (62). Nausea, vomiting, abdominal pain, and perforated viscus with peritonitis have been reported following ingestion of multiple peppers at a single sitting (63, 64). Inhalation has been reported to result in cough in occupationally exposed *Capsicum*-processing workers (65) and in laryngospasm (66). Involvement with the eyes causes pain, tearing, erythema, and blepharospasm; this effect has led to use of "pepper sprays" to ward off would-be attackers. Both acute and chronic dermatologic manifestations can also occur when handling *Capsicum*. Possible acute effects include skin irritation, erythema, and burning pain without vesiculation. In chronic exposures, severe dermatitis with vesiculation can

occur (67). A case of Sweet's syndrome in a pepper preserver has also been attributed to capsaicin (68).

Ethanol

Ethanol, the most widely abused pharmacologic substance in the world, exerts diverse effects on several body systems. The most prominent effects of ethanol consumed in moderate amounts involve the CNS. Ethanol can also act as a peripheral vasodilator and diuretic. It exerts its effects on the brain by dissolving in neuronal plasma membranes, thereby altering the movement of chloride and calcium ions involved in regulation of electrical signals and neurotransmitter release. Ethanol's diuretic effect is thought to relate to its ability to inhibit posterior pituitary secretion of antidiuretic hormone (69). Both the diuretic and CNS effects of ethanol are well known and not commonly mistaken for allergic reactions. The histamine-releasing ability of ethanol was discussed earlier. Consequently, this section will focus on other responses to ethanol that depend on its peripheral vasodilator properties, sometimes mistaken for ethanol "allergy."

The mechanism of ethanol-induced peripheral vasodilatation remains incompletely understood. Both direct effects—possibly mediated through increases in nitric oxide synthase activity (70, 71)—and centrally mediated effects (72) have been suggested. Both normal individuals and those with metabolic deficiencies can experience ethanol's vasodilator effects. In normal subjects, nasal congestion with increases in upper airway resistance (73) and mild cutaneous flushing reactions have been noted within minutes of ethanol ingestion. Alcohol sensitivity is a symptom complex that can consist of cutaneous flushing, tachycardia, hypotension, somnolence, nausea, and vomiting. This response is thought to be mediated by increased levels of acetaldehyde resulting from diminished or inhibited aldehyde dehydrogenase (ALDH) enzymatic activity. It can occur following ethanol interaction with disulfuram, metronidazole, griseofulvin, quinacrine, hypoglycemic sulfonylureas, phenothiazines, or phenylbutazone in normal individuals or in individuals deficient in one of the mitochondrial isoenzymes of ALDH, designated $ALDH_2$.

$ALDH_2$ deficiency is common in certain Asian groups (affecting about 50% of Chinese, Japanese, and Koreans) and has been reported to protect against alcoholism (74, 75). It appears only rarely among non-Asian ethnic groups. The inactive $ALDH_2$ allele is dominant, so that both homozygotes and heterozygotes exhibit $ALDH_2$ deficiency and alcohol sensitivity. Affected individuals experience symptoms to varying degrees within minutes of ingestion, responding with elevations in serum cortisone (76). Extreme cases of ethanol sensitivity presenting with coma have been reported (77). Treatment is supportive. A cutaneous ethanol patch test has been suggested as a more reliable indicator of the $ALDH_2$ phenotype than self-reported ethanol-induced flushing (78).

Myristicin

The spice nutmeg is derived from the dried fruit of the nutmeg tree (*Myristica fragrans*). Taken in moderation as a flavoring for foods, nutmeg is innocuous. Consumption of large quantities can precipitate psychosis, however. The active ingredient in nutmeg thought to be responsible for this adverse effect is myristicin. Structurally, myristicin is similar to mescaline (Fig. 30–1) (79). It has been proposed that myristicin may be metabolized in vivo to an amphetamine-like compound with effects similar to those of lysergic acid diethylamide (LSD) (80). It remains unclear whether myristicin or one or more metabolites accounts for its psychoactive properties, as synthetic myristicin does not always precipitate hallucination (81). Some investigators questioned nutmeg's psychoactive properties and have reviewed various medicinal uses of this spice (82). One tablespoon of grated nutmeg (roughly 7 g) contains about 2% myristicin by weight (83). Symptoms generally appear 3–8 hours after ingesting more than one tablespoon. The most prominent effects involve the central nervous and cardiovascular systems. Apprehension, fear of impending death, anxiety, and visual hallucinations accompanied by regular tachycardia are common (84, 85). Patients may also experience palpitations, nausea, vomiting, and chest pressure. Because dry mouth, fever, cutaneous flushing, and blurred vision can occur, acute nutmeg intoxication is sometimes mistaken for anticholinergic intoxication. One differentiating physical examination feature is that myristicin usually, although not always, causes miosis rather than mydriasis (86, 87).

Treatment for acute nutmeg intoxication is supportive. Emesis induction of an unknown ingestion is controversial. Many patients ingesting a toxic quantity of nutmeg are nauseated and will

vomit spontaneously. Activated charcoal with sorbitol may decrease the systemic absorption, thereby mitigating the duration and severity of symptoms. Various psychotropics have also been employed, including diazepam and haloperidol for anxious and hallucinogenic features (86, 87).

Psoralen

Psoralens are naturally occurring compounds belonging to a group of compounds known as furocoumarins. Furocoumarins are tricyclic hydrocarbons consisting of a furan ring condensed on benzopyrone (Fig. 30–1) (88). Synthetic psoralens are used commonly for the treatment of certain dermatological diseases, including psoriasis. In PUVA (psoralen + ultraviolet A radiation) therapy patients receive psoralen with the addition of UVA light which causes the photoaddition of psoralen to pyrimidine bases of DNA, resulting in a cross-linking between DNA strands (88). PUVA-induced cross-linking of DNA is thought to mediate the observed antiproliferative effects of psoralen on psoriasis. Sunlight with addition of psoralen also leads to the generation of reactive oxygen species, free radicals that can damage cell membranes, cytoplasmic constituents, and cell nuclei, resulting in a photodermatitis (88). Naturally occurring psoralens have been found to be present in celery, parsley, limes, lemons, bergamot, and parsnips. Celery field workers and handlers frequently develop photosensitization problems as a result of celery furanocoumarins (89). Photocontact dermatitis of the skin has also been demonstrated to occur following external contact with the fig tree (*Ficus carica*) in conjunction with exposure to the sun. Contact with the fig leaf sap and shoot sap is required in fig-induced photodermatitis; the fruit sap does not contain significant amounts of psoralen (90).

Patients exposed to food psoralens typically develop clinical symptoms within 24 hours of skin contact with furocoumarins. The initial presentation usually includes sunburn, linear bullae, and/or blisters, which may persist for up to 1 week. Hyperpigmentation usually follows and may remain for several weeks to months (91). In children, phytophotodermatitis may be confused with child abuse (91). Awareness of this condition in pediatric patients may prevent an unpleasant situation when questioning parents or caretakers, as well as unnecessary diagnostic procedures. Most cases of phytophotodermatitis do not require treatment. Marked pain and discomfort may be treated with cool, moistened dressings for several days. Topical corticosteroids may also be used, and in severe cases the use of systemic steroids has been recommended (91). The use of aspirin and other prostaglandin inhibitors has been proposed but there is no scientific evidence that this therapy is helpful (91). The prognosis is usually excellent, although severe, life-threatening burns occur rarely.

Solanine

Solanine is a general term used to describe the glycosidic alkaloids present in the common potato (*Solanum tuberosum*). Structurally, these glycoalkaloids are complex molecules consisting of three sugars attached to a nitrogen-containing steroidal skeleton (Fig. 30–1) (92, 93). Potato plant synthesis of solanine is thought to be a defense mechanism against fungus growth on potatoes; the compound α-solanine has been shown to be fungitoxic and is synthesized at cut (wound) surfaces (93). The production of α-solanine is also stimulated by mechanical injury, exposure to light in the field (green potatoes) or in the marketplace and with aging of the potato (93). In addition to its fungicidal properties the compound α-solanine is also a moderate inhibitor of specific and non-specific cholinesterases. The highest total solanine levels in the potato plant are present in the foliage, blossoms, and sprouts, followed by the peel, potato sprouts, and the tuber flesh. The US Department of Agriculture (USDA) potato breeding program has an accepted guideline for α-solanine content in commercial potatoes at below 200 μg/g fresh weight (93). Unfortunately, the levels of α-solanine under certain weather conditions can rise to far above that level. Several outbreaks of illness have been traced to the consumption of potatoes with α-solanine contents ranging from 100 to 400 μg/g (93).

The symptoms of solanine poisoning may occur 2–20 hours after a meal. They can include vomiting, diarrhea, and severe abdominal pain, and more severe cases present with neurological symptoms, including headaches, dizziness, drowsiness, confusion, visual disturbances, dilated pupils, and weakness, sometimes followed by unconsciousness. The vital signs include fever, rapid weak pulse, low blood pressure, and rapid respiration—not unlike the vitals seen in patients experiencing anaphylaxis (94). Recovery from solanine poisoning is usually complete, but coma and

death have been reported in cases of severe solanine poisoning.

Baking, boiling, or microwaving does not affect the α-solanine content of potatoes. The contents are only slightly reduced by frying. Fried potato peels are a source of large quantities of solanine. In one study, fried potato peels had glycoalkaloid levels that ranged from 1390 to 1450 μg/g, which is more than seven times the recommended upper safety limit (93).

Treatment of solanine poisoning is mostly supportive once a history of potato consumption has been obtained. The best way to avoid solanine poisoning is to avoid excessive potato consumption, especially the eating of potato peels. One simple test of solanine levels is to chew a small piece of the raw peel. Potato skins with levels of total glycoalkaloid higher than 100 μg/g of tuber cause a slow developing, hot burning, persistent irritation of the sides of the tongue and back of the mouth. Potato skins that contain more than 200 μg/g give an immediate burning sensation (93).

Glycyrrhetinic Acid

Glycyrrhetinic acid is the pharmacologically active constituent of licorice that is extracted from the sweet root of the plant *Glycyrrhiza glabra* (Fig. 30–1) (95). The use of licorice dates back to at least 1000 BC when stores of the root were placed in the tombs of Egyptian pharaohs. Its therapeutic activity for a wide variety of ailments was extolled in the writings of the ancient Greeks, Romans, and Chinese (96). More recently licorice has been shown to have the pharmacologic properties of a gastric mucosal protectant, and antitussive agent, a sedative, and an anti-inflammatory agent (96). The largest importer of licorice in the US is the tobacco industry for use as a conditioning and flavoring agent. Licorice cures tobacco and thus has been used for a century in cigars, pipe tobacco, cigarettes, and chewing tobacco (97).

When licorice is ingested habitually or in excess patients develop symptoms that share most of the clinical and biochemical features of primary hyperaldosteronism. Clinical manifestations include those of sodium retention (pulmonary and peripheral edema, breathlessness, and hypertension) and hypokalemia (cardiac dysrhythmias, polyuria due to nephrogenic diabetes insipidus, proximal myopathy, lethargy, paresthesias, muscle cramps, headaches, and tetany) (95, 98). Biochemical markers for excessive activation of mineralocorticoid receptors in the distal renal tubules include hypokalemic alkalosis and suppression of plasma renin activity (95). It is thought that glycyrrhetinic acid acts by inhibiting renal 11β-hydroxysteroid dehydrogenase activity, thereby diminishing the conversion of cortisol to cortisone and resulting in high renal levels of cortisol (99). Because cortisol binds to mineralocorticoid receptors with the same affinity as aldosterone, there is a resulting hypermineralocorticoid effect of cortisol (99).

Treatment of patients with licorice-induced hypermineralocorticoidism includes the administration of spironolactone, which acts as a competitive inhibitor of mineralocorticoid receptors. Since most sodium is reabsorbed in the proximal renal tubules, concomitant administration of a thiazide diuretic, which blocks reabsorption of sodium proximal to the distal portion of the nephron, is required for maximal diuretic effect. The suppression of 11β-hydroxysteroid dehydrogenase activity, as well as many of the changes in electrolyte balance, may persist for almost 2 weeks after licorice intake is discontinued. The prolonged suppression of 11β-hydroxysteroid dehydrogenase activity appears to be due to the continued action of glycyrrhetinic acid, because as urinary glycyrrhetinic acid levels fall, the suppression of 11β-hydroxysteroid dehydrogenase activity is reversed (98). Unfortunately it takes 2–4 months following cessation of licorice consumption for the function of the renin-aldosterone system to return completely to normal (99).

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31

Management of Food Allergy

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Introduction

Allergic reactions to foods encompass a spectrum of symptoms ranging from mild to life-threatening to fatal anaphylactic reactions. The relationship of a food to a reaction may be very clear, as in an acute IgE-mediated reaction following peanut ingestion. In such cases, elimination of the food should prevent the onset of symptoms. The overall contribution of a food to the production of atopic dermatitis (AD) or eosinophilic gastroenteritis may be less well understood, however, and elimination of the offending antigen may not result in complete resolution of the disease.

For patients who have food allergies, avoidance of the offending food is the key to preventing an allergic reaction. Unfortunately, complete avoidance is difficult to achieve, because food allergens can be hidden in other foods. Therefore, all patients need written instructions for emergency management of a reaction. Treatment of food allergy may include attempts to prevent sensitization, medications to prevent or palliate symptoms associated with ingestion of the antigen, and possibly immunotherapy—a recent development.

Allergen Avoidance

The best strategic approach to the management of true food hypersensitivity is complete avoidance of the allergen. It is critical to provide patients with adequate information about the allergen, including the types of food in which it may be found and the various terms that are used to identify it on an ingredient statement.

Allergen Identification

The foods most commonly implicated in food allergy are egg, peanut, milk, soy, wheat, fish, shellfish, and tree nuts (pecans, almonds, walnuts, pistachio nuts, cashews, hazelnuts, Brazil nuts, etc.) (1). However, any food has the potential to cause an allergic reaction. Although avoidance of the offending food with careful menu planning and label reading would appear reasonably possible, it is actually quite challenging. The literature is replete with reports of accidental exposures of food-sensitive individuals to the very antigen they are striving to avoid. Even minute quantities of an allergen may provoke serious reactions in extremely sensitive patients. The following discussion identifies potential problem areas and provides suggestions for educating patients in avoidance strategies.

Label Reading

Food-allergic individuals should read the ingredient label on all foods. This step ought to be repeated every time they shop, because ingredients may change without warning. Label reading for some can take as much as 2 hours each time they go to the grocery store. Ingredient statements should also be read for bath products and cosmetics, as some contain extracts from common food allergens such as almonds or milk. Pet food sometimes contains wheat, eggs, milk, or peanuts. Children have had reactions after being licked by a dog that has ingested food that contains one of the foods to which the child is allergic. Because toddlers may eat things they find on the floor, in-

cluding pet food, extra care and attention should be given to these situations.

As an extra precaution, some families read the ingredient label three times: at the store, before they put the groceries away at home, and before they serve the food to the allergic child. Some report that they only noticed the allergen they are avoiding during the third reading, thus justifying to themselves the need for this extra-cautious approach.

To properly avoid the food to which they are allergic, patients must learn the scientific and technical names for foods that may appear on labels. For example, the presence of milk protein may be indicated as whey or ammonium caseinate; eggs as albumin or globulin (Table 31–1). Joshi reported that of 91 sets of parents participating in a label reading study in an allergy clinic, less than 10% of those avoiding milk where able to spot the "milk words" on a label, only 54% of those avoiding peanuts correctly identified peanuts on a label, and 22% correctly answered soy (2). Ninety percent of the parents with near perfect scores in label reading were members of the Food Allergy & Anaphylaxis Network (FAAN), supporting the need for proper education of label reading for patients and their families.

FAAN (Fairfax, VA; 800-929-4040; www.foodallergy.org) provides wallet-size laminated or magnetic cards to make label reading easier. These "How to Read a Label" cards contain lists of synonyms and ciphers under which milk, egg, wheat, peanut, soy, shellfish, and tree nuts may masquerade. The cards are updated regularly as new terms are identified.

Understanding kosher rules and markings can make label reading easier (3). A "D" indicates that a product contains dairy products, even if its presence is not disclosed on the ingredient statement. Products that list a "D" on the front but may not list milk on the ingredient statement include some brands of tuna, sliced bread and bread sticks, breakfast cereals, cookies, imitation butter flavor, pancake syrup, pretzels, fruit snacks, cake mixes, and frostings. In some cases, milk may be present in the natural flavors. The designation "D.E." (dairy equipment) on a label signifies that the product was manufactured on equipment also used to produce dairy-containing food. As a result, the product might possibly contain trace amounts of milk protein (3).

"Pareve" or "Parve" on a label indicates that a rabbinical agency has determined that this product does not contain dairy products. However, under Jewish law, a food product may contain a small amount of milk and still meet religious specifications for "pareve" (3). Anaphylaxis was reported in one milk-sensitive child after ingestion of pareve-labeled food (4). As a result, products labeled "pareve" may not be safe for those with milk allergy.

Under current food labeling laws, casein or caseinates are considered additives even though they are milk-derived proteins. Thus, foods that contain these ingredients can be legally advertised as "nondairy" products. Examples of foods listed as nondairy that may contain milk include coffee whiteners, whipped toppings, and imitation cheeses. A number of reactions have occurred to children whose family members believed "nondairy" to mean "no dairy" and did not read the ingredient statement on the back of the package.

Ingredients categorized as flavors are often used in small quantities in commercially processed foods and are not required to be listed individually on the label. Many food manufacturers buy flavors from suppliers that consider the contents of their flavors to be a trade secret (5). Natural flavors are often derived from products including milk, wheat, and soy. Reactions have been reported from allergens in flavors. In 1996 the US Food & Drug Administration (FDA) issued a letter to the food industry stating, "an amount of a substance that may cause an adverse reaction is not insignificant" and must be declared on the label even if it is a color, flavor, or spice, which typically are not required to be listed by name (6). Unfortunately, some companies, especially small- to mid-size companies, have not adopted this recommendation. For now, patients should call the manufacturer to inquire about the presence of al-

Table 31–1.
Partial List of Synonyms for Common Food Allergens

Milk Protein
Ammonium caseinate
Casein
Curds
Whey
Non-dairy
Soy Protein
Edamame
Wheat Protein
Semolina
Peanuts
Valencias
Monkey nut
Ground nut

lergens in products with "flavors" noted on the package.

To get the information they are looking for, they must be as specific as possible, for example, asking "Does this product contain soy?" rather than, "What is in the natural flavors?" Most large manufacturers willingly provide this information. Products from companies that do not provide this information to families with food allergies are to be avoided. Imported foods should also be avoided, because labeling standards in other countries may not be as strict as those in the US; often ingredient declarations for these products are incomplete and US distributors take no responsibility for tracking down that information from foreign sources (5).

The use of allergen advisory or "may contain" labeling has proliferated in recent years. These allergen advisory statements are voluntary; as a result, each manufacturer has their own decision tree for when and what statement to use on a product. Examples of these statements include "may contain peanuts"; "contains the occasional nut"; "manufactured in a plant that also processes milk"; and "manufactured on shared equipment with nuts." Many patients report frustration at their diminishing food choices as the proliferation of products with these advisory statements appears on the market; others have chosen to ignore these statements completely. This should be discouraged, as there is a chance that the product may contain the allergen listed on the advisory statement. The FDA has notified the industry that these allergen advisory statements are not to be used in place of good manufacturing practices (6).

Another labeling situation that poses a threat to patients is the listing of the food allergen as the last ingredient. Some patients have reported ingesting products with the allergen as the last ingredient and having suffered no reaction, determining that the ingredient is not present or that they are no longer allergic. In one case, a young teen ate a baked good that listed peanut flour as the last ingredient, allegedly telling her friend that she'd done this before without having a reaction. Unfortunately, the product did contain peanuts and she died a short time later from her reaction.

Most of the products that cause reactions are desserts or candy, particularly chocolate candy. Patients, particularly those with peanut or tree nut allergy, should be advised to avoid these types of products, unless they are prepared at home, as a precaution.

Lists of commercially prepared "safe" foods are a popular request of busy parents looking for shortcuts in labeling reading. Such lists should be avoided because manufacturers change ingredients without warning. A list of "safe" products can quickly become outdated and the incorrect information on the list can lead to a reaction, particularly since these lists are often copied and shared with caregivers, teachers, and others, and old lists may not be retrieved and replaced. In one example, a school and day care center published such a list and included a "peanut and tree nut safe" donut shop. Months later the establishment introduced a nut-containing product made on shared equipment with "plain" donuts.

Occasionally, a manufacturer makes a labeling or packaging error by including an undeclared allergen in a product, putting a product in the wrong packaging, or using an out-of-date label with incomplete ingredient information. These situations pose a special hazard to those with food allergies. The FDA requires products whose labels are incorrect to be recalled from the market. To quickly get the word out to the allergic community when these situations arise, FAAN has developed a Special Allergy Alert System. Information about the mistake, product name, code, and other criteria are sent via mail (to FAAN members only) and e-mail (to anyone), and are placed on FAAN's website (www.foodallergy.org). All patients who have food allergies should be encouraged to sign up for these special allergy alert notices.

Some foods may appear to be so straightforward that the patient may not feel it necessary to scrutinize the label for hidden allergens. Alternatively, the food product is considered so unlikely to be allergenic that the label is never reviewed at all (Table 31–2). Ingredient labels should be reviewed for all products.

Cross-Contact

Even with careful labeling, concealed allergens may still adulterate a food. Cross-contact can occur during the processing of foods. Although the production lines are cleaned thoroughly between each product run, mistakes are sometimes made. It has been reported that some dark chocolate may be manufactured on the same line as milk-containing products (e.g., milk chocolate), making contact with milk allergens possible (7).

Granola bars are often produced on the same line as products that contain peanuts or a variety of

Table 31–2.
Unexpected Sources of Common Allergens

Food	*Ingredient*
Worcestershire sauce	Anchovies, sardines
Soy sauce	Wheat
Imitation butter flavor	Milk protein
Water-added ham, deli meats, some sausages and hot dogs	Milk or soy
Sweet and sour sauce	Wheat or soy
Egg substitutes	Egg white
Low fat peanut butter	Soy
Pet food*	Eggs, wheat, milk, peanuts, and soy
Cosmetic and bath products	Milk, tree nuts, eggs
Imitation crab legs	Wheat, fish, crab
Veggie burgers	Nuts

*Toddlers may sample pet food off the floor.

nuts, which could allow the granola bars to become contaminated with substances not listed on the label (5). Products can sometimes incorporate a stray ingredient from another product. For example, small pieces of peanuts or nuts that remain in the equipment after thorough cleaning may become dislodged during the next production run (5).

Various types of nut butter, including peanut butter, are commonly run on the same production line, allowing contamination of subsequent products (5). Ice cream containing nuts may be sieved to remove the nuts so that the base can be used for another flavor of ice cream. This policy may result in unsuspected contamination with nut allergen (8). Food industry experts now recommend that companies put "like into like" when reusing materials. Although large ice cream manufacturers heed this advice, small ice cream companies may not.

Other potential sources of cross-contact may occur in the grocery store. Bulk food bins may be used for a variety of products with little or no cleaning of the bins in between each change over, and shoppers may inadvertently transfer a scoop from one bin to another (5). Cheese is often sliced on the same equipment as deli meats, making cross-contact possible (5). It is common practice to place various types of donuts, croissants, and muffins together in display cases, where they are likely to come into contact with one another or where the same serving tool is used for all.

Avoiding these types of high-risk foods will help minimize the patient's chances of suffering an accidental ingestion of the food to which they are allergic.

Cooking

Families must learn how to adapt recipes and make appropriate allergy-safe substitutions. Often the entire family elects to follow the restricted diet so that home can be a safe place for the child. This also minimizes the amount of cooking needed and the chances for cross-contact between allergen-containing and allergen-free foods.

Some families choose to bring the allergen into the home and use it as an opportunity to role-play situations that the child may encounter in avoiding the allergen away from home. These families often have designated areas of the pantry and refrigerator for the allergen-free foods, some place stickers on all food: green stickers to indicate "safe" foods and red stickers symbolizing "unsafe" foods (9). This strategy helps the child and other family members avoid unsafe foods. Other families have used colored dishes, spoons, and glasses for the allergic child, thus keeping food allergy top-of-mind at all times.

If the allergen is present in the home, extra care should be taken during cooking. The allergen-free meal should be prepared first, covered, and removed from the cooking area to be sure it is not accidentally contaminated with the allergen. One mother reported causing a reaction to her son who was allergic to milk after mistakenly using the same serving spoon for his food after using it to serve cheese-containing food to the rest of the family.

Keeping an extra supply of "safe" foods ready ensures that there is always something available for the allergic child, especially on harried days or when a babysitter or other family member takes over the cooking responsibility.

There is no one way to manage food allergies; each family must decide what strategies work best for them. Their decisions will have to be revisited as the child ages and takes more control of his or her food allergy management.

Psychosocial Impact

The constant vigilance required to avoid a reaction can be a source of stress to the family. In a study of the impact of food allergy on the quality of life, Sicherer (10) reported that "childhood food allergy had a significant impact on general health perception, emotional impact on the parent, and limitation on family activities."

Stress on the family may come from a number of sources. Families may have to work around

family members or friends who do not believe food allergies are real and cause a reaction to the child when they slip some of the restricted food to the child in an attempt to "prove" their theory to the child's parents.

In the school setting, children with food allergies can be the targets of class bullies. Some have had reactions as a result of this bullying. In one case a child who was allergic to milk was sprayed with milk and suffered an allergic reaction. Schools have a responsibility to keep all children safe and to hold those who harass or tease others accountable.

Sometimes, a family that has adjusted to living with food allergy may experience a setback when their child has a reaction. If the parent served the food that caused the reaction, the parent may experience guilt or loss of confidence in their ability to care for their child. Children who have suffered a severe reaction sometimes develop disordered eating. Some eat only one or two foods for long periods of time after a reaction. Others become withdrawn and extremely fearful, not trusting anyone else to read the ingredient label on their behalf. It is not uncommon for these children to experience panic attacks. Their siblings may also express anxiety, fearing that their brother or sister will die; some become jealous of the attention the parents give to the "at risk" child.

Mothers of young children who have been diagnosed with food allergies have a unique set of stressors. Frequently they report feeling guilty for causing their child's food allergies, particularly children that have been breastfed. These feelings are more intense in families where the mother reports eating peanuts or tree nuts while pregnant or nursing and the child subsequently develops a peanut or tree nut allergy. Mothers also express remorse for the pain their child may have suffered before a diagnosis was made.

Parents need to know how serious a reaction could be and what they should to if one were to occur. However, statements such as, "This child is so allergic he won't be safe in school and must be home schooled" or "This is the worst case I've ever seen" create an atmosphere of fear and dread. Some parents become so fearful they cannot function; they home school their child and minimize contact with others in an effort to avoid a possible deadly reaction.

Messages that empower the parents ultimately benefit the child. The family must work to find a balance for their child between caution and living a normal life. Knowing that there are thousands of students with food allergies in the school systems across the country who participate in class activities, team sports, go to camp, attend sleepovers, etc., shows parents that food allergies are manageable and they needn't restrict their child's social activities. Allowing the child to be part of the decision making for food allergy management builds confidence in the child and prepares the child to successfully manage his or her food allergies later on in life.

It is clear that food allergies affect the entire family. The psychological impact on the family can be intense, will change according to family events, and will differ between the parents, siblings, and the child who is allergic.

A follow-up visit with a physician and a registered dietitian a month or so after diagnosis and after severe allergic reactions may provide families the opportunity to ask questions and get information for handling situations that may have come up since the diagnosis. Parents who find their fears are impeding their day-to-day activities or whose children are showing signs of acute stress should be encouraged to speak with a professional counselor.

Teens and Young Adults: High-Risk Patients

Studies of fatal food-induced anaphylactic reactions have shown that high-risk patients include adolescents and young adults with food allergy (particularly peanut or tree nut allergy), and asthma. One study of 32 fatalities reported, 17 (54%) were in the 10- to 19-year old age range. Individuals in this age group pose unique challenges because they spend more time away from home in the company of their friends, often don't carry their EpiPen, try to treat anaphylaxis with asthma inhalers, and tend to go off alone when a reaction occurs.

In one tragic fatal anaphylaxis case, the teen went to the restroom alone and was discovered some time later by his friends. He was found clutching his asthma inhaler. In another case, the teen collapsed in front of her friends, who stood by and watched not knowing what they could do to help.

These tragedies point to some critical lessons for us: adolescents and young adults should be given specific information for managing their food allergies in a variety of new situations, we must reinforce that epinephrine is the medication of

choice for handling a severe reaction, and that reactions are never planned. They should be encouraged to tell their friends what do to if a reaction occurs. FAAN's Be A PAL: Protect A Life from Food Allergies program and the *Friends Helping Friends* video are designed to simplify this educational task. There are several EpiPen carriers on the market, which will make it easier for teens, especially boys, to carry their EpiPen. Pictures of these carriers appear on FAAN's website (www.foodallergy.org).

FAAN's *Stories from the Heart: A Collection of Essays from Teens With Food Allergies* is a good resource for teaching teens that their concerns are universal, and they can learn from what others have done to balance their food allergies and active social calendar.

Management of Food Allergy at School

Reactions in schools are commonly caused by foods used in school projects or class celebrations. Lack of written emergency action plans and insufficient staff training in recognition of symptoms have been attributed to a delay in treatment in some cases (11).

How a child's food allergies are managed in the child's school is to be determined by the student's parents and the school staff. There is no one way to manage a student's food allergies. Each child's needs are different; therefore, schools must customize their plans and change them as the child ages. For younger students, some schools elect to designate a "peanut- or milk-free" table in the cafeteria; others allow the children to eat in the library or another room outside the cafeteria with a few friends. Some schools require that foods sent in for class celebrations must be commercially prepared and contain preprinted ingredient statements, and others request that the student's parents send in "safe" snacks for their child, or they provide non-food treats (pencils, stickers, etc.) in lieu of food.

In older students, reactions have been caused by food exchanged with well-meaning friends who believe the food to be safe. Schools have implemented a "no food trading" policy to prevent these types of reactions.

Children with food allergies meet the criteria of being disabled according to Public Law 93–112, The Rehabilitation Act of 1973, Section 504 (12). Such children are eligible for modifications in the school program even if they do not require special education. Modifications may include health services, such as administration of medication at school, and supplementary aids, such as menu information and ingredient substitution in school meal programs. These substitutions must be provided at no additional cost to the student's parents (12). In addition, schools cannot require parents to sign a liability waiver or come to the school themselves to administer emergency medications (13). Schools cannot legally manage food allergies by preventing students with food allergies from participating in field trips, class projects, or other school activities.

School staff should work in partnership with the child's parents, receive written information from the child's physician, and develop a plan of action for handling a reaction. Special care must be taken to provide appropriate education for school personnel (14–16). Often school staff calls the patient's parent before administering medication, or they rely on asthma inhalers for treating an anaphylactic reaction. Both should be avoided.

A written Food Allergy Action plan should be placed on file at the school for each student with a true food allergy (Fig. 31–1) (13). The school must keep epinephrine available for a child; be willing and able to administer it; and call the rescue squad, even if a school nurse is present on the premises (12, 17). National guidelines describing the school staff, parent, and student's responsibility are included in FAAN's School Food Allergy Program that contains a binder with information and a video about managing food allergy at school. Parents should be given instructions for follow-up with their primary care physician or specialist after a reaction occurs, and they should be reminded to refill medications after a reaction.

Eating Away From Home

When food is consumed that is not personally prepared and served in one's home, the risk of encountering a hidden allergen increases. As an example, a peanut-sensitive teenager made her own jam sandwich while on a camping trip (18). She was not aware that the knife had been used earlier to spread peanut butter and had been wiped but not washed. She died minutes after eating the sandwich. Another individual suffered a reaction after eating ice cream that should not have contained nuts. It was later discovered that the wait staff mistakenly put the wrong flavor ice cream on the child's ice cream cone.

Food Allergy Action Plan

Place Child's Picture Here

ALLERGY TO:_____

Student's Name:_____ D.O.B:_____ Teacher:_____

Asthmatic Yes* ☐ No ☐ *High risk for severe reaction

◆ SIGNS OF AN ALLERGIC REACTION ◆

Systems:	**Symptoms:**
•**MOUTH**	itching & swelling of the lips, tongue, or mouth
•**THROAT***	itching and/or a sense of tightness in the throat, hoarseness, and hacking cough
•**SKIN**	hives, itchy rash, and/or swelling about the face or extremities
•**GUT**	nausea, abdominal cramps, vomiting, and/or diarrhea
•**LUNG***	shortness of breath, repetitive coughing, and/or wheezing
•**HEART***	"thready" pulse, "passing-out"

The severity of symptoms can quickly change. *All above symptoms can potentially progress to a life-threatening situation.

◆ ACTION FOR MINOR REACTION ◆

1. If **only symptom(s)** are:_____, give_____
medication/dose/route
_____

Then call:

2. Mother_____, Father _____, or emergency contacts.
3. Dr. _____ at _____

If condition does not improve within 10 minutes, follow steps for Major Reaction below.

◆ ACTION FOR MAJOR REACTION ◆

1. **If ingestion is suspected and/or symptom(s) are:**_____,

give_____IMMEDIATELY!
medication/dose/route

Then call:

2. Rescue Squad (ask for advanced life support)
3. Mother_____, Father _____, or emergency contacts.
4. Dr. _____ at _____

DO NOT HESITATE TO CALL RESCUE SQUAD!

A Parent's Signature_____Date____ Doctor's Signature_____ Date____

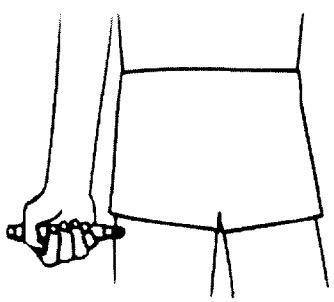
Figure 31–1. Continues.

EMERGENCY CONTACTS	TRAINED STAFF MEMBERS
1. _____ Relation: _____ Phone: _____ 2. _____ Relation: _____ Phone: _____ 3. _____ Relation: _____ Phone: _____	1. _____ Room _____ 2. _____ Room _____ 3. _____ Room _____

EPIPEN® AND EPIPEN® JR. DIRECTIONS

1. Pull off gray activation cap.

2. Hold black tip near outer thigh (always apply to thigh).



3. Swing and jab firmly into outer thigh until Auto-Injector mechanism functions. Hold in place and count to 10. The EpiPen® unit should then be removed and taken with you to the Emergency Room. Massage the injection area for 10 seconds.

For children with multiple food allergies, use one form for each food.

B

The Food Allergy & Anaphylaxis Network

Figure 31–1. Continued.

Food-allergic patients must be on heightened alert when dining away from home. Common ingredients can appear in unexpected places, for example, eggs in meat loaf or peanut butter in enchilada sauce. Convincing the wait staff that food allergies are real, and that it is critical that they give accurate information about ingredients, are just some of the obstacles patients must be prepared to address. From the restaurateur's perspective, high staff turnover and part-time staff make training or standardization of food allergy policies difficult to implement. When dining in a restaurant, patients should address food allergy queries to the restaurant manager. The manager is often more seasoned and less distracted than harried wait staff, increasing the chances that the patient will receive accurate information.

Furlong et al reported that reactions in restaurants were caused by a number of factors: the food-allergic individual not telling the wait staff about the food allergy; cross-contact between foods (primarily from shared ice cream equipment, from cooking surfaces, and serving utensils); and establishment error (e.g., switching ingredients and not notifying the wait staff) (19). Half of the reactions were caused by allergens in unexpected places, for example, in sauces, dressings, or in egg rolls. Desserts accounted for 43% of the reactions, followed by entrees (35%), appetizers (13%), and other (9%).

Individuals who are allergic to peanuts or tree nuts should not eat in Chinese, Thai, Indian, or other Asian-type restaurants, because these ingredients are commonly used and cross-contact between foods during meal preparation and cooking is likely. Peanut-allergic individuals have reported reactions after eating Mexican food. These restaurants are now using peanut butter in some dishes, for example in enchilada sauce. Patients should exercise caution when eating these types of foods.

Any patient who is allergic to fish or shellfish should be advised to avoid eating at seafood restaurants even if they order a non-fish meal, because the oil, grill, and other cooking areas are likely to contain small amounts of fish or shellfish protein that could come into contact with the fish-free meal. Some individuals are so sensitive to a food that simply breathing the aerosolized protein in steam can cause a severe or even fatal reaction. A child who was allergic to beans reportedly died after inhaling fumes released from a pressure cooker filled with garbanzo beans (20), and a shrimp-sensitive woman is said to have suffered fatal anaphylaxis minutes after a waiter in a restaurant walked past her carrying a sizzling shrimp dish (21).

Buffet-style service offers another potentially high risk for cross-contact, because the food is often placed in serving dishes that are close to each other, and small amounts of one food may fall into another serving dish. Diners often dip one spoon into several dishes, causing cross-contact. Finally, dishes and their ingredients are rarely identified. One woman learned after she had a reaction that the food she ate contained walnuts.

Avoidance of high-risk foods, such as sauces, desserts, foods prepared in a pastry covering, combination foods (such as stews), and fried foods, may help patients avoid a reaction. For example, almonds may be used in dressings for chicken entrees, in sauces used on fresh fruit, and in baked goods (5). Eggs may be used to create foam for milk toppings on specialty coffee drinks, as a binder in meatballs or meatloaf, and as a glaze on baked goods (5). Peanut butter is used to thicken chili, Mexican salsa, spaghetti sauce, hot chocolate, and brown gravy (5). It can also be used as the "glue" to hold egg rolls and Rice Krispies treats together, to add crunch and texture to piecrusts and cheesecakes, and to add flavor to brownies.

It is a common industry policy for restaurants to cook several types of foods in the same deep fat fryer. This can pose a risk to the allergic individual who has no way of knowing what other foods were fried in that cooking oil. In one case, an individual with a fish allergy reacted to french fries that had been cooked in the same oil as the fish.

Nuts and other toppings are often accidentally dropped into containers of ice cream. Furthermore, the scoopers for the various flavors are often placed in a common tub of water, which may contain protein from all of the different flavors.

In spite of their precautions, however, mistakes can occur in the kitchen during meal preparation, as well. Several reactions have occurred after the kitchen staff simply removed the allergen rather than making a new dish. To avoid this risk, if a food-allergic individual is served an allergen-containing dish at a restaurant (a cheeseburger instead of a plain burger), the individual should keep the original dish at their table to ensure that a new dish is prepared.

While eating in a fast serve or fast food restaurant, it is not prudent to assume that what is safe in one restaurant will necessarily be safe in another. Although food preparation at chain restaurants is usually standardized, regional differences may exist in products served or ingredients used

(22). Franchise owners may not follow corporate policy regarding separation of various foods during cooking and preparation.

When eating in restaurants, individuals with food allergies should always identify themselves to the wait staff and manager, ask questions about ingredients used, cooking methods (for example, is the grill greased with butter?), and ask for advice on selecting menu items. Patients should order simply prepared foods with as few ingredients as possible, for example, a baked potato without the toppings.

To discreetly and consistently convey information to the restaurant staff, some teens and young adults prefer to use a "chef card" (Fig. 31–2). These personalized cards usually include the list of synonyms for the allergen, a caution about food preparation, and the symptoms of a reaction (to convey the seriousness of the food allergy). Some use a brightly colored laminated card; others have business cards printed with this information.

As a rule, if the patient has any doubt that his or her questions and concerns are being taken seriously, the individual should eat elsewhere. A peanut-sensitive teenager died after eating an egg roll at an Asian restaurant (23). He apparently had asked the waiter if any of the food was cooked in peanut oil, and was assured that the restaurant did not use any peanut oil. He may not have inquired about the use of peanut butter, which the restaurant used in its egg rolls.

Special Occasions

Preparation, planning ahead, and minimizing risks are the key ingredients for success during special occasions such as birthday parties, family gatherings, vacations, and air travel.

Before attending a birthday party or visiting a relative's house, the hostess should be alerted of the food allergy. Some families prefer to bring their own "safe" food for their peace of mind. For vacations, many rent condominiums or cottages with kitchens so they can prepare the child's foods themselves. Those who choose this option often bring food with them or ship staples such as bread and cereals to their vacation destination. For sleep-away camps the options may include providing the child's food or reviewing the menu to determine what foods the child can eat. Careful attention should be given to camp activities that will require the children to be in remote areas to be sure emergency medical services are available if needed.

Regarding air travel, the best policy is to avoid eating any food served by the airline, as ingredient lists are not usually available and the meals are prepared in large warehouses with many opportunities for mistakes or cross-contact. Some families of children with peanut allergy request peanut-free flights. No airline can guarantee a peanut-free flight. There may be peanut ingredients in meals; other passengers may carry peanuts on the plane with them. Some airlines will serve a non-peanut

Sample Chef Card

To the Chef:

WARNING! I am allergic to peanuts. In order to avoid a life-threatening reaction, I must avoid the following ingredients:

artificial nuts
beer nuts
cold pressed, expelled, or extruded peanut oil
ground nuts
mandelonas
mixed nuts
monkey nuts
nut pieces
peanut
peanut butter
peanut flour

Please ensure any utensils and equipment used to prepare my meal, as well as prep surfaces, are thoroughly cleaned prior to use. Thanks for your cooperation.

Figure 31–2.

snack upon request; others make no such accommodations. Families need to check with the airline when booking their flights, confirm the arrangements before the trip, and keep in mind that airlines change their policy without warning. As a precaution, all families should keep their child's medications stored in a carry-on bag and be prepared to treat a reaction should one occur.

When traveling outside the US, other problems may arise. In some parts of Europe, for example, product labels do not have to list all ingredients and emergency services differ from country to country. FAAN's booklet entitled *Travel Guide: Tips for Traveling with Food Allergy* includes information and advice for managing meals while traveling (24).

Treatment of a Food-Allergic Reaction

Because ingestion of a food allergen can occur even with stringent avoidance measures, and time is a critical factor in successful treatment of a reaction, a treatment protocol must be immediately available in case of inadvertent ingestion of the offending allergen. FAAN's booklet *Just One Little Bite Can Hurt* and video "It Only Takes One Bite" are references that can be recommended to patients to raise their own awareness, and that of their families, friends, and teachers, to the potential severity of food allergy (20).

Treatment of a Mild Reaction

No clear clinical distinction is generally drawn in food allergy between mild and severe reactions analogous to the classification of generalized reactions to insect stings in children (25, 26). For the purposes of this discussion, a mild reaction is considered to be urticaria only with no other systemic symptoms appearing in a patient who is not at high risk for serious anaphylaxis, i.e., has never had a severe reaction before and/or does not have asthma. Alternatively, it might consist of mouth itching only in a subject who has the oral allergy syndrome (27) and no risk factors. Risk factors for serious reactions to foods include asthma (28), peanut or tree nut allergy (28–31), previous history of severe reaction to any food (32), extreme atopy (with elevated IgE and multiple positive skin tests, AD, food allergy, and asthma) (32–35), and use of β-blocking medications (33) (Table 31–3).

For an individual who presents with a mild reaction to a food and has none of the risk factors listed above, treatment may be limited to an antihistamine, preferably liquid diphenhydramine. It should be clearly understood that antihistamines possess no antianaphylactic activity and are never a substitute for epinephrine. Whether a subject who has experienced a mild allergic reaction to a food should routinely carry epinephrine remains the subject of some debate (32–35). However, all patients with IgE-mediated food allergy should be warned about the possibility of developing a more severe anaphylactic reaction. In addition, parents of young children with food allergy should be advised to contact their physician if their child develops wheezing from any cause (e.g., viral infection), because evidence of airway hyperreactivity moves the child into a higher risk group. Parents of children with mild reactions may prefer to keep epinephrine available for use, but reserve actual administration to occasions on which more severe symptoms develop. All individuals should be instructed in the signs and symptoms of anaphylaxis (Table 31–4) and warned to use epinephrine immediately and seek emergency care if significant symptoms develop (see Table 15–1). A written food allergy action plan (Fig. 31–1) clearly describing what symptoms to look for and what to do if a reaction occurs should be provided to these patients and their caregivers.

Table 31–3.
Risk Factors for a Severe Allergic Reaction to a Food

Asthma
Extreme atopy
History of anaphylaxis to any food
β-Blocker treatment
Peanut or tree nut as the allergen
Adolescent or young adult
Lack of readily available epinephrine

Treatment of Moderate to Severe Reactions

Any individual who has a history of an IgE-mediated reaction to a food, especially if it was more severe than urticaria or mouth itching only, and/or who has any of the risk factors listed above, should be considered at risk for a more serious subsequent reaction. Some of the most severe reactions may not, in fact, have urticaria associated with the symptom complex (32–36). The treatment of choice in such cases is epinephrine administered by intramuscular injection (32, 35, 37,

Table 31–4.
Signs and Symptoms of Anaphylaxis

Organ System	*Signs and Symptoms*
Skin	Flushing
	Pruritus
	Urticaria
	Angioedema
	"Goose-bumps"
Respiratory	
Upper	**Hoarseness***
	Stridor
	"Lump in throat"
Lower	**Chest tightness**
	Dyspnea
	Wheezing
Cardiovascular	Dizziness
	Syncope
	Hypotension
	Loss of consciousness
Gastrointestinal	Abdominal cramping
	Nausea and vomiting
	Diarrhea
Genitourinary	Uterine cramping
	Uterine bleeding
Neurological	Feeling of "impending doom"
	Headache

*Epinephrine should be used immediately in any patients presenting with **bolded** symptoms.

38). The point at which to administer epinephrine remains controversial. Traditionally, administration was delayed until the onset of serious symptoms, but evidence suggests that it may be a poor policy to wait for severe symptoms to develop in high-risk subjects (14, 32, 37, 39). *In any patient with a history of a severe reaction, epinephrine should be administered as soon as it is realized that the allergenic food has been ingested.*

Epinephrine is currently available in premeasured doses for patient use from only one source. The EpiPen (Dey, Napa, CA) is a unit-dose device for use in adults and children weighing 28 kg or more (40). It delivers 0.3 mg of epinephrine as 0.3 mL of a 1:1000 solution in an automatic syringe preloaded for intramuscular injection. The EpiPen Jr. is intended for smaller children; it delivers 0.15 mg epinephrine in 0.3 mL of 1:2000 dilution of epinephrine. In children weighing less than 10–15 kg, one may either administer the EpiPen Jr. or dispense a needle and syringe and vials of epinephrine for accurate dosing of smaller amounts. For children less than 10 kg, a needle, syringe, and vials of epinephrine should be prescribed. The usual dose of epinephrine is 0.01 mg per kg of body weight (mg/kg) up to a maximum of 0.3 mL, but larger doses may be well tolerated. Epinephrine kits ideally should be stored between 59°F (15°C) and 86°F (30°C).

Inhaled epinephrine delivered by metered-dose devices has been compared with injected epinephrine (41–43). Theoretically, this treatment could allow more rapid deposition of epinephrine at the site of laryngeal edema. Doses of 10–20 puffs of metered-dose inhaler-delivered epinephrine are comparable to those provided with the injection of 0.3 mL of 1:1000 epinephrine, although the duration of effect may be somewhat shorter. An extensive literature on this approach as a treatment for acute anaphylaxis does not exist (33, 44), but anecdotal reports of successful use of this treatment are known (34). However, recently Simons (45) showed that children are not able to inhale adequate amounts of epinephrine by this method.

A rapidly absorbed antihistamine (H1 antagonist) should be prescribed for all patients with IgE-mediated food allergy. Liquid diphenhydramine at 1 mg/kg up to 75 mg, is rapidly absorbed and may ameliorate some symptoms of anaphylaxis. However, antihistamines should never be considered a substitute for epinephrine in the treatment of anaphylaxis.

Corticosteroids provide no immediate effect, but are usually recommended for use early in the treatment of moderate to severe anaphylaxis in the hope that they will prevent or ameliorate a prolonged or biphasic reaction (46). Furthermore, they restore the responsiveness of β-receptors to their agonists. Patients who have severe anaphylaxis or who have received corticosteroid therapy during the previous 6 months should receive pharmacologic doses of corticosteroids (47).

Individuals who have food allergy and asthma may be at higher risk for severe allergic reactions than those without asthma and food allergy (32). Bronchodilators may be used during a reaction, but these or other asthma medications should never be used as a substitute for epinephrine.

Treatment of Extremely Severe Reactions

Life-threatening anaphylaxis from food ingestion typically involves severe compromise of the upper and lower respiratory tract, although cardiovascular reactions can develop, including dysrhythmias and shock (36, 48; see also Chapter 15). The treatment approach should be tailored to the condition of the patient (Table 31–5). If any question arises about the adequacy of cardiopulmonary function, the caregiver should administer supplemental oxygen, secure an intravenous line, and begin cardiac monitoring.

Table 31–5.
Management of Anaphylaxis

Assessment
Check airway; secure if necessary
Assess level of consciousness
Obtain vital signs
Estimate body weight
Initial Treatment
Epinephrine
Further Treatment Based on Evaluation of Clinical Condition
General
H1 antihistamines
Corticosteroids
O_2
Trendelenburg position
Respiratory symptoms
Nebulized β-agonist
Aminophylline
Nebulized epinephrine
Cardiovascular symptoms
Intravenous fluids
Colloid
Crystalloid
H1 and H2 antihistamines
Inotropic agents
Vasopressors
Glucagon
Assisted ventilation

In the hypotensive patient, a combination of H1 and H2 antihistamines may be an additional treatment strategy. Studies suggest that this combination could protect against the decrease in diastolic blood pressure linked to histamine (49, 50). For example, 1 mg/kg diphenhydramine and 4 mg/kg cimetidine could be infused slowly (51). The effectiveness of this therapy as a prophylactic agent in preventing histamine-induced hypotension is generally accepted (52). Its use in the treatment of acute anaphylaxis remains more controversial (53–55).

Respiratory Symptoms

Food allergy may provoke severe asthmatic attacks that result in death or require mechanical ventilation (28, 32). In addition to intramuscular epinephrine, the treatment approach to severe bronchospasm resembles that for any asthma attack, with the understanding that recurrent or prolonged severe obstruction may occur, necessitating observation for at least 4 hours after a satisfactory response to treatment (32). A β-agonist such as albuterol nebulized with oxygen is the basis of treatment. If upper airway edema develops, nebulized racemic epinephrine may be administered. In the face of severe bronchospasm, intravenous aminophylline may be used, although albuterol, aminophylline, and hypoxia carry some risk of additive cardiac toxicity (47). Intubation and assisted ventilation may be necessary (32, 36). Edema of the upper airway, a less common effect, may also occur (48). Intubation or cricothyroidotomy may be necessary.

Advice to Patients for Treating a Reaction

All patients with food allergy should be instructed in avoidance of the incriminated food. However, in spite of best efforts to avoid the food allergen, reactions are likely to occur from "hidden" ingredients. Vander Leek (56) reported that 50 (60%) of 83 peanut-allergic children had a total of 115 documented adverse reactions caused by accidental exposure to peanuts during follow-up.

Patients need to be taught the early warning symptoms of a reaction to a food. Even if they have previously had only mild reactions, they should be educated about all possible symptoms that may develop in more severe allergic reactions. Each food-allergic subject must maintain constant scrutiny of his/her diet. All food-allergic individuals should receive information concerning emergency medical identification systems such as MedicAlert (www.medicalert.org).

Some debate has arisen about which patients should receive a prescription for epinephrine, although all agree that subjects at risk for a severe food allergic reaction should carry epinephrine (32, 34). The medical record should include documentation of patient instruction in the identification and treatment of an allergic reaction.

The patient must be given written instructions for when and how to use the prescribed epinephrine kit, and be urged to carry epinephrine at all times and to use it early in the course of a reaction, because lack of epinephrine administration can prove catastrophic (28, 32). *Up to a third of food-allergic patients experiencing moderate to severe reactions may develop biphasic reactions.* Therefore, all patients must seek professional medical care after using epinephrine, even if symptoms appear to have resolved (32). They must remain under observation for 4–6 hours as a precaution. Patients should be warned that, although an H1 antihistamine may ease symptoms of itching and urticaria, this medication is not a substitute for epinephrine. Likewise, asthma medications should not be used in place of epinephrine.

In the US, the majority of states do not allow Emergency Medical Technical (EMT) Basics, who represent 72% of all emergency medical service personnel, to carry and administer epinephrine. The EMT Basics are usually the first to arrive in response to a 911 call. Patients should be advised to call their rescue squad ahead of time, warn them of their need for epinephrine in case of a medical emergency, and set up an acceptable safety net. In some cases, if they tell the 911 dispatcher that they need epinephrine, an Advanced Life Support (ALS) vehicle and paramedics will be sent. All ALS vehicles in the US are staffed by paramedics who can carry and administer epinephrine (57).

Prevention of Food Allergy

In addition to allergen avoidance, prevention of food allergy may include preventing sensitization to allergens by means of early allergen avoidance, as discussed further in the next chapter, administering drugs to allow ingestion of the food culprit, and altering established food sensitivity through immunological modification.

Prevention of Sensitization

Since 1936, when Grulee and Sanford (58) reported that infants who were breast-fed developed less eczema than those who received cow's milk, the idea of manipulating the infant's diet to decrease the development of allergic disease has drawn great attention. This topic has been extensively reviewed by Zeiger (59). The picture that emerges does not present a strong argument for the success of this approach (60).

Carefully performed studies in Sweden have shown that neither avoidance nor ingestion of large amounts of cow's milk or egg during the third trimester of pregnancy affect the development of atopic disease from birth to 5 years of age (61–63). Many studies have found that dietary intervention after delivery may result in a lower incidence of food allergy and AD by age 12–24 months (61, 64–69). Such dietary intervention has included modalities such as strict breast-feeding combined with avoidance of highly allergenic foods by the lactating mother, or the use of protein hydrolysate formula instead of breast-feeding and diet regulation. Zeiger and Heller (70) published an outcome study on 165 children aged 7 who were at high risk to develop atopic disease. These children had been followed since birth in a prospective randomized, controlled study of food allergen avoidance. In the prophylaxis group, the mother had avoided cow's milk, egg, and peanut in the last trimester of pregnancy and throughout lactation, and the infant had avoided cow's milk until age 1, eggs until age 2, and fish until age 3. The control infants followed standard infant feeding practices. Although a significant reduction in food allergy and AD was noted in the prophylaxis group by age 1, this effect had faded by age 2 and disappeared by age 4 (64, 71). By age 7, no difference was found in the development of food allergy or any other atopic disease in either group (70). Even in carefully designed studies, it appears that dietary manipulation might lessen the frequency of food allergy during infancy—a time when it may be quite troublesome—but it does not seem to affect the eventual outcome of food allergy and has not resulted in any decrease in the frequency of respiratory allergic diseases (64–68, 71, 72). Prophylactic feeding with soy formula has not been found to be effective in preventing the development of food allergy (59).

An American Academy of Pediatrics policy statement (73) recommends that "Infants at high risk for developing allergy, identified by a strong (biparental: parent, and sibling) family history of allergy may benefit from exclusive breastfeeding or hypoallergenic formula or possibly a partial hydrolysate formula." For these infants, solid foods should not be introduced until 6 months of age. Dairy products should not be introduced until age 1; eggs at age 2; and peanuts, tree nuts, and fish at age 3.

Drug Treatment

Although the major emphasis in preventing food allergy involves avoidance with instructions for treatment if accidental ingestion occurs, it is not always possible to avoid a food completely. It would be desirable to have a drug that could be taken either before deliberate ingestion of a food or on a regular basis to decrease reaction to an accidentally or episodically ingested food, but no such drug is available at the present time. As discussed further in Chapter 42, traditional Chinese herbal medicines may provide a means of treating patients prophylactically in certain "high-risk" situations.

Allergen Immunotherapy

Allergen immunotherapy is a time-honored treatment for allergy. In view of the severe and

persistent nature of peanut allergy, it was hoped that a trial of immunotherapy could alter the natural course of this condition. Oppenheimer et al (74) reported the results of a double-blind, placebo-controlled (DBPC) study in three subjects with anaphylactic sensitivity to peanuts that underwent rush desensitization with peanut allergen. A follow-up study of this group 1 month after rush immunotherapy revealed a 10- to 100-fold reduction in skin prick test (SPT) sensitivity and a 2- to 20-fold increase in antigen dose on DBPC challenge (75). Both rush and maintenance immunotherapies were associated with a significant frequency of generalized reactions; 23% during the build-up phase and 37% during maintenance. It was concluded that traditional immunotherapy was not feasible for the treatment of food allergy. Consequently, a number of novel immunotherapeutic strategies, including anti-IgE therapy and use of recombinant "engineered" proteins are being investigated, as discussed in Chapter 42.

A number of "alternative" therapies have been claimed to be efficacious in the treatment of food allergy, including the use of sublingual drops or intradermal injections of suspected allergens to provoke a symptom and then administer a different dose of this allergen to "neutralize" the reaction (76, 77). Several studies have evaluated this technique with negative results (78, 79), including a DBPC study conducted in 1990 (80). This study showed that the provocation-neutralization technique "lacks scientific validity" and any previously reported success "appears to be the result of suggestion and chance" (80).

Summary

A diagnosis of food allergy affects not only the patient, but also his or her family or other caregiver. Education about label reading and identification of symptoms, and a written emergency action plan, should be given to all patients with food allergies. Access to newsletters such as *Food Allergy News* (published by FAAN), warning jewelry (MedicAlert, Turlock, CA; www.medicalert.org), and careful planning are all essential in managing food allergy and allowing the patient to receive the education and emotional support necessary for managing food allergies. At present, efforts to prevent sensitization to foods or to allow the deliberate ingestion of food allergens with drug pretreatment remain at the experimental stage, as does the use of allergen immunotherapy to desensitize the food-allergic patient.

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Natural History and Prevention of Food Hypersensitivity

Robert A. Wood

Introduction

The natural history of food allergy refers to both the acquisition of allergic sensitivities and their natural course over time. Food allergy most often begins in the first 1–2 years of life with the process of sensitization by which the immune system responds to specific food proteins, most often with the development of allergen-specific immunoglobulin E (IgE). Over time, most food allergies are lost, although allergy to some foods is often long-lived. For example, whereas most milk and egg allergies are outgrown, most peanut and tree nut allergies are not. Although the natural course of food allergy is probably genetically determined in most children, specific measures may help prevent food allergy in some children. This chapter reviews the development of food allergy, the natural history of food sensitivities over time, and prospects for the prevention of food allergy in at-risk children.

When considering the natural history of food allergy, the criteria used to define food allergy must be carefully considered. Some studies report solely the rates of sensitization, and others focus on clinical reactivity to specific foods. The definition of clinical reactivity is also not consistent between studies; some rely solely on parental reports of food reactions, others utilize food challenges and other more objective evidence of true food allergy. These details are important because a history of an adverse food reaction, or even evidence of sensitization, does not necessarily mean that a patient will exhibit a clinical reaction upon exposure to that food. The specific criteria used to diagnose food allergy may therefore have a significant effect on the results of these studies, especially those used to measure the prevalence of food allergy.

Studies on the Development of Food Allergy

Most food allergy is acquired in the first 1–2 years of life. The prevalence of food allergy peaks at 5%–8% at 1 year of age, then falls progressively until late childhood, after which the prevalence remains stable at 1%–2%. In this section, studies on the development of food allergy will be reviewed.

Bock (1) prospectively followed 480 children recruited from a single pediatric practice for the development of food allergy from birth through the age of 3 years. Foods that were suspected of causing adverse reactions were eliminated from the diet and then reintroduced in either open or blinded challenges at regular intervals. Limited allergy testing was performed, so it was not possible to characterize the proportion of reactions that were IgE-mediated. Overall, 28% of the children were reported to have an adverse food reaction, and the reactions were confirmed by challenge in 8%. Eighty percent of these reactions occurred in the first year of life, and the majority of the foods could be successfully reintroduced into the diet within 1 year of the onset of the allergy.

Another prevalence study was conducted in Finland in a cohort of 866 children who were followed for the occurrence of food allergy at ages 1, 2, 3, and 6 years (2). The diagnosis of food allergy was based on a history of either rash or vomiting, and all suspected reactions were confirmed by elimination and home rechallenge. Allergy testing

was not otherwise conducted. By these criteria, the prevalence of adverse food reactions was 19% at age 1, 22% at age 2, 27% at age 3, and 8% at age 6. In order of prevalence, the foods most commonly implicated at all ages were citrus fruits, tomato, egg, strawberry, and fish.

An even larger cohort study was recently conducted in Norway (3–5). For the first part of the study, a population-based cohort of 3623 children was followed from birth until the age of 2 (3), during which parents completed questionnaires regarding adverse food reactions at 6-month intervals. The cumulative incidence of adverse food reactions was 35% by age 2, with milk being the single food item most commonly incriminated at 12%. The duration of the reactions was usually short, with approximately two thirds of the reactions resolving within 6 months of their onset.

In the second phase of the study, those children who had persistent complaints of milk or egg allergy underwent a more detailed evaluation at the age of 2–2½ years (4, 5), including skin testing and open and double-blind oral challenges. The point prevalences of cow's milk and egg allergy or intolerance at the age of 2½ years were estimated to be 1.1% and 1.6%, respectively. Most milk reactions were not IgE-mediated, and only 33% of parental reports of adverse milk reactions were confirmed, whereas most egg reactions were IgE-mediated and 56% of parental reports were confirmed.

Host and Halken (6) sought to determine the prevalence of milk allergy by prospectively following 1749 Danish children from birth through age 3. The children were carefully evaluated by history, milk elimination, oral challenge, and skin tests or radioallergosorbent tests (RAST). Milk allergy was suspected in 117 children (6.7%) and confirmed in 39 (2.2%). Of the latter, 21 had IgE-mediated allergy and the remaining 18 were classified as non-IgE-mediated. All milk allergy developed in the first year of life, and most of the allergic children were able to tolerate milk by age 3 years (56% by age 1, 77% by age 2, and 87% by age 3). All children with non-IgE-mediated allergy were tolerant by age 3 years, compared to 75% with IgE-mediated allergy. Also, of those with IgE-mediated allergy, 35% had other food allergies by age 3 and 25% had other food allergies at age 10 (7). Those children were also more likely to develop inhalant allergies over time.

Tariq and colleagues (8) followed a cohort of children for the development of peanut and tree nut sensitization through the age of 4 years. All children born on the Isle of Wight in a 1-year period were recruited and evaluated at ages 1, 2, and 4 years. Fifteen (1.2%) of the 1218 children were sensitized to peanut or tree nuts. Thirteen were sensitive to peanut and 6 had allergic reactions to peanut (0.5% of the population), and one child each had a reaction to hazelnuts and cashews.

One final study of importance followed the development of sensitization to common food allergens in a large cohort of children without clinical confirmation of food sensitivity. From a birth cohort of 4082 children in the Multicenter Allergy Study conducted in Germany, 216 were assessed for allergy by RAST at 1, 2, 3, 5, and 6 years of age (9). The overall annual incidence rates for food sensitization decreased from a peak of 10% at age 1 year to 3% at age 6. Sensitization to egg and milk were most common at all ages, followed by wheat and soy. This study also found that there was a high rate of aeroallergen sensitization in children who began with food sensitivities, especially to egg (10, 11). Remarkably, if a child had both a positive family history of allergy and an egg-specific IgE level above 2 kU_A/L at the age of 12 months, there was a 78% positive predictive value (PPV) and a 99% specificity for the development of inhalant allergen sensitivity by the age of 3 years (10).

Several points are worth emphasizing from these studies. First, suspected food allergy is extraordinarily common in early childhood, with at least one fourth of all parents reporting one or more adverse food reactions. Second, adverse food reactions can be confirmed in 5%–10% of young children with a peak prevalence at around 1 year of age. Third, most food allergy is lost over time. And finally, children who begin with one food allergy, especially if it is IgE-mediated, have a very high chance of developing additional food allergies, as well as inhalant allergies. It is therefore critical that children with food allergy be identified as early as possible, both to initiate an appropriate diet for their existing allergies and to institute preventative measures that may help to reduce their chance of developing additional food allergies, asthma, and allergic rhinitis.

Studies on the Loss of Food Allergy

Most food allergy is lost over time. The process of outgrowing food allergies, by which a patient becomes completely tolerant to a food that had previously caused a reaction, varies a great deal for different foods and among individual pa-

tients. Outgrowing a food allergy may be helped by strict avoidance of the offending food, in that repeated exposures to even small quantities may delay the development of tolerance in some patients.

In the Bock study (1), almost all adverse food reactions had been lost by the age of 3 years. Among these, 11 children with confirmed milk allergy and 14 children with probable milk allergy were able to tolerate milk by the age of 3. The median duration of adverse reactions to milk was, in fact, only 9 months. In a second study by Bock, nine children who had had severe reactions to milk, egg, and/or soy at 2–15 months of age were followed for 3–9 years (12). Over time, three of the nine children were able to fully tolerate the offending food, four could tolerate small amounts, and two continued to have reactions with small exposures.

Dannaeus and Inganas (13) followed 82 children between the ages of 6 months and 14 years who had a variety food allergies for a period of 2–5 years. Of the 12 children who were allergic to milk, four (33%) developed complete tolerance, seven (58%) had reduced sensitivity, and only one (8.3%) remained unchanged by the completion of their follow-up. Fifty-five children had egg allergy, of which 20 (36%) developed complete tolerance, 24 (44%) had reduced sensitivity, and 11 (20%) remained unchanged. The results were very different for fish and peanut and tree nut allergy, with only five (16%) of 32 patients with fish allergy developing tolerance. Of 35 patients with peanut or tree nut allergy, none developed tolerance.

Sampson and Scanlon (14) followed a group of 75 patients between the ages of 3 and 18 years with atopic dermatitis (AD) and food allergy that had been diagnosed by skin testing, RASTs, and double-blind, placebo-controlled food challenges (DBPCFC). Patients were rechallenged yearly to each of the foods that had previously elicited a positive challenge. After 1 year, 19 (25%) of the 75 had lost all food allergy, including 15 (33%) of 45 patients allergic to one food and 4 (19%) of 21 allergic to two foods. A total of 38 of 121 specific food sensitivities had been lost after 1 year. After 2 years, an additional 4 (9.1%) of 44 patients lost their food allergy, while none of the 20 patients rechallenged after 3 years had a negative challenge. The results for specific foods after 1–2 years of follow-up are represented in Table 32–1, showing that egg allergy had been lost in 24%, milk in 19%, soy in 50%, wheat in 33%, and peanut in 20%. In a similar study by Sampson, follow-up data was provided on 40 of 113 patients with food allergy and AD 1–2 years after their original diagnosis (15). In that study, egg allergy had been outgrown in 14 (70%) of 20 patients, compared to 4 (57%) of 7 with milk allergy, 1 (25%) of 4 with wheat allergy, and 2 (67%) of 3 with soy allergy.

Table 32–1.
The Persistence or Loss of Specific Food Sensitivities Over 1–2 Years in Children with AD

Allergen	*Total*	*Positive Challenge*	*Negative Challenge*
Egg	59	45 (76%)	14 (24%)
Milk	21	17 (81%)	4 (19%)
Soy	10	5 (50%)	5 (50%)
Wheat	6	4 (67%)	2 (33%)
Peanut	10	8 (80%)	2 (20%)
Other	15	3 (33%)	10 (66%)

From (14).

Milk Allergy

The natural history of milk allergy has been most extensively studied (16–25). However, as summarized in Table 32–2, the results of these studies do not provide a completely clear and consistent picture.

Dannaeus and Johansson (17) followed 47 infants with milk allergy for 6 months to 4 years. In children with immediate-type, IgE-mediated reactions, 14 (29%) developed complete tolerance to milk over the course of the study, compared to 35 (74%) of those with delayed-type, non-IgE-mediated reactions. The trend for non-IgE-mediated milk allergy to be outgrown more quickly than IgE-mediated allergy has been demonstrated in most studies, including the study by Host and Halken (6), in which the vast majority of all children were milk-tolerant by age 3.

Several studies on milk allergy have been published by Hill and colleagues (18–21). In their first natural history study (18), 47 children 3–66 months of age with challenge-confirmed milk allergy were followed for a median of 16 months (range 6–39 months). Overall, 38% of the children were able to tolerate milk by the completion of the study. When the children were divided into groups based on having immediate, intermediate, or late milk reactions, tolerance occurred in 40%, 42%, and 25%, respectively. Milk-specific IgE, IgA, IgM, and IgG levels were measured and no specific immunological changes were clearly associated with the development of milk tolerance.

In the second study from this group, a cohort of 100 children with challenge-confirmed milk al-

Table 32–2.
Studies on the Natural History of Milk Allergy

Author (Reference)	*N*	*Age at Diagnosis*	*Duration of Follow-Up*	*Percent Tolerant at Completion of Study*	
				IgE-Mediated (or Immediate-Type) Reactions	*Non-IgE- Mediated (or Delayed-Type) Reactions*
Dannaeus (17)	47	14 days to 20 months	6 months to 4 years (mean 28 months)	29%	74%
Host (6)	39	0–12 months	To age 3 years	76%	100%
Hill (18)	47	3–66 months	6–39 months (mean 16 months)	40%	38%
Bishop (20)	100	1–98 months (mean 16 months)	5 years	67%	86%*
Hill (21)	98	4–100 months (median 24 months)	6–73 months (median 24 months)	22%	59%
James (23)	29	3–14 years (median 3 years)	3 years	38%	NA

* Combines immediate and late reactors.

lergy were followed for 5 years (20). Overall, milk tolerance had occurred in 28% of patients by age 2 years, 56 (56%) by age 4 years, and 78 (78%) by age 6 years. When the children were divided into groups on the basis of immediate, intermediate, or late reactions, tolerance had occurred by the completion of the study in 67%, 87%, and 83%, respectively. Adverse reactions to other foods were also common in this cohort, occurring to egg in 58 (58%), soy in 47 (47%), and peanut in 34 (34%). Most children also developed one or more other atopic disease; at the completion of the study 40% had asthma, 43% had allergic rhinitis, and 21% had eczema.

Another study from this group followed 98 children with milk allergy for a median of 2 years (range 6–72 months) (21). In this study, the children were divided into two groups; 69 had IgE antibodies to milk with immediate-type reactions, and 29 had delayed-type reactions. Over the period of follow-up, 15 (22%) of 69 with IgE-mediated disease developed tolerance, compared to 17 (59%) of the 29 with non-IgE-mediated reactions. For those children with IgE-mediated milk sensitivity, the development of tolerance was associated with lower milk-specific IgE levels at the time of diagnosis and at study completion, as well as a significant reduction in their milk skin test reactivity. However, it is also important to note that eight of the 15 who developed tolerance still had strongly positive skin tests at that time.

Three additional studies focused specifically on the immunological changes associated with the development of milk tolerance. From a group of 80 milk-allergic children, James and Sampson (23) reported on a subset of 29 who were followed for a minimum of 3 years. Evaluations included annual DBPCFCs, skin tests, and measurement of casein-specific and β-lactoglobulin specific IgE, IgG, IgG1, and IgG4 antibody concentrations. All children had specific IgE to milk as well as positive skin tests, and 80% had AD. The median age at the time of study entry was 3 years with a range from 1 month to 11 years. Of the 29 children, 11 (38%) developed tolerance at a median age of 7 years. In those who became tolerant to milk, specific IgE and IgE:IgG ratios to both milk proteins were lower initially and decreased significantly over time.

Two detailed studies on antibody responses to milk proteins and the development of milk tolerance were reported by Chatchatee et al (24, 25). In the first study, IgE- and IgG-binding epitopes on α_{s1}-casein were identified using the sera of 24 milk-allergic children, and the patterns of epitope recognition were analyzed to determine if they might help predict the natural history of milk allergy. When comparing epitope recognition of patients with persistent milk allergy to that of younger children likely to outgrow their allergy, they found that two IgE binding regions were recognized by all of the older children with persistent milk allergy but none of the younger children. In the second study, a similar analysis was performed of IgE- and IgG-binding epitopes on β- and κ-casein in milk-allergic patients. Three IgE binding regions on β-casein and six on κ-casein were recognized by the majority of patients in the older age group but none of the younger patients. In addition to a clearer definition of the antibody responses to specific milk proteins or epitopes, these studies suggest that it may eventually be possible

to develop clinical tests—in essence, epitope-specific RASTs—that could identify children at risk for more persistent milk allergy.

A summary of studies on the natural history of milk allergy is presented in Table 32–2. As one examines this information, a confusing picture emerges. For example, in the study by Host and Halken (6), which, in many ways, is the best study on milk allergy yet performed, 76% of those with IgE-mediated milk allergy and 100% of those with non-IgE-mediated milk allergy were milk-tolerant by the age of 3 years. These numbers are far higher than those presented in the other studies. The only numbers that approach those are from the study by Bishop et al (20), although it took until age 6 for 78% of those children to become milk-tolerant. The differences in these studies are almost certainly a result of selection biases. The study by Host and Halken was population-based, so it would therefore include all degrees of milk sensitivity, whereas the other studies included children who were under the care of an allergy specialist, indicating that they may have had a more severe form of milk allergy. Thus, for the primary care physician it is likely that the more optimistic numbers will be correct, whereas the allergist might expect a slower rate of loss of milk allergy in their patients over time and a higher percentage of patients with persistent milk allergy.

Egg Allergy

Only one study has specifically focused on the natural history of egg allergy. Ford and Taylor (26) followed 25 children from 7 months to 9 years of age (median 17 months) with challenge-confirmed egg allergy for 2–2½ years. Egg allergy resolved in 11 (44%) of 25 and persisted in the other 14. Skin tests were negative or diminished in size in those who lost their egg reactivity, compared to those with ongoing reactivity. This result is similar to that in the 36% of children in the Dannaeus study (13) who became egg-tolerant, although they also reported that an additional 44% had become less sensitive over time. Those data would agree with the clinical observation that the vast majority of egg allergy is outgrown by the school-age years.

Peanut Allergy

Until recently, the dogma had been that peanut allergy is rarely, if ever, outgrown, and studies had in fact suggested that that was the case. For example, Bock and Atkins (27) followed 32 children 1–14 years of age with challenge-confirmed peanut allergy over a period of 2–14 years, and found that 24 (75%) had had accidental peanut exposures and reactions and no patients appeared to outgrow their allergy.

However, evidence that a subset of children with peanut allergy may lose their sensitivity was first reported by Hourihane et al (28). They evaluated 230 children with a diagnosis of peanut allergy and performed oral challenges in 120. A total of 22 children between the ages of 2 and 9 years had a negative challenge, equaling 18% of those challenged or 9.8% of the total group. They found that a negative challenge was associated with a smaller skin test size and fewer allergies to other foods compared to those with persistent peanut allergy.

Spergel et al (29) retrospectively reviewed 293 patients with a diagnosis of peanut allergy. All families were offered a peanut challenge to confirm the diagnosis, and a total of 33 children between the ages of 18 months and 8 years with a convincing history of peanut allergy and a positive skin test were actually challenged. Of those, 14 passed their challenge and were felt to have resolved their peanut allergy. None of the five patients with a history of peanut anaphylaxis developed tolerance, compared to 9 (53%) of 17 with a history of urticaria and 4 (40%) of 10 with a history of AD. In addition, those developing tolerance had significantly smaller skin test responses than the 19 with a positive challenge.

Skolnick et al (30) performed a detailed evaluation of 223 children with a diagnosis of peanut allergy, including an oral peanut challenge in those who had not had a reaction in the past year and who had a peanut-specific IgE (PN-IgE) < 20 kU_A/L. As shown in Table 32–3, 97 children were not challenged because they were considered to still be peanut-allergic based on either a history of a recent reaction or a PN-IgE level > 20 kU_A/L (31), and an additional 41 children were eligible to be challenged but declined. Of the 85 children who were challenged, 48 (21% of the total group) passed the challenge and were felt to have outgrown their peanut allergy. The PN-IgE level as measured by RAST was the best predictor of a negative challenge, with 61% of those with a PN-IgE level < 5 kU_A/L and 67% with a level < 2 kU_A/L passing their challenge. The presence of other atopic diseases and the severity of initial peanut reactions did not predict the chance of losing peanut allergy, and even one patient who had

Table 32–3.
Characteristics of Patients with Persistent and Resolved Peanut Allergy

	Passed Challenge (N = 48)	*Failed Challenge (N = 37)*	*Unable to be Challenged (N = 97)*	*Refused Challenge (N = 41)*	*Total (N = 223)*
Age at diagnosis					
Range	8 months to 12 years	6 months to 4 years	2 months to 10 yr	8 months to 15 years	2 months to 15 years
Median (years)	1.5	1.5	1.5	2	1.5
Current age (years)					
Range	4–17.5	4–13	4–20	4–16.5	4–20
Median	6	6.5	7	7	6.5
PN-IgE at diagnosis*					
Range	< 0.35–52.9	1.8–24.4	4.5–> 100	0.64–>100	< 0.35–>100
Median	2.2	2.91	>100	6.27	19.8
Current PN-IgE*					
Range	< 0.35–20.4	< 0.35–18.2	16.8–> 100	< 0.35–16.9	< 0.35–> 100
Median	0.69	2.06	> 100	4.98	10.7

*PN-IgE, peanut specific IgE level in kU_A/L. A level < 0.35 is considered negative, and any level over 100 is reported as > 100.
From (30).

had severe anaphylaxis with his initial reaction outgrew his allergy.

A final study on the natural history of peanut allergy was reported by Vander Leek et al (32). Eighty-five children with peanut allergy were studied, including 55 who were followed for at least 5 years. Among those patients, 31 (58%) of 55 who had been followed for 5 years and nine (75%) of 12 who had been followed for at least 10 years had had at least one reaction due to an accidental exposure. In addition, the majority of these reactions were more severe than initial reactions, and 31 (52%) of 60 included potentially life-threatening symptoms. Severe reactions were associated with higher PN-IgE levels compared to those with purely cutaneous reactions. The only positive note from this study was that four children did outgrow their peanut allergy.

Peanut allergy is therefore likely to be lifelong for most but not all patients. Because a substantial minority of patients do appear to lose their sensitivity over time, it is appropriate to reevaluate children with peanut allergy on a regular basis. Those patients who have not had reactions in the past 1–2 years and who have a low PN-IgE level should be considered for an oral challenge in a supervised setting. If a patient is still peanut-allergic by late childhood or adolescence, it is very unlikely that they will subsequently lose their allergy, and regular retesting may no longer be warranted.

Other Foods

Far less has been published about the natural history of other food allergies. Among the other most common food allergens, it is clinically recognized that soy and wheat allergy are typically outgrown in the preschool age years, but no large studies have focused on the natural course of these food allergies. In the studies by Sampson of children with food allergy and AD (14, 15), soy allergy was outgrown in 50% and 67% of children over a 1- to 2-year follow-up, compared to 25% and 33% for wheat. The few children in the studies of Bock (1) and Host and Halken (6) who had soy and wheat allergy had lost these allergies by the age of 3. Hill et al (33) did report on 18 infants with intolerance to both soy and extensively hydrolyzed infant formulas through the age of 3. However, although they report that two children were tolerant of soy by age 3 years, the true frequency of soy tolerance could not be determined, because soy had still not been reintroduced to 13 children at the completion of the study.

As was noted above in a number of studies, adverse reactions to fruits, vegetables, and other cereal grains are typically very short-lived (1, 2, 13). Although some children do have severe, IgE-mediated allergies to these foods that may persist over time, for most children they can be successfully reintroduced into the diet within a period of 6–12 months. Many of these may, in fact, represent intolerances or irritant reactions rather than true allergy.

In contrast, although actual studies are limited, allergies to tree nuts, fish, shellfish, and seeds are usually not outgrown. The study by Dannaeus (13) did include 26 patients with tree nut allergy, none of whom lost their sensitivity in a 2–5 year follow-up, and 32 children with fish allergy, of whom 5 became tolerant. One additional study

followed 11 patients with shrimp allergy over a 2-year period and found that allergen-specific antibody levels did not change significantly over that period of time (34).

Food Allergy in Adults

Most studies on the natural course of food allergy have logically involved children. The most common food sensitivities in adults include peanut, tree nuts, fish, and shellfish, all of which are most often life-long. In fact, it is their persistent nature that makes them the most common food allergies in adults, in that most of these allergies are actually acquired in childhood and persist into adulthood.

One study, however, did focus on the natural history of food allergies in adults (35). Twenty-three adults with allergies to a variety of foods underwent baseline DBPCFCs, in which clear reactions in 10 patients to a total of 13 foods were identified. The patients were then placed on strict dietary avoidance of the offending food for 1–2 years and rechallenged. Five (38%) of the 13 previously offending foods were well tolerated, including milk in two patients, and wheat, egg, and tomato in one patient each. The two patients with nut allergy continued to react, as did two patients with milk allergy and one patient each with allergies to potato, garlic, and rice.

Follow-up of the Food-Allergic Child

It is imperative that food-allergic children undergo regular follow-up. This is necessary to monitor growth, signs and symptoms of ongoing food allergy, adherence to the recommended avoidance diet, and objective measures of food allergy. Any reactions that have occurred must be reviewed with particular attention to how the reaction might have been prevented and whether the treatment provided was appropriate.

All children with food allergy should also be re-evaluated at regular intervals to determine if the allergy has been outgrown. This typically should be done annually, although for some food allergies a shorter or longer interval might be appropriate. For example, an infant with adverse reactions to fruits or vegetables might deserve re-evaluation after 3–6 months, whereas an older child who clearly has persistent peanut or tree nut allergy may no longer need repeat testing, although regular follow-up is still important to review avoidance procedures and treatment protocols.

The re-evaluation process may include skin testing, RAST analyses, and/or oral food challenges, depending on the specific clinical scenario. It is very important to note, however, that a positive skin test or RAST does not necessarily mean that the food allergy has not been outgrown, since these tests can remain positive even when the patient is no longer clinically sensitive. CAP-RASTs (CAP-System FEIA) have increasingly become the test of choice to monitor food allergies over time and to help guide decisions about the timing of oral food challenges. In the end, a food challenge will usually be necessary to prove that an allergy has been outgrown. These must be performed with caution because severe reactions may occur even when the testing suggested that the food allergy had most likely been outgrown.

Until an allergy has been outgrown, it is recommended that a strict avoidance diet be maintained. However, while the clinical impression has been that strict avoidance increases the chance of outgrowing a food allergy and may even hasten the process, very little data supports this notion (23, 35). In addition, it is clear that some children rapidly outgrow their food allergies without strict avoidance, whereas others fail to lose their allergies even with the most stringent diets. Because strict avoidance is so difficult, it would be ideal if we could somehow identify, such as with epitope mapping, those children who might be equally likely to outgrow their food allergies with or without a strict diet. However, until we have further information on this issue, it is still likely that the majority of children with food allergy will benefit from very strict avoidance, at least to avoid symptoms, and, hopefully, to promote the outgrowing process.

Strategies for the Prevention of Food Allergy

In the future, it will likely be possible to modulate the infant's immune system to reduce or prevent the development of allergy. Currently, however, food allergen avoidance is the primary strategy available that can reduce the development of food allergy. Preventative strategies can be categorized into three stages: primary prevention, which blocks the initial process of sensitization; secondary prevention, which reduces the development of further disease in a patient who has already developed allergy; and tertiary prevention, which reduces symptoms following disease ex-

pression (36). Although the primary prevention of allergy would be ideal, it is difficult to accomplish because at-risk children need to be identified at, or even before, birth. This strategy is particularly feasible for families who already have one or more food-allergic children and may be especially motivated to reduce the odds of food allergy in subsequent offspring. Secondary and tertiary prevention are more realistic possibilities and should be included in the treatment plan for all children who have presented with food allergy, eczema, or other early signs of atopic disease.

Although it could be argued that all families should practice primary prevention, this approach is so difficult that it is typically recommended only for those at high risk of developing allergy. A number of laboratory approaches for the identification of high-risk children have been studied, including cord blood IgE levels, cytokine levels, eosinophil counts, and specific genetic markers (37). However, none of these have proven to be sufficiently superior to the family history to be recommended for use in clinical practice. The family history therefore remains the most useful and practical method to identify the allergy-prone infant, although no complete agreement exists as to the specifics of the family history that should prompt the institution of primary prevention (38, 39). It is clear, however, that even allergic disease in one parent increases the likelihood of allergy in a child, and that allergic disease in both parents or a parent and a sibling further increases those odds to between 40% and 70% (40, 41).

A number of approaches have been studied for the primary prevention of food allergy in high-risk children. Guidelines summarizing these approaches were recently presented by the American Academy of Pediatrics (AAP) (38) and by the European Society for Paediatric Allergology and Clinical Immunology (ESPACI) and European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) (39). These guidelines, which are summarized in Table 32–4, will serve as the basis for the following discussion.

Food Avoidance During Pregnancy

Although the developing fetus can mount immunological responses to food allergens (42, 43), it is not clear that exposure to food allergens in utero, or even these immunological responses, are related to the subsequent development of specific food allergies. Several studies on milk and egg avoidance in the third trimester of pregnancy have not shown any reduction in food allergy or other atopic disease (41, 44–46). These findings have been confirmed in a recent meta-analysis (47). The European guidelines do not recommend maternal food allergen avoidance during pregnancy, whereas the AAP guidelines recommend no avoidance with the possible exception of peanut. The AAP guidelines suggest that peanut be avoided on the basis of limited evidence that peanut consumption in pregnancy may be associated with an increased risk of developing peanut allergy (48).

Breast-Feeding and Maternal Avoidance of Allergenic Foods During Lactation

Both the American (38) and European (39) guidelines strongly recommend exclusive breast-feeding for the allergy-prone infant, only differing in duration (at least 6 months vs 4–6 months, re-

Table 32–4.
Summary of Recommendations for Primary Prevention of Food Allergy by the AAP and ESPACI and ESPGHAN

Parameter	*AAP 2000 (38)*	*ESPACI/ESPGHAN 1999 (39)*
Definition of high-risk infants	Both parents or parent plus sibling	Affected parent or sibling
Maternal pregnancy diet	Not recommended with possible exception of peanut	Not recommended
Exclusive breast-feeding	6 months	4–6 months
Maternal lactation diet	Eliminate peanuts and nuts (consider eliminating eggs, cow's milk, fish)	Not recommended
Avoid soy formulas	Yes	Yes
Hypoallergenic formula for bottle-fed high-risk infants	Yes: use an extensive or possibly partial hydrolysate when not breast-feeding	Yes: use formula with confirmed reduced allergenicity
Hypoallergenic formula for supplementation	Yes: use extensive or possibly partial hydrolysate	Yes: use formula with confirmed reduced allergenicity
Delayed introduction of solid foods to infant	Delay all for at least allergenic 6 months; cow's milk for 12 months; eggs for 24 months; peanuts, nuts, and fish for 36 months	Start at fifth month of life

spectively). The AAP further recommends continued breast-feeding, although not exclusively, to at least age 12 months, for all infants. In addition to the nutritional, immunological, and physiologic benefits of breast milk, data also support the potential value of breast-feeding in the prevention of food allergy and other atopic disorders (49–55). Although the results of these studies are not entirely consistent, most have shown a reduction in eczema and some have demonstrated reduced food allergy, especially when comparing breast-feeding to the use of cow's milk-based formulas. A meta-analysis (56) that reviewed 18 studies comparing breast-feeding with cow's milk formula feeding found an overall reduction in eczema with at least 3 months of exclusive breast-feeding, especially in children with a family history of atopy. In addition, studies have also demonstrated a reduction in asthma in the first 2–5 years of life with exclusive breast-feeding for at least 3 months (57–59).

Although the recommendation to breast-feed is straightforward, the more difficult question is whether the lactating mother should restrict allergenic foods from her diet. It has been shown that food allergens from the mother's diet are excreted in breast milk (60–65) and it is clear that some children do react to these allergens (66–68). Data demonstrate that some children may become sensitized to food antigens via breast milk exposure (69, 70). Studies conflict, however, as to whether maternal avoidance can reduce the development of allergic sensitization (47, 71–74). The AAP guidelines recommend that peanuts and tree nuts be eliminated from the mother's diet during lactation, and that avoidance of milk, egg, and fish be considered for particularly high-risk infants. The European guidelines, however, do not recommend any maternal avoidance during lactation.

Formula Selection for the Allergy-Prone Infant

An appropriate formula must be selected for infants who are not breast-fed or when a supplement to breast milk is needed. Both the AAP and European guidelines recommend that cow's milk and soy formulas be avoided in allergy-prone infants. Although soy is somewhat less allergenic than cow's milk (75), no evidence indicates that soy formula leads to a reduction in food allergy or eczema compared to cow's milk-based formula (76–79).

Other formula options include protein hydrolysates, defined as being either partially or extensively hydrolyzed, and elemental products. Elemental (free amino acid-based) formulas have the least allergenic potential but are not routinely recommended for prophylaxis because of their high cost. Studies have demonstrated that feeding with both partially and extensively hydrolyzed formulas can reduce the development of food allergy and eczema (79–89), although the benefit of extensively hydrolyzed formulas may be greater than that of partial hydrolysates (88, 89). Based on the available data, the AAP guidelines recommend that allergy-prone infants be fed an extensively, or possibly a partially, hydrolyzed formula, and the European guidelines recommend use of a formula with "confirmed reduced allergenicity." Both groups recommend that partial hydrolysates not be used in patients with confirmed milk allergy because of their greater allergenicity.

Introduction of Solid Foods

Data from several studies suggest that an early introduction of solid foods may lead to an increase in eczema, and possibly food hypersensitivity, in allergy-prone infants (90–93). The AAP guidelines recommend that solid food introduction be delayed until 6 months of age, compared to 5 months in the European guidelines. In addition, the AAP guidelines specify that milk be withheld through 12 months, egg through 24 months, and peanut, tree nuts, and fish through 36 months in allergy-prone infants. It should be noted that these restrictions do not just mean avoiding milk and eggs, but rather that all derivatives of these foods be avoided by carefully examining ingredient labels of all foods given to the child.

Secondary Prevention of Food Allergy

There are no controlled studies on the secondary prevention of food allergy or any other atopic disorder. However, as described above, there is a very high risk that children who present with food allergy will go on to develop additional food sensitivities as well as asthma and allergic rhinitis (7, 10, 11, 20). It is therefore logical to assume that, in children who have presented with food allergy or AD, an opportunity may exist to reduce their chances of developing additional food and inhalant allergies.

At the very least, the AAP guidelines regarding the introduction of solid foods for allergy-prone infants should be applied to infants and

young children who have already presented with food allergy or significant AD. For example, in an infant who has presented with milk allergy, the introduction of egg, peanut, tree nuts, fish, and shellfish should be delayed in an effort to prevent the development of allergies to these foods. In addition, it may be prudent to perform testing for these major food allergens before they are introduced into the diet.

Data are even less clear on preventing the development of inhalant allergies. Studies on the primary prevention of inhalant allergies suggest that the early institution of dust mite control measures may help to reduce the development of asthma and specific sensitivities to environmental allergens (94, 95). It would therefore be reasonable to advise that dust mite controls be instituted as a preventative measure in children with food allergy. In addition, given the evidence that home dampness and exposure to environmental tobacco smoke promote the development of both allergy and asthma (96–99), these exposures should be avoided. The issue of animal allergens is less clear. While some studies have shown that early cat and dog exposure increases the risk of asthma and allergy (99, 100), other studies have shown that these exposures may have a protective effect (101, 102). Further studies will be needed to fully answer this question, although from the standpoint of tertiary prevention, it is clear that once a child has become sensitized to an indoor pet, the pet is likely to do more harm than good and it is advisable to remove the pet from the home.

Tertiary Prevention of Food Allergy

As noted, tertiary prevention refers to the treatment of existing food allergy. This approach, including the following recommendations, is summarized in Table 32–5. First, the causal food should be strictly avoided, both in the child's diet and in the mother's diet if breast-feeding. Detailed educational materials on food avoidance must be provided. Second, exclusive breast-feeding should be continued, and the mother should also avoid the other highly allergenic foods as reviewed for primary and secondary prevention. Extensively hydrolyzed formulas may be used for supplementation of milk-allergic infants, although a trial on a soy formula may also be appropriate, especially in older infants and those with IgE-mediated milk allergy (103). Elemental formulas may be needed in those milk-allergic infants who are unable to tolerate soy or protein hydrolysate formulas, especially those with allergic (eosinophilic) enteropathy. Milk from other mammals such as goats and sheep should be avoided in cow's milk-allergic children due to a high rate of cross-reactivity. Introduction of new foods to the infant with food allergy should be extremely conservative as described for the primary and secondary prevention of allergy.

Table 32–5.
Strategies for the Tertiary Prevention of Food Allergy

1. Complete exclusion of causal food
   a. Provide educational materials on food avoidance.
2. Continue exclusive breast-feeding with:
   a. Maternal avoidance of the causal food.
   b. Maternal restriction of peanuts, tree nuts, fish, shellfish, and possibly cow's milk and egg (secondary prevention).
3. If not breast-fed, use a hypoallergenic formula.
4. In milk-allergic infants:
   a. Begin with an extensively hydrolyzed formula.
   b. If allergic symptoms persist, use an amino acid-based formula.
   c. A trial on a soy-based formula may be appropriate, especially for older infants with IgE-mediated milk sensitivity.
   d. Avoid partially hydrolyzed protein formulas.
   e. Avoid unmodified proteins of goat's or sheep's milk.
5. In infants with allergic enteropathy, use an extensively hydrolyzed formula or, if allergic symptoms persist, an amino acid based formula.
6. Cautiously introduce new foods.
7. Provide medications to treat food-allergic reactions and detailed instructions for their use.

In addition to allergen avoidance, patients and their families must be provided with medications to treat allergic reactions and detailed instructions for their use.

Potential Risks and Disadvantages of Allergy Prevention

Although most of the recommended measures appear benign, some potential risks and disadvantages need to be considered and weighed against the possible advantages. The first and foremost risk is malnutrition, both for the child and the breast-feeding mother. Great care must be taken to prevent this complication, including supplementation with calcium and other specific nutrients when necessary. For the infant and small child with milk allergy, it may be necessary to continue to use a formula product to ensure adequate intake of both fat and protein. The second major disadvantage is the high cost that may be incurred through both avoidance diets and the use of hypo-

allergenic formulas. The cost is so substantial that these strategies may be prohibitively expensive for some families. Finally, there is also a certain degree of anxiety and social isolation associated with the rigorous diets that are recommended. Bearing these risks in mind, however, this is information that should be provided to all at-risk patients. This is especially the case for those with a previously affected child, due to both the increased risk of atopy and the high level of motivation that these families are likely to have.

Conclusions

An understanding of the natural history and prevention of food hypersensitivity is extremely important to the management of food-allergic patients. Although the various studies on these topics are not completely consistent, trends in the data provide several clear messages. First, food allergy is very common. Second, the vast majority of food allergy has its onset in the first 1–2 years of life. Third, most food allergy is outgrown, although there are notable exceptions to this generally positive outcome. Fourth, food allergy is often the first of the atopic diseases, with most children going on to develop respiratory allergies over time. Finally, at least some food allergy can be prevented by avoiding major food antigens in infancy and early childhood.

It is also important to stress the importance of making early, accurate diagnoses of childhood food allergy. Only this will allow for the initiation of the key elements necessary for the care of the food-allergic patient, including education about avoidance diets and the development of emergency care plans for the treatment of allergic reactions. Avoidance diets are complex and require detailed education, without which the child will be at risk for accidental reactions and possibly even more persistent food allergy. In addition, measures that might help to prevent the development of additional food allergies, as well as inhalant allergies, should be initiated at the time of the initial diagnosis.

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33

Diets and Nutrition

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Over the past several decades a variety of desensitization routines and medications have been reported to be useful in the treatment of food hypersensitivity. A recent study in patients with a history of peanut-induced anaphylaxis suggested that rush immunotherapy was efficacious in diminishing the severity of the reactions to peanut following an oral food challenge (1), but the high rate of side effects associated with this form of immunotherapy make it unlikely that it will be utilized in any general clinical setting. Other claims of therapeutic modalities have not been substantiated in patients with challenge-documented food allergy utilizing appropriately controlled trials. Consequently, strict elimination of the offending food antigen remains the only proven therapy for food hypersensitivity.

Elimination of a food allergen from the diet is best accomplished by teaching patients and parents to limit the use of commercially prepared foods, to read food labels, and to watch for unsuspected sources of food antigens (e.g., peanut butter may be used in chili sauce, egg rolls, and other foods). In the US, food manufacturers are required by law to list all ingredients (even trace amounts) of the food product except incidental additives (e.g., processing aids). Ingredients are listed in rank order with those constituting the highest weight percentage first. Ingredients that constitute 2% or less of the weight of the product are not required to be listed in order of weight. In some cases, these types of ingredients are listed in generic terms such as starches, flavorings, and spices, thus forcing parents or patients to contact manufacturers to inquire about these ingredients that are not clearly described on the label. Labeling requirements vary throughout the world and are generally less stringent than those in the US. Complete elimination diets have been shown to lead to the loss of clinical reactivity to many foods (development of clinical tolerance) in about one third of children and adults after 1–2 years (2, 3).

Instituting a food elimination diet should be considered comparable to prescribing a medication, which always carries a definite risk-benefit ratio. Consequently, appropriate diagnostic measures must be undertaken before special diets are implemented. Unfortunately, broadly restricted diets have all too frequently been prescribed on the basis of history, standard allergy tests (e.g., skin tests, radioallergosorbent tests [RASTs]), or unsubstantiated tests (e.g., cytotoxic tests, food immune complexes, food-specific IgG or IgG4) and have resulted in severe malnutrition (4–6) or delayed diagnosis of severe underlying disorders (7, 8).

As specialists in the diagnosis and management of adverse food reactions, allergists and other health care professionals must recognize the enormous task and emotional burden placed on patients and families by the prescription of elimination diets. The time required to purchase groceries and prepare these special meals is drastically increased, eating at restaurants becomes difficult and in some cases impossible, and eating at friends' homes or at school often needs to be curtailed. Consequently, social isolation and eating disorders may result from implementation of food restrictions.

Although it is often helpful to think of foods in certain botanical families, there is no clinical evidence to support broad intrabotanical or intraspecies cross-reactivity (9–11). In addition, most patients are allergic to only one or two foods; wide-

spread dietary restrictions, therefore, are rarely necessary (12, 13). Every effort must be made to diagnose food allergies correctly and to educate patients about proper elimination diets. A parent's obsession with a child's food allergy is only reinforced when physicians fail to validate historical claims or laboratory studies. In extreme cases, this obsession may present as Munchausen syndrome by proxy, and should be considered a form of child abuse (14).

Basic Nutritional Requirements

Energy (kilocalories [kcal]) and protein (grams [g]) requirements of children are based on their age and weight (15) are listed in Table 33–3.

A child's rate of growth is typically used to assess the adequacy of energy and protein in their diets. A child with food hypersensitivity may have increased energy and protein requirements, necessitating more frequent evaluation of their growth. A yearly evaluation of growth is recommended for children over 1 year of age who are not experiencing any exacerbations of their allergic disease or changes in dietary restrictions. For infants or children recently diagnosed with food hypersensitivity for which dietary restrictions are being implemented, growth should be monitored every 1–3 months until the child's food hypersensitivity is under good control and normal growth established.

Vitamin and mineral requirements are based on recommendations of the Food and Nutrition Board of the National Academy of Sciences (NAS). The NAS regularly updates these requirements on the basis of current research. In 1998, the NAS released a new system of defining vitamin and mineral requirements called the Dietary Reference Intakes (DRI) (16). The DRI includes four categories of nutrient requirements: 1) Estimated Average Requirement (EAR), which is used to assess the nutrient intakes of population groups, not individuals; 2) Recommended Dietary Allowance (RDA), which is the estimate of the daily requirements for an individual; 3) Adequate Intake (AI), which is the level of nutrient intake that has supported normal growth in defined populations; these references are used when no RDA has been established; and 4) Tolerable Upper Intake Level (UL), which is the maximum daily nutrient intake that can be tolerated safely and is unlikely to cause adverse effects in most individuals. When assessing children's diets, 100% of the RDAs or AIs is considered optimal for all vitamins and minerals. Typically, supplementation is recommended when a child's intake of a nutrient is less than two thirds of the RDA or AI and attempts at increasing intake by dietary modification have been unsuccessful. The vitamin and mineral supplement should be chosen on the basis of the child's needs. Very large doses of supplements are unnecessary; no evidence supports intake in excess of the RDAs or AIs. Children consuming combinations of formulas, fortified foods, and supplements should have their vitamin and mineral intakes evaluated for potential intakes above the ULs.

Children's diets should contain a wide variety of foods, because no "perfect" foods exist. The macronutrient breakdown of a child's diet is generally 15%–20% protein, 45%–55% carbohydrates, and 30%–35% fat. About 65%–70% of a child's protein intake should be as high quality protein, i.e., animal products or complimentary proteins that provide all of the essential amino acids (15). The carbohydrate intake should emphasize complex carbohydrates because of their nutrient contribution is preferable to that of simple sugars. Fats in the diet should be varied so that the child's intake consists of an equivalent blend of saturated, monounsaturated, and polyunsaturated fats. Typically, the diet of a child who consumes animal products includes adequate amounts of saturated fats. Consequently, added fats should take the form of vegetable oils and margarines that provide mono- and polyunsaturated fats.

Assessing Nutritional Status

Strict elimination diets may cause deficiency disorders in individuals of any age. Since a growing child is the most susceptible to dietary deficiency from restricted diets, however, the emphasis of this section will be placed on assessment of the pediatric patient. Before one can ascertain whether a restricted diet has affected a child's growth, expected growth for the child must be defined. A child is generally expected to maintain or improve his or her growth rate consistent with previously established growth patterns. Table 33–1 defines normal growth rates for height and weight based on National Center for Health Statistics/ Centers for Disease Control (NCHS/CDC) statistics at the 50th percentile (17). If a child varies significantly from these growth channels, further investigation of the child's growth is warranted.

Waterlow has developed a classification system that defines both stunting and wasting (18).

Table 33–1.
Median Height and Weight Gains

	Height Expected Change		*Weight Expected Change*	
	Boy	*Girl*	*Boy*	*Girl*
0–6 mos	17.3 cm	16.0 cm	4.5 kg	4.0 kg
6–12 mos	8.3 cm	8.4 cm	2.4 kg	2.3 kg
12–18 mos	6.3 cm	6.6 cm	1.3 kg	1.3 kg
18–24 mos	5.2 cm	5.6 cm	1.1 kg	1.1 kg
24–30 mos	4.8 cm	4.3 cm	1.2 kg	1.2 kg
30 mos–3 yrs	4.5 cm	4.1 cm	1.1 kg	1.1 kg
3–3 6/12 yrs	4.2 cm	3.8 cm	1.1 kg	1.0 kg
36/12–4 yrs	3.8 cm	3.7 cm	1.0 kg	0.9 kg
4–4 6/12 yrs	3.7 cm	3.4 cm	1.0 kg	0.8 kg
46/12 yrs–5 yrs	3.3 cm	3.4 cm	1.0 kg	0.9 kg
5–5 6/12 yrs	3.2 cm	3.2 cm	1.0 kg	0.9 kg
56/12–6 yrs	3.0 cm	3.0 cm	1.0 kg	0.9 kg
6–6 6/12 yrs	2.9 cm	3.0 cm	1.0 kg	1.1 kg
66/12–7 yrs	2.7 cm	3.0 cm	1.2 kg	1.2 kg
7–7 6/12 yrs	2.7 cm	2.9 cm	1.1 kg	1.5 kg
76/12–8 yrs	2.6 cm	2.9 cm	1.3 kg	1.5 kg
8–8 6/12 yrs	2.6 cm	2.9 cm	1.4 kg	1.8 kg
86/12–9 yrs	2.6 cm	2.9 cm	1.4 kg	1.9 kg
9–9 6/12 yrs	2.6 cm	3.0 cm	1.6 kg	2.0 kg
96/12–10 yrs	2.7 cm	3.1 cm	1.7 kg	2.0 kg
10–10 6/12 yrs	2.8 cm	3.2 cm	1.9 kg	2.2 kg
106/12–11 yrs	3.0 cm	3.3 cm	2.0 kg	2.3 kg
11–11 6/12 yrs	3.1 cm	3.4 cm	2.2 kg	2.2 kg
116/12–12 yrs	3.3 cm	3.3 cm	2.3 kg	2.3 kg
12–12 6/12 yrs	3.3 cm	3.1 cm	2.5 kg	2.3 kg
126/12–13 yrs	3.5 cm	2.5 cm	2.7 kg	2.3 kg
13–13 6/12 yrs	3.4 cm	1.9 cm	2.8 kg	2.2 kg
136/12–14 yrs	3.2 cm	1.4 cm	3.0 kg	2.0 kg
14–14 6/12 yrs	3.1 cm	0.8 cm	3.0 kg	1.8 kg
146/12–15 yrs	2.8 cm	0.6 cm	2.9 kg	1.6 kg
15–15 6/12 yrs	2.5 cm	0.3 cm	2.8 kg	1.3 kg
156/12–16 yrs	2.0 cm	0.3 cm	2.6 kg	0.9 kg
16–16 6/12 yrs	1.7 cm	0.3 cm	2.3 kg	0.5 kg
166/12–17 yrs	1.0 cm	0.4 cm	1.9 kg	0.3 kg
17–17 6/12 yrs	0.5 cm	0.3 cm	1.5 kg	0
176/12–18 yrs	0.1 cm	0.3 cm	1.1 kg	0

Expected change is rate of gain seen in children growing along the 50th percentile over the designated period. For example, a 6-month-old girl would be expected to have gained 16 cm in length and 4.0 kg in weight from birth to 6 months of age.
Modified from (17).

"Height for age" is a measure of "stunting." Stunting is classified as being "mild," "moderate," or "severe." Increments of 5% are used because they represent approximately 2 standard deviations (SD) around the mean for "height for age."

"Weight for height" is utilized as a measure of "wasting," and like stunting, is also classified as "mild," "moderate," or "severe." In this case, increments of 10% are used because they represent approximately 1 SD around the mean. Table 33–2 shows Waterlow's classification of stunting and wasting.

To determine how the Waterlow classification is utilized, consider the following example: A 4-year-old boy whose height is 96 cm and whose weight is 13.5 kg would have the following plot (see Example A in Figs. 33–1 and 33–2).

Table 33–2.
Waterlow Classification of Wasting or Stunting

	Normal	*Mild*	*Moderate*	*Severe*
Stunting* (Low weight for age)	> 95%	90%–95%	85%–90%	< 85%
Wasting* (Low weight for height)	> 90%	80%–90%	70%–80%	< 70%

*Percent of height for age and weight for height based on the 50th percentile of the Boston growth standard. Source: (18).

Height: 96 cm is 5th–10th percentile for age.

Weight: 13.5 kg is 3rd–5th percentile for age.

"Height for Age" =

$$\frac{\text{Actual height}}{\text{Height for age at the } 50^{\text{th}} \text{ percentile} \times 100}$$

OR

$$\frac{96 \text{ cm}}{102.9 \text{ cm}} \times 100 = 93\%$$

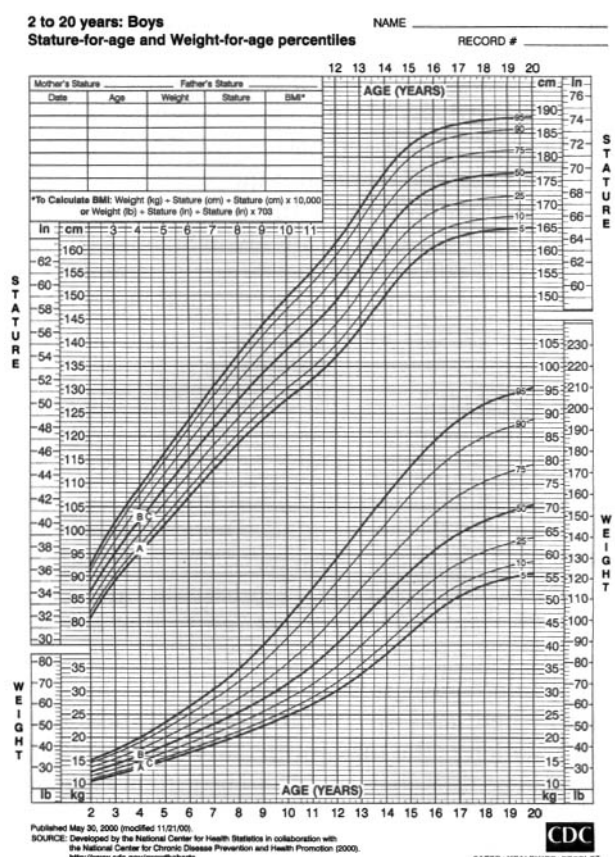


Figure 33–1.

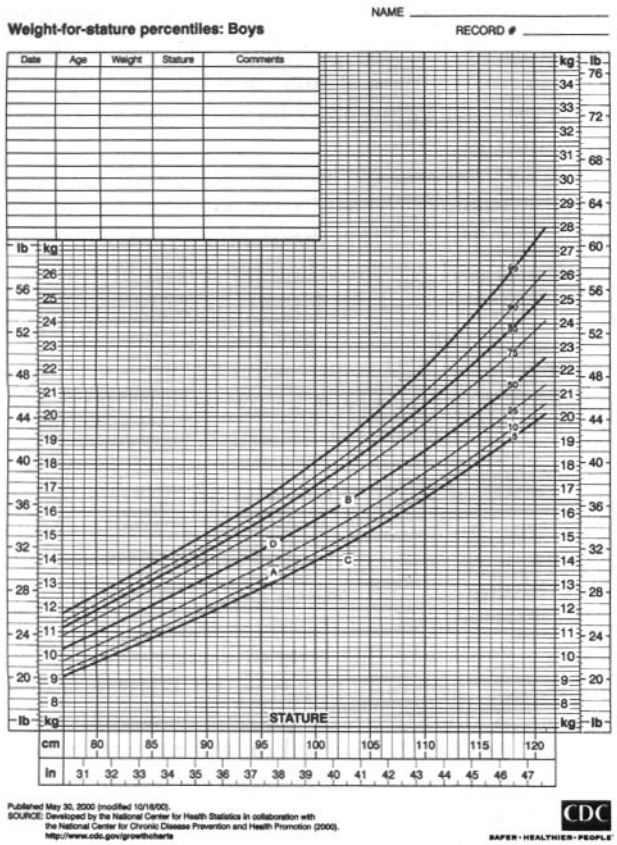


Figure 33–2.

"Weight for Height" =

$$\frac{\text{Actual weight}}{\text{Weight for height at 50th percentile}} \times 100$$

OR

$$\frac{13.5 \text{ kg}}{13.9 \text{ kg}} \times 100 = 97\%$$

His growth would be considered mildly stunted but show no signs of wasting. This condition may indicate an acute nutritional insult, i.e., one that could occur when a severely restricted diet is implemented for an extended period of time. This degree of undernutrition could eventually evolve into wasting if dietary modification is not initiated.

If a change in growth rate occurs in a child on a restricted diet, the first area of dietary intake to evaluate is the average daily caloric intake. For children who are attempting to maintain normal growth, the RDA may be used as the yardstick with which to measure their intake (Table 33–3). For example, a 4-year-old boy who is at the 50th percentile of height for his age would be approximately 103 cm in height, and should weigh 16.5 kg

Table 33–3.
Recommended Dietary Allowances

Age (Years)	*Kcal per Kg of Ideal Weight*	*Protein (g) per Kg of Body Weight*
0–0.5	108	2.2
0.5–1	98	1.6
1–3	102	1.2
4–6	90	1.1
7–10	70	1.0
11–14 Males	55	1.0
15–18 Males	45	0.9
19–22 Males	40	0.8
11–14 Females	47	1.0
15–18 Females	40	0.8
19–24 Females	38	0.8

Modified from (15).

to be at the 50th percentile for weight for height (See Example B in Figs. 33–1 and 33–2). His average daily energy intake should be 1485 kcal on the basis of his weight × RDA for calories (16.5 kg × 90 kcal/kg).

These same standards can be utilized to determine caloric needs for a child who is wasted or stunted. For a wasted child who is 103 cm tall, but weighs only 14 kg, the following equation would be used to determine caloric requirements for catch-up weight gain (see Example C, Figs. 33–1 and 33–2):

$$\frac{\text{Weight for height at the } 50^{\text{th}} \text{ percentile}}{\text{Actual body weight}} \times 90 \text{ (calories for age)}$$

Or

$$\frac{16.5 \text{ kg}}{14 \text{ kg}} \times 90 = 106 \text{ cal/kg for catch-up weight gain}$$

This child would, therefore, require 1484 kcal (14 kg × 106 kcal/kg) for catch-up growth.

For the stunted child (e.g., a 4-year-old whose height is 96 cm) the following equation can be used to determine calories for catch-up growth (see Example D in Figs. 33–1 and 33–2):

Weight for actual height at the 50^{th} percentile × calories for age = calories for catch-up growth

Or 14.6 kg × 90 cal/kg = 1314 calories

If the child's average caloric intake matches this level, it should be increased to satisfy the caloric requirements for a child who is at the 50th percentile for both height for age and weight for height.

Caloric requirements for catch-up growth can also be calculated by utilizing the child's estimated basal metabolic rate (BMR) for his or her actual weight. Caloric requirements are estimated at two times the BMR. For a 14 kg child, the BMR would be 680 calories and 1360 calories would be required per day for catch-up growth (Table 33–4) (19).

Protein requirement is the second area of dietary intake that should be evaluated. Sixty-five percent to 70% of a child's protein requirements should be as high-quality protein, or protein of high biological value (16). A high-biological-value protein comes from an animal source, thus one or more of the following foods should be included in the child's diet unless contraindicated by the child's food hypersensitivity elimination diet: eggs, milk products, poultry, meat, or fish. Another exception should be made if the child is following a "vegan" vegetarian diet (which excludes all animal products, even milk and eggs). In such a case, the diet must be analyzed to ensure that it includes complimentary proteins that provide adequate amounts of all the essential amino acids. The NAS has established recommended daily dietary allowances for protein (15). Protein requirements are based on age and determined by body weight; for example, a 4-year-old requires 1.1 g of protein per kg of body weight. Thus, a 4-year-old boy weighing 16.5 kg should consume a minimum of 18 g of protein per day, of which about 12 g should consist of high-biological-value protein (15). See Table 33–3 for recommended protein intakes.

Table 33–4.
Standard Basal Calories Based on Patient's Weight

Weight (Kg)	*Kcal per 24 Hours (Both Sexes)*	
3	140	
5	270	
7	400	
9	500	
11	600	
13	650	
15	710	
17	780	
19	830	
21	880	
	Kcal per 24 Hours Male	*Kcal per 24 Hours Female*
25	1020	960
29	1120	1040
33	1210	1120
37	1300	1190
41	1350	1260
45	1410	1320
49	1470	1380
53	1530	1440
57	1590	1500
61	1640	1560

Source: (19)

The final area of the diet that should be evaluated involves the child's intake of vitamins and minerals. This intake should be compared with the RDAs and AIs for vitamins and minerals [see DRIs (20); Table 33–5]. The RDAs include a safety factor to accommodate variation in bioavailability and individual requirements (20). If a child's intake of a nutrient falls below 65% of the established standards, it can be considered to be deficient. The child's family should be provided with a list of foods that are good sources of the deficient nutrient or nutrients. If these foods cannot be incorporated into the child's diet, then a multivitamin and mineral supplement should be prescribed.

To undertake this type of dietary evaluation, an allergist generally requires the assistance of a registered dietitian. The patient or parents of a child should be instructed on the proper recording of dietary intake, and asked to keep a record for a period of 3–7 days. The completed dietary record may then be analyzed by one of many available computer programs. The Food and Nutrition Information Center (FNIC) provides a complete listing of nutrient analysis software made available on-line at www.nal.usda.gov/fnic. The patient's average daily intake can be compared with the standards described above for calories, protein, vitamins and minerals, and a nutritional intervention program established.

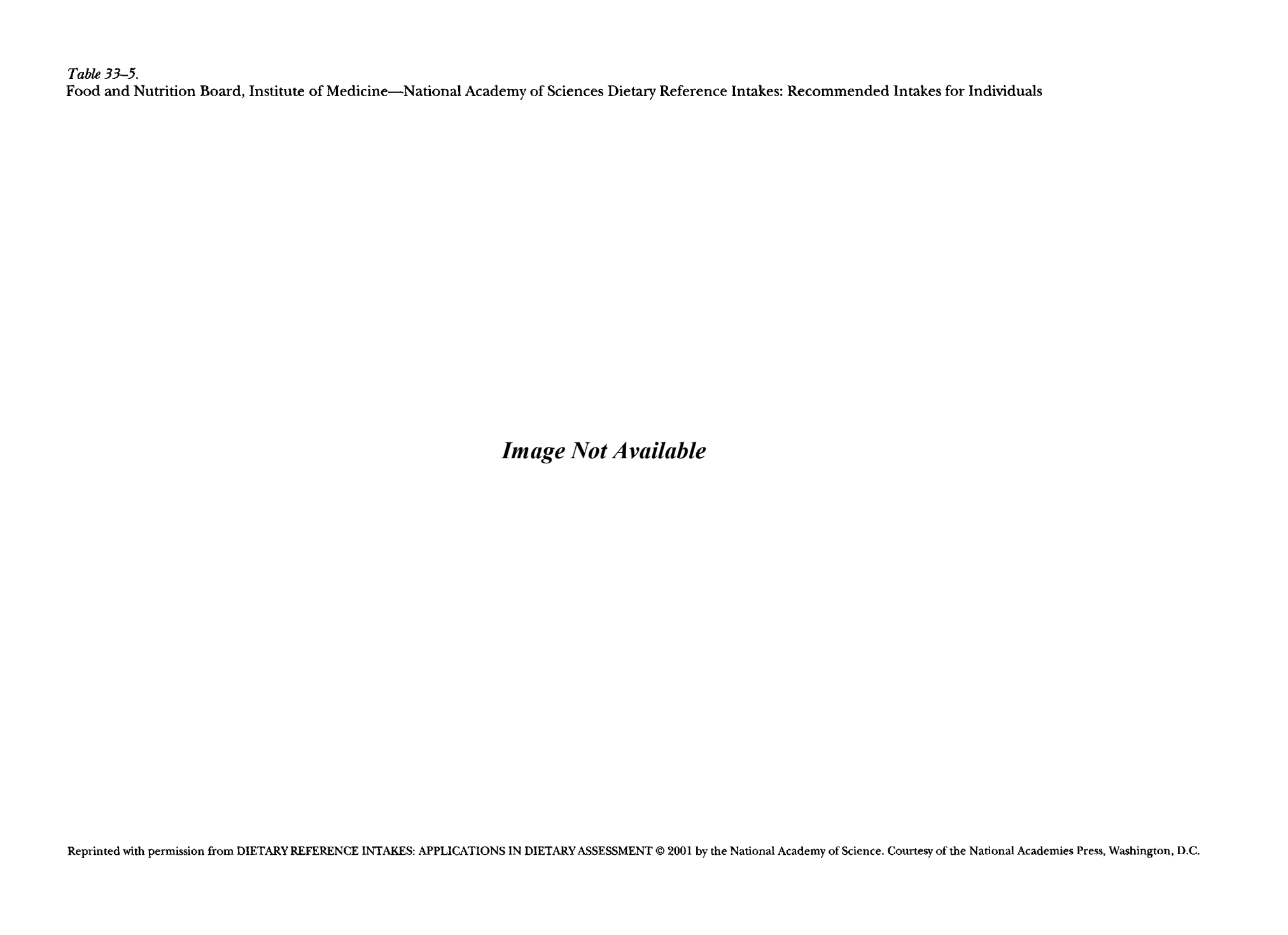
Modifying Diet for Diagnosis

Double-blind placebo-controlled food challenges (DBPCFCs) are the "gold standard" by which to diagnose food allergy. In the early stages of the diagnostic workup, however, elimination diets are often utilized. If a limited number of foods are suspected, they may be totally eliminated from the diet for as long as 2 weeks prior to anticipated challenge. When food allergy is suspected but no specific foods are implicated, highly restrictive diets are sometimes employed. Such diets might include the following:

Infants < 4 months: casein hydrolysate or amino acid formula (e.g., Neocate and EleCare)

4–8 months: infant diet + rice cereal + pears

Table 33–5.
Food and Nutrition Board, Institute of Medicine—National Academy of Sciences Dietary Reference Intakes: Recommended Intakes for Individuals



9–24 months: 4–8 month diet + rice + squash + lamb

>24 months: 9–24 month diet + fresh lettuce + potato + safflower oil + tea + sugar

OR

Amino acid formula (e.g., Neocate One Plus or EleCare)

Restricted diets are not without risk, and if prolonged, they can lead to malnutrition and growth retardation. A restricted diet that has been implemented without confirmation by a DBPCFC should be instituted for only a brief (1–6 weeks) period. With IgE-mediated disorders, symptomatic improvement should appear within 1–2 weeks; in contrast, with several of the non-IgE-mediated gastrointestinal hypersensitivities (e.g., food-induced enteropathy or allergic eosinophilic gastroenteritis), significant symptomatic improvement may not be seen for 4–6 weeks. No evidence exists that more prolonged restrictive diets are necessary for any of the well-substantiated food hypersensitivities.

When only one or two foods are "suspect," the patient or parents of a child may eliminate these foods and substitute other foods from similar food categories. For example, if eggs and wheat are the "suspect" foods, the diet can be supplemented with other sources of animal protein and other grains to replace the nutrients contributed from eggs and wheat. A common mistake is to eliminate foods from the diet without replacing them with alternative nutrient sources. For example, a child who is egg- and wheat- restricted and consumes no breads or other baked goods may have a diet that is deficient in certain B vitamins and iron, because fortified breads and baked goods contribute significant amounts of these nutrients. Milk presents a more difficult nutritional restriction. Attempts should be made to replace cow's milk with a soy milk (Alsoy, Isomil, Prosobee, Soy Dream), a protein hydrolysate (Alimentum, Nutramigen, or Pregestimil; but *not* Good Start, which is a partial whey hydrolysate), or an amino acid formula (Neocate, Neocate One Plus powder, or EleCare) that will provide the nutrients typically found in milk and milk products.

If a strict elimination diet is instituted, one should attempt to provide a nutritionally complete diet. Table 33–6 provides sample meal patterns that would meet greater than 75% of a 4-year-old child's RDAs. The enriched rice milk provides calcium and vitamin D at levels found in milk. If en-

Table 33–6.
Sample Meal Patterns

Sample Meal Pattern 1

Breakfast
1 c enriched rice milk
1 c grits, enriched
2 homemade pork sausage patties
2 egg-, milk-, wheat-free banana rice muffins

Lunch
1 broiled lamb chop
1 serving French-fried potatoes
½ c cooked carrots
1 c enriched rice milk

Dinner
1 broiled pork chop
1 boiled potato
½ c cooked squash
1 serving canned pears
1 c enriched rice milk

Vegetable oils or unsalted, milk-free margarine can be used to provide additional calories.

Sample Meal Pattern 2

Breakfast
1 c enriched rice milk
1 c puffed rice cereal
2 oz grilled fresh ham slice
2 rice pancakes with pure maple syrup

Lunch
3 oz ground lamb patty
2 slices egg-, milk-, and wheat-free bread
1 oz potato chips
½ c applesauce
1 c enriched rice milk

Dinner
1 broiled pork chop
1 c white rice
1 serving cooked spinach
1 serving canned pineapple
1 c enriched rice milk

Vegetable oils or unsalted, milk-free margarine can be used to provide additional calories.

Sample Meal Pattern 3

Breakfast
1 c enriched rice milk
1 c cream of rice cereal
4 slices bacon
2 egg-, milk-, and wheat-free corn muffins

Lunch
2 slices fresh ham
2 slices of egg-, milk-, and wheat-free bread
1 oz potato chips
1 banana
1 c enriched rice milk

Dinner
1 broiled lamb chop
1 baked potato
1 serving cooked broccoli
1 serving canned peaches
1 c enriched rice milk

Vegetable oils or unsalted, milk-free margarine can be used to provide additional calories.

riched rice milk is unavailable, these two nutrients need to be added to the diet (Table 33–7).

A highly restricted diet is monotonous and should be "opened" with the introduction of one previously restricted food every 5–7 days. Symptom records should be maintained during periods of both complete elimination and reintroduction. Long-term dietary restriction of foods requires confirmation of hypersensitivity by DBPCFCs, because sole reliance on an elimination diet can lead to erroneous diagnosis of food allergy. Often, an excessive number of foods are eliminated because environmental factors confound the evaluation, resulting in an erroneous diagnosis of hypersensitivity. Equally likely is that key food allergies will be missed because the patient maintains the same level of disease activity throughout the trial period, and the patient (or his or her parents) is unable to detect any significant changes.

Formula-only diets are occasionally instituted. If a child can tolerate a protein hydrolysate formula (such as Nutramigen, Pregestimil, or Alimentum), it should be used rather than an amino acid formula such as Neocate or EleCare. A protein hydrolysate formula is preferable because amino acid formulas are very costly and should be used only in documented cases of failure on protein hydrolysate formulas. If an amino acid formula becomes necessary, products are available for various age groups from infants through adults (Table 33–8 contains a chart comparing pediatric products.) Amino acid formulas should be analyzed for their protein, essential fatty acid, vitamin, and mineral content, and modified as needed.

Table 33–7.
Calcium-Fortified Foods and Supplements

Fortified Food	*Elemental Calcium*
6 fl oz calcium-fortified orange juice	200–225 mg
6 fl oz Gerber Graduate juices	200 mg
6 fl oz Sunny Delight Calcium Orange Drink	250 mg
8 fl oz Enriched Rice Dream Rice Milk	300 mg
1 oz calcium-fortified cereal	100–200 mg
Supplement	*Elemental Calcium*
Calci-Mix	500 mg/capsule
Cal Quik	400 mg/tsp
Neo-Calglucon syrup	115 mg/tsp
Rolaids	220 mg/tablet
Extra Strength Rolaids	270 mg/tablet
Tums	200 mg/tablet
Tums E-X	300 mg/tablet
Tums Ultra	400 mg/tablet

Table 33–8.
Comparison of Nutrient Content of Pediatric Elemental Formulas (per 1000 kcal)

Nutrient	*Neocate*	*Neocate One Plus Powder*	*EleCare*
Energy (kcal/g)	4.21	4.00	4.75
Protein equivalent, g	31	25	30.1
Fat, g	45	35	47.6
Carbohydrate, g	117	146	107
Calcium, mg	1240	620	1084
Phosphorus, mg	931	620	811
Magnesium, mg	124	90	84
Iron, mg	18.5	7.7	17.7
Zinc, mg	16.6	7.7	11.2
Manganese, mg	0.9	1.0	1.05
Copper, mg	1.24	1.0	1.26
Iodine, μg	154	60	71.6
Sodium, mg	373	200	453
Potassium, mg	1551	930	1505
Chloride, mg	772	350	600
Selenium, μg	37.3	15.4	23.2
Chromium, μg	35.6	30	23.2
Molybdenum, μg	47.5	35	25.3
Vitamin A, μg RE	1227	350	821
Vitamin D, μg	21.75	7.8	10.5
Vitamin E, mg α-TE	7.65	5.5	14.2
Vitamin K, μg	87.9	15	63.2
Vitamin C, mg	92.6	31	90.5
Thiamine, mg	0.926	0.6	2.1
Riboflavin, mg	1.378	0.7	1.05
Vitamin B_6, mg	1.235	0.8	1.01
Vitamin B_{12}, μg	1.70	0.7	4.21
Niacin, mg	15.44	9.0	16.8
Folic Acid, μg	102	60	295
Pantothenic Acid, mg	6.2	2.4	4.21
Biotin, μg	31	20	42.1
Osmolality at standard dilution (mOsms/kg water)	375	610	596

Management of Food-Allergic Patients

A few foods are responsible for the majority of allergic reactions (21). In adults, these foods include nuts, peanuts, fish, and shellfish. In children, the main culprits include egg, milk, peanuts, soy, wheat, and fish. The elimination of each of these foods and the potential nutritional consequences will be addressed separately.

Cow's Milk

Once a patient has been diagnosed as cow's milk allergic, milk must be completely removed from the diet. No milk or milk by-products are allowed, not even in small amounts. Table 33–9 (22) lists words found on food product labels that indicate the presence of milk proteins (i.e., "milk words"). Any product that contains one or more of

Table 33–9.
Label Ingredients That Indicate the Presence of Milk Protein

Artificial butter flavor
Butter, butter fat, butter oil
Buttermilk and buttermilk solids
Casein (casein hydrolysate)
Caseinates (in all forms including ammonium, calcium, magnesium, potassium, sodium)
Cheese
Cottage cheese
Cream
Curds
Custard
Ghee
Half and half
Hydrolysates (casein, milk protein, protein, whey, whey protein)
Lactalbumin, lactalbumin phosphate
Lactoglobulin
Lactulose
Milk (in all forms including condensed, derivative, dry, evaporated, goat's milk and milk from other animals, low-fat, malted, milkfat, nonfat, powder, protein, skimmed, solids, whole)
Nougat
Pudding
Rennet casein
Sour cream and sour cream solids
Sour milk solids
Whey (in all forms including delactosed, demineralized, protein concentrate)
Yogurt
Label Ingredients That May *Indicate the Presence of Milk Protein*
Brown sugar flavoring
Caramel candies
Caramel flavoring
Chocolate
Flavoring (including natural and artificial)
High protein flour
Lactic acid starter culture
Lactose
Luncheon meats, hot dogs, sausages
Margarine
Non-dairy products

Modified from (22).

these words on its label ingredient list should not be consumed on a milk-restricted diet. In addition, patients should be instructed to contact food manufacturers about listed ingredients such as caramel, brown sugar and natural flavors, margarine, chocolate, and high protein flours, which may contain milk or milk by-products. Deli meats may be cross-contaminated with dairy products by cheese products or other meat products containing milk having been sliced on the same equipment. Processed meats, including hot dogs, sausages, and luncheon meats, may contain milk proteins, particularly the low- and reduced-fat product varieties. In some products, milk proteins may be labeled as "natural flavoring." Other hidden sources of milk products may be found in "carryover contamination" during processing of the food product (e.g., non-milk-based desserts, and fruit juices and drinks in single serving tetra packs [vacuum-sealed cardboard boxes]). Kosher labeling may help identify products that contain milk proteins. A "D" (dairy) next to the rabbinical agency's symbol indicates the possible presence of milk protein, which may or may not be found in the ingredient statement. A "DE" (dairy equipment) indicates that the product has been produced on equipment that is also used to produce milk-containing foods.

A child on a milk-restricted diet may not be consume adequate amounts of the following nutrients: vitamin D, vitamin B_{12}, riboflavin, pantothenic acid, calcium, and phosphorus. Milk is a nutrient-dense food and a primary source of calcium and vitamin D. To emphasize the importance of milk as a source of nutrients for a growing child's diet, consider the following example. A child 4–6 years of age who typically consumes three 8-oz glasses of milk per day receives the following nutrients from the milk intake alone: 100% of the vitamin B_{12}, riboflavin and calcium requirements; 75%–85% of vitamin D and phosphorus requirements; and 55%–60% of the pantothenic acid requirements. Alternative sources of these nutrients may be provided in milk-free formula preparations such as casein hydrolysates and soy formulas. Children on "infant" milk-free formulas should continue on these formulas as long as they remain acceptable to the child. Such formulas need not be discontinued on the basis of the child's age. If a formula has been discontinued, attempts should be made to reintroduce milk-free formulas or enriched soy or rice milk beverages into the diet to provide a good source of calcium, phosphorus, vitamin A, and vitamin D. A child on a milk-restricted diet who does not receive a milk substitute will require calcium and vitamin D supplementation.

Alternative sources of the other major nutrients found in milk include meats, legumes, nuts, and whole grains. Children who are milk allergic may sometimes react to one or more of these nutrient sources as well, which makes balancing their nutritional intake more difficult. Obtaining regular dietary intake records for patients can assist in identifying possible nutrient deficiencies. Nutrient intake is considered inadequate when the patient consumes less than two thirds of the various RDAs and AIs as determined from a minimum of a 3-day diet record average. Supplementation should be provided either by dietary modification, vitamin and mineral supplementation, or

provision of a nutritionally complete milk-free formula.

Egg

A patient who has been diagnosed as having a food hypersensitivity to eggs must avoid all forms of eggs. Table 33–10 lists words that indicate the presence of egg protein. A patient on an egg-restricted diet should not consume any product with one or more of these words on the label ingredient list.

Eggs alone are not an essential food in the diet of an adult or child. They are a dietary source of vitamin B_{12}, pantothenic acid, folacin, riboflavin, selenium, and biotin. One egg provides between 10% and 20% of a 4- to 6-year-old child's requirements for these nutrients. Typically, these nutrients can easily be supplied by other foods in the patient's diet.

Eggs are incorporated in a wide variety of products because of their excellent physical properties in food processing (e.g., coagulation, stabilization, emulsification). They may be used to form the custard base of ice creams and yogurts. Egg whites may be used to give pretzels, bagels, and other baked goods a shiny outer finish. (Labels may not indicate the presence of an egg glaze on some bakery products such as breads and rolls.) Because eggs are used in coating batters for fried foods, the egg-allergic patient should avoid buying fried foods from vendors who do not maintain separate vats for frying each type of food sold because of the possibility of food protein carry-over in the frying oil. Egg whites or shells may be used as clarifying agents in soup stocks, consommés, bouillons, and coffees. Eggs are used in imitation shellfish and institutional pureed foods to improve texture and appearance. Egg albumin stabilizes whipped fruit products used in desserts and drinks. Eggs are used as a binder in meatloaf, meatballs, and pasta.

For each egg required in cooking, the following substitutions can be made:

1. Mix 1 packet of unflavored gelatin with 1 cup (c) boiling water. Substitute 3 tablespoons of this liquid for each egg. Refrigerate the remainder for as long as 1 week, and microwave it to liquefy it for reuse. Use this mixture in recipes with another source of leavening (i.e., baking powder or baking soda).
2. 1½ tablespoons water plus 1½ tablespoons oil plus 1 teaspoon baking powder.
3. 1 teaspoon baking powder plus 1 tablespoon water plus 1 tablespoon vinegar (add vinegar separately at the end for rising).
4. 1 teaspoon yeast dissolved in ¼ c warm water.
5. 1 tablespoon apricot puree as a binder, not a leavening agent.

One problem that may occur on an egg-restricted diet is the unintentional limitation of grain products, because many such foods contain egg. A child's diet that is limited in eggs and grain is apt to be inadequate in some of the B vitamins and possibly iron. Thus, it is important to teach families how to prepare a variety of foods without eggs.

Table 33–10.
Label Ingredients That Indicate the Presence of Egg Protein

Albumin (also spelled albumen)
Egg (dried, powdered, solids, white, yolk)
Eggnog
Lysozyme (used in Europe)
Mayonnaise
Meringue, meringue powder
Surimi
Label Ingredients That May *Indicate the Presence of Egg Protein*
Flavoring (including natural and artificial)
Lecithin
Macaroni
Marzipan
Marshmallows
Nougat
Pasta

Modified from (22).

Peanuts

Peanut sensitivity is fairly common in both children and adults, but somewhat easier to manage than sensitivity to its legume relative, soybeans. Allergies to more than one legume are rare and allergists generally do not recommend a generalized legume restriction; however, researchers in France have reported a high degree of cross-reactivity between peanuts and lupine (23). Thus, caution is advised in the consumption of lupine in peanut-allergic individuals. Approximately one third of individuals who are allergic to peanuts are also allergic to at least one type of tree nut such as pecans, walnuts, or almonds (24, 25). Increasingly, peanut products are mixed with or substituted for

tree nut products in the manufacturing process. For these reasons peanut-allergic patients are generally advised to avoid tree nuts as well as peanuts.

Peanut protein (especially peanut butter) is used in many foods. Some foods known to contain peanuts or peanut products include marzipan, chili, spaghetti sauces, shish kabobs, egg rolls, ethnic dishes, cereals, crackers, soups, baked goods, frozen desserts, and candy. Table 33–11 lists words that indicate the presence of peanut protein. Hydrolyzed plant and vegetable protein is typically derived from soybean in food products from the US, but imported foods frequently use peanuts as a source of this ingredient. The generic term "flavoring" should also be regarded with caution, because it encompasses any food component added to impart a particular flavor and can include any of the major allergenic foods.

An important issue that remains unresolved is the quantity of peanut antigen necessary to provoke an allergic response. In a study by Walzer (26), the investigator induced a wheal-and-flare reaction at a passively sensitized skin site with an intravenous injection of the protein nitrogen equivalent of 1/44,000 of one peanut kernel. Fries (27) reported a case of wheezing and urticaria after a jar of peanut butter was opened in front of the patient. Utilizing DBPCFCs, 50–100 mg of peanut flour elicited allergic symptoms in some children. It should be remembered, however, that the most sensitive patients—those experiencing life-threatening anaphylaxis—were not challenged. A study of 75 peanut allergic adults demonstrated no reactivity to refined peanut oil (28), but crude peanut oils that are either cold-pressed, expressed, or expelled elicited allergic reactions in peanut-sensitive individuals (29). Some reported reactions to peanut oil may relate to the presence of other food protein in oil used for frying (e.g., fish and other seafood), but some caution may still be necessary.

Peanuts provide the following nutrients: niacin, magnesium, vitamin E, manganese, and chromium in significant amounts and smaller amounts of potassium, vitamin B_6, folacin, phosphorus, copper, and biotin. Fortunately, many other foods can provide these same nutrients. Thus, a peanut restriction alone would not negatively affect a growing child's diet.

Table 33–11.
Label Ingredients That Indicate the Presence of Peanut Protein

Artificial nuts
Beer nuts
Cold-pressed, expelled, or extruded peanut oil
Ground nuts
Goobers
Mandelonas
Mixed nuts
Monkey nuts
Nut pieces
Peanuts
Peanut butter
Peanut flour
Peanut oil, cold-pressed, expelled, or extruded

Items and Label Ingredients That May *Indicate the Presence of Peanut Protein*

African, Chinese, Indonesian, Mexican, Thai, and Vietnamese dishes
Baked goods (pastries, cookies, etc.)
Candy (including chocolate)
Chili
Egg rolls
Enchilada sauce
Flavoring (including natural and artificial)
Marzipan
Nougat
Sunflower seeds
Notes
• Arachis oil is peanut oil
• Studies show that most allergic individuals can safely eat peanut oil (but not cold-pressed, expelled, or extruded peanut oil)
• Experts advise peanut-allergic patients to avoid tree nuts

Modified from (22).

Tree Nuts

A variety of nuts can cause severe anaphylactic reactions: almond, Brazil nut, cashew, chestnut, filbert/hazelnut, hickory, macadamia, pecan, pine nut, pistachio, and walnut. Tree nuts are added to an increasing variety of foods such as barbecue sauces, cereals, crackers, and frozen desserts. Ethnic foods, commercially prepared baked goods, and candy can be cross-contaminated with nuts, because they are frequently used in some varieties of these foods. Foods that list "flavoring," including both artificial and natural, must be checked for the possible use of nuts as a flavoring agent. Table 33–12 provides a list of tree nuts and ingredients that contain tree nuts. Absent from this list are coconut, water chestnut, nutmeg, and mace, which are not restricted on a diet that eliminates tree nuts.

Tree nut sensitivity one is of the most common food hypersensitivities, affecting 0.7%, or 1.5 million, of adults in the US (30). Patients suffering from an anaphylactic reaction to one nut are often told to avoid other nuts because of potential cross-reactivity. Although immunologic

Table 33–12.
Label Ingredients That Indicate the Presence of Tree Nuts

Almonds, almond paste
Artifical nuts
Brazil nuts
Caponata
Cashews
Chestnuts
Filberts/hazelnuts
Gianduja (a nut mixture found in some chocolate)
Hazelnuts/filberts
Hickory nuts
Macadamia nuts
Mandelonas
Marzipan/almond paste
Nan-gai nuts
Natural nut extracts (e.g., almond, walnut)
Nougat
Nut butters (e.g., cashew butter)
Nutmeal
Nut oil
Nut paste (e.g., almond paste)
Nut pieces
Pecans (mashuga nuts)
Pesto
Pine nuts (also referred to as Indian, piñon, pinyon, pignolia and pignon nuts)
Pistachios
Pralines
Walnuts
Items That May *Contain Tree Nuts*
Mortadella may contain pistachios
Natural and artificial flavoring may contain tree nuts

Modified from (22).

Table 33–13.
Label Ingredients That Indicate the Presence of Soy Protein

Edamame
Hydrolyzed soy protein
Miso
Natto
Shoyu sauce
Soy (soy albumin, soy fiber, soy flour, soy grits, soy milk, soy nuts, soy sprouts)
Soya
Soybean (curd, granules)
Soy protein (concentrate, isolate)
Soy sauce
Tamari
Tempeh
Textured vegetable protein (TVP)
Tofu
Items and Label Ingredients That May *Indicate the Presence of Soy Protein*
Asian cuisine
Flavoring (including natural and artificial)
Vegetable broth
Vegetable gum
Vegetable starch
Note
• Studies show most soy-allergic individuals may safely eat soy lecithin and soybean oil.

Modified from (22).

cross-reactivity (or multiple asymptomatic sensitivity) may be common, it is unlikely that nut-allergic patients will react to a variety of different nuts. Until this subject has been carefully addressed with double-blind challenge studies, however, caution must be exercised in recommending the ingestion of different nuts in nut-allergic patients.

Soybean

Soybean hypersensitivity is much less common than peanut sensitivity, but avoidance of soy products can be much more difficult. Soybeans constitute a major component of processed food products in the US and other parts of the world. Soybeans and soybean products are incorporated into infant formulas, baked goods, canned tuna, cereals, crackers, soups, and sauces. Table 33–13 lists "soy words" denoting foods that contain soy protein. In addition, soy is often utilized as a carrier protein for flavorings, because it is relatively flavorless and readily absorbs the flavors of other foods. Individuals on soy-exclusion diets must contact the manufacturers of foods containing artificial and natural flavorings to ensure that the product does not contain soy protein.

The processing of most soybean oils removes the protein portion. A study conducted in seven soy-allergic individuals found no reactions to soy oil when the subjects were blind-challenged with soy oil (31). However, an insufficient number of patient were studied to unequivocally state that soy oil is safe in all highly sensitive soy patients. Currently, children with soybean sensitivity are allowed to consume soybean oil and soy lecithin. If a child's allergic symptoms continue or show signs of re-exacerbation, however, all soybean products should be eliminated during a trial period.

Soybeans contribute the following nutrients to a patient's diet: thiamin, riboflavin, vitamin B_6, folacin, calcium, phosphorus, magnesium, iron, and zinc. Like the foods previously discussed, soybeans alone do not make major contributions to overall nutrition, but the exclusion of foods that contain soybean proteins may have a dramatic dietary impact.

The classification of foods by botanical family has inspired considerable confusion about the importance of intra-botanical cross-reactivity, especially in relation to legumes. A study in challenge-proven legume-allergic patients demonstrated that symptomatic reactivity to multiple members of the legume family is rare, despite evidence for broad antibody cross-reactivity provided by skin prick tests (SPTs) or RAST results (9, 32). This study reported that among 69 patients who had one or more positive SPTs to legumes, 41 patients (59%) had legume sensitivity documented via blinded challenge, or a convincing history of severe anaphylaxis. Of the 41 legume-sensitive patients, only 2 (5%) exhibited symptomatic reactivity to more than one legume. Both of these individuals had a history of severe anaphylaxis following peanut ingestion and a positive challenge to soybeans. When they were maintained on a peanut- and soybean-restricted diet, both lost their symptomatic soybean reactivity within 1–3 years of the initial challenge. Both patients were able to consume other legumes without any problems. This study indicates that a symptomatic reactivity to one legume does not necessitate the elimination of the entire legume food family unless a clinical hypersensitivity to each legume is individually confirmed by blinded oral challenges.

Fish

Fish is one of the most common causes of food-allergic reactions in adults, and one of the most common causes of food allergy at all ages in countries where large quantities of fish are consumed. The protein Gad c 1, a parvalbumin, has been well characterized and is believed similar in many species of fish (33). Consequently, it is generally recommended that fish-allergic individuals avoid all species of fish. In a study of fish-allergic patients undergoing double-blind challenges to a number of different fish species, most patients allergic to one species of fish could safely ingest a different species (10). However, results of skin tests and RASTs indicate extensive cross-reactivity among fish species and do not help determine which fish can be ingested safely (34). Although allergy to tuna seems to be uncommon, one should assume extensive cross-reactivity among fish species and eliminate all fish from the diet of fish-allergic patients unless blinded challenges are conducted to determine which fish species may be eaten safely. If a fish-allergic patient can tolerate certain fish species, he or she must be very careful when eating at restaurants to ensure that the fish species ordered is not substituted with another species.

A food allergy to fish may be welcomed by some children in the US, for whom fish is typically not a favorite food. Fish can provide some key nutrients to a child's diet, however, in addition to providing an alternative source of high-biological-value protein. A 3.5-oz serving of fish provides significant amounts of niacin, vitamin B_6, vitamin B_{12}, vitamin E, phosphorus, and selenium. To a somewhat lesser degree, it also serves as a dietary source of potassium, magnesium, and iron.

Typically fish is not a hidden ingredient in foods. However, a number of foods containing fish or fish products that are not always appreciated include Worcestershire sauce (if it contains anchovy), Caesar salad, caviar, and roe. In addition, fish is sometimes flavored and sold as imitation lobster or other shellfish.

Shellfish

Allergic reactions to various crustaceans (shrimp, crabs, lobster, and crawfish) and mollusks (clams, oysters, scallops) reportedly are common in adults. Extensive work is focusing on the characterization of various crustacean and mollusk antigens. In practice, individuals allergic to one shellfish are told to avoid other shellfish because skin testing or RASTs commonly demonstrate cross-reactivity. Little data are available to support or refute this practice, so caution must continue to be exercised in recommending consumption of other seafood in shellfish-allergic patients. Table 33–14 provides a list of the various shellfish and products that contain shellfish. Shellfish are generally not "hidden" in foods, but occasionally different shellfish may be included in dishes unbeknownst to a waiter in a restaurant.

Wheat

Because wheat is a predominant food product in the US and other countries, wheat elimination diets are particularly difficult for a patient and his or her family to maintain. Children on a wheat-restricted diet are severely limited in their selection of foods. Cereals, breads, pastas, crackers, and cookies are obviously limited, as are some sauces, lunch meats, snack foods, and candy. Families complying with this restriction generally can utilize products made from amaranth, barley, buckwheat, corn, oats, quinoa, rice, and rye that may be

Table 33–14.
Label Ingredients That Indicate the Presence of Shellfish

Abalone
Clams (cherrystone, littleneck, pismo, quahog)
Cockle (periwinkle, sea urchin)
Crab
Crawfish (crayfish, ecrevisse)
Lobster (langouste, langoustine, scampo, coral, tomalley)
Mollusks
Mussels
Octopus
Oysters
Prawns
Scallops
Shrimp (crevette)
Snails (escargot)
Squid (calamari)
Ingredients That May *Indicate the Presence of Shellfish*
Bouillabaisse
Fish stock
Flavoring (including natural and artificial)
Seafood flavoring (such as crab or clam extract)
Surimi
Notes
• Any food served in a seafood restaurant may be cross-contaminated with fish or shellfish.
• For some individuals, a reaction may occur from cooking odors or from handling fish or shellfish.

Modified from (22).

Table 33–15.
Label Ingredients That Indicate the Presence of Wheat Protein

Bran
Bread crumbs
Bulgur
Couscous
Cracker meal
Durum
Farina
Flour (all purpose, bread, durum, enriched, graham, high gluten, high protein, instant, pastry, self-rising, soft wheat, steel ground, stone ground, whole, wheat)
Gluten
Kamut
Matzoh, matzoh meal (also spelled matzo)
Pasta
Seitan
Semolina
Spelt
Triticale
Vital gluten
Wheat (bran, germ, gluten, malt, starch)
Whole wheat berries
Label Ingredients That May *Indicates the Presence of Wheat Protein*
Flavoring (including natural and artificial)
Hydrolyzed protein
Soy sauce
Starch (gelatinized starch, modified starch, modified food starch, vegetable starch)
Surimi

Modified from (22).

available in grocery or health food stores. One study indicates that as many as 20% of wheat-allergic children will react to another cereal grain (11). Additionally, caution is advised with the use of two products: kamut, an ancient wheat grain, and triticale, a hybrid of wheat and rye. These foods have not yet been clinically evaluated in wheat-sensitive individuals. In addition, wheat-allergic patients may use specialty food products intended for people with gluten-sensitive enteropathy (celiac disease), who must avoid all the gluten-containing grains (i.e., wheat, oats, barley, and rye). These foods may be found in special dietary shops or ordered from mail order companies. A large number of wheat-free recipes are available from which to prepare a variety of baked goods, thereby permitting greater variety in the diet and improved nutrient density.

A child who must follow a wheat elimination diet should avoid all types of wheat products. Table 33–15 lists foods that contain wheat protein. Any food item that contains one of these ingredients should not be consumed on a wheat exclusion diet. A child on a wheat-avoidance diet faces a risk related to insufficient intake of the following nutrients: thiamin, riboflavin, niacin, iron, selenium, and chromium. Four servings of wheat products will provide 20%–40% of the requirements for these nutrients in a 4- to 6-year-old child's diet. The thiamin, riboflavin, niacin, and iron in baked goods derives from the fortification of wheat flours to levels found in whole grains. Wheat products also contribute to a patient's intake of magnesium, folacin, phosphorus, and molybdenum. Given that wheat contributes several important nutrients, a child on a wheat elimination diet should have his or her intake evaluated for possible nutritional inadequacies.

Rice

Rice allergy is much less common than wheat allergy. Because rice is not a prominent food in the typical US diet, it is considerably easier to avoid than wheat or corn. A rice-exclusion diet alone should not cause any dietary problems. If rice avoidance is combined with elimination of another cereal grain, however, evaluation of nutrient intake may be more important. Rice contributes thiamin, riboflavin, niacin, and iron primarily via fortification of these nutrients. "Rice words" found on food

labels that indicate its presence as an ingredient include rice flour, rice starch, rice noodles, and rice bran. Foods that contain these items should be avoided on a rice elimination diet.

Corn

Fortunately, a true allergy to corn is very rare. Corn elimination diets are very difficult to manage because corn and corn products constitute ingredients in a large number of processed food products, primarily in the form of corn sweeteners or cornstarch. Table 33–16 lists words that indicate the presence of corn in the product (i.e., "corn words"). Corn oil is not listed as a food to avoid because the allergenic portion (protein) is removed in the processing of corn oil. A small study challenging documented corn-allergic patients with corn oil, corn sugar, and corn syrup provoked no reactions, whereas blinded challenge with cornstarch did provoke one reaction (35). One corn-allergic patient also reacted to cornstarch on blinded challenge.

As can be seen from the list of "corn words" listed above, a corn elimination diet would restrict a variety of foods, including baked goods, beverages, candy, canned fruits, cereals, cookies, jams, jellies, lunch meats, snack foods, and syrups. A patient on a corn elimination diet must rely on alternative sweeteners, thickeners, and leavening agents, such as fruit juices, beet or cane sugar, maple syrup, honey, aspartame, wheat starch, potato starch, rice starch, tapioca, baking soda, and cream of tartar. As with a wheat exclusion diet, many of the commonly available convenience items must be avoided. The patient may, however, be able to find some convenience foods that have either eliminated sugar from the food or substituted an allowed sweetener such as natural fruit juices or honey. Health food stores typically carry a variety of baked goods and some candies that are made with safe ingredients. The family may also use homemade foods.

Corn elimination affects nutrient intake primarily through the exclusion of other food products containing corn protein, rather than because of the nutrient contribution of corn alone. Corn contributes thiamin, riboflavin, niacin, and iron, via fortification of corn products; it also provides chromium. These nutrients are found in similar amounts in other grain products such as wheat or rice, so any other fortified grain could be substituted for corn. A child who is being strictly maintained on a corn elimination diet may benefit from a dietary evaluation, because the nutritional adequacy of his or her diet depends on the family's ability to provide varied alternative foods.

Table 33–16.
Label Ingredients That Indicate the Presence of Corn Protein

Baking powder
Corn (corn alcohol, corn flour, cornstarch, cornmeal, corn sweetener)
Dextrates, dextrins
Flavoring (caramel, natural and artificial)
Grits
Hominy
Maize
Maltodextrins
Marshmallow
Powdered sugar
Label ingredients that may *indicate the presence of corn protein*
Corn syrup solids
Starch (food, modified food, vegetable)
Vegetable gum

Modified from (22).

Other Grains

The remaining grain—oats, barley, and rye—will not be discussed individually because they provoke food sensitivities less commonly than most of the foods discussed earlier. It should be noted, however, that most "all-purpose flours" contain barley, and should, therefore, be taken into consideration in patients with suspected wheat sensitivity. Information on gluten-free diets may be obtained from the Celiac Sprue Association USA, P.O. Box 31700, Omaha, NE, 68131-0700, (402) 558-0600; or Ener-G Foods, P.O. Box 84487, Seattle, WA 98124-5787, (800) 331-5222.

Conclusions

In caring for the patient with adverse food reactions, specific food hypersensitivities should be appropriately identified and an elimination diet instituted that not only restricts the offending allergens, but also provides the patient with sound nutrition. Fortunately, most patients are allergic to only one or two foods, so prescribed diets are not too restrictive. When multiple food sensitivities are identified, however, the patient is at risk for nutritional inadequacies and it is imperative to

enlist the assistance of a dietician to formulate an appropriate diet. In addition, the dietician can provide a variety of recipes (such as those listed in the appendix of this chapter) that allow patients with multiple food sensitivities to maintain some variety in their diet.

The key to a food elimination diet is the avoidance of known allergens at all times. For this reason, it is recommended that patients use "allowed" fresh fruits, vegetables, meats, poultry, and whole grains to prepare homemade foods. When commercially prepared foods are used, the patient (or patient's parents) must read the labels and contact the manufacturer to ascertain whether certain allergens are present. Patients must be reminded to recheck labels regularly because the ingredients in various products (especially store brands) may change over time as the food manufacturer obtains new suppliers. In some cases, regional differences have been reported in product ingredients of national brands. Many questions will arise regarding specific food allergies for which no definite answers exist: for example, whether various vegetable oils (e.g., soy, peanut, corn, etc.) are safe for all highly sensitized patients, or whether soy lecithin or corn syrup solids are safe for soy- or corn-allergic patients. In cases of inadequate information, the severity of the patient's allergic history must be considered when selecting the dietary restrictions. For example, some caution in the use of peanut oil may be recommended in a patient who experiences life-threatening anaphylaxis, whereas products with soy lecithin would not be restricted in a soy-allergic patient with mild abdominal complaints.

Patients with severe food hypersensitivities must be taught to self-administer emergency medications. In addition, they must be reminded to carry emergency medications (EpiPen) for use in case they accidentally ingest a food to which they are allergic. In three series of fatal anaphylactic reactions secondary to food allergen ingestion, virtually all victims knew they were allergic to the allergen they unknowingly ingested, but in most cases failed to take adequate emergency treatment measures (36–38).

Another problem that is becoming increasingly recognized involves "cross-contaminants" in processed foods. For example, most tofu ice creams are manufactured in dairy plants where machinery is supposed to be carefully cleaned. Nevertheless, several cases of milk-sensitive children experiencing allergic reactions after ingesting tofu ice creams have been reported in which the tofu ice creams were found to contain significant levels of cow's milk proteins (39). Similarly, a child experienced an anaphylactic reaction after ingesting a small amount of a Popsicle that came off the same line used to make Creamsicles. Sensitive assays for detecting small concentrations of food proteins are now being developed in several laboratories may eventually prove useful in screening ostensibly "safe" foods to which patients react. If patients react to a food that should not cause an allergic reaction, they should be instructed to save a portion of the food so that a laboratory capable of measuring small quantities of contaminating food proteins may analyze it.

Restaurants and other public eating places continue to be high-risk environments for food-allergic patients. Patients must be counseled to be very assertive and precise about their specific food allergies when questioning the wait staff about the contents of various dishes. For example, a peanut-allergic patient should ask whether there is any peanut, peanut butter, or peanut oil in a dish and not just whether it includes peanuts. He or she should explain the seriousness of the allergy, and if uncomfortable about the response given by the server, insist on speaking to the chef. If some doubt remains, the best strategy is not to order the dish in question.

With the use of the DBPCFC to establish a firm diagnosis of food hypersensitivity and the careful monitoring of the dietary content and nutritional requirements, food-allergic patients can thrive and maintain a normal lifestyle.

Appendix

The following recipes have been contributed by the Food Allergy and Anaphylaxis Network and patient families. The Food Allergy and Anaphylaxis Network provides an extensive resource of educational materials and other resources for individuals with food allergies and their caregivers. For a detailed listing of available information contact the network at (800) 929-4040 or through the Internet (*http://www.foodallergy.org*) or e-mail (faan@foodallergy.org). All of these recipes are free of milk, egg, peanut, nut, fish, and shellfish; in addition some recipes will also be free of other allergens as noted.

M, E, P, S, N Free
Deep Dish Pizza

2 packages Quick Rise dry yeast
2 c warm water (90°)
½ c vegetable oil
4 tbsp olive oil
½ c corn meal
5½ c flour

In a food processor or heavy duty mixer dissolve yeast in water; add oils, corn meal and 3 c flour. Beat 10 minutes. Attach dough hook and add the remaining 2½ c flour. Knead for several minutes with the machine. Let rise until doubled in bulk, and then punch down. Let rise again and punch down.

Using olive oil, oil large (10 in) round cake pans. Place dough in center of pan; use your fingers to push dough out to the edge and up the sides of the pan. Dough should be about ⅛ inch thick. Place meat toppings on the bottom of the pie. Next, add tomatoes or tomato sauce and other toppings as desired

Bake at 475° for 20–40 minutes, checking frequently.

Makes crust for 2–3 pizzas.

M, E, P, S, N Free
Wacky Cake

1½ c flour
1 c sugar
½ tsp salt
3 tbsp cocoa powder
1 tsp baking soda
1 tsp vanilla extract
1 tbsp vinegar
5 tbsp oil
1 c cold water
Confectioner's sugar

Preheat oven to 350°. Sift dry ingredients into mixing bowl. Add vanilla, vinegar, oil, and water. Blend well, pour into ungreased 9-inch square pan. Bake at 350° for 25–30 minutes. Sprinkle with confectioner's sugar.

Alternative: Omit cocoa powder, and add one mashed banana after adding water.

M, E, P, S, N Free
Gingersnaps

¾ c milk-free margarine
1 c brown sugar
¼ c molasses
2 tbsp orange juice
2¼ c flour
2 tsp baking soda
½ tsp salt
1 tsp ground ginger
1 tsp ground cinnamon
½ tsp ground cloves

Cream together the margarine, brown sugar, molasses, and orange juice. Sift together in a separate bowl the flour, baking soda, salt, ginger, cinnamon, and cloves. Stir the dry ingredients into the molasses mixture.

Form dough into small balls. Roll in granulated sugar; place 2 inches apart on greased cookie sheet. Bake in moderate oven (375°) for 12 minutes. Makes about 5 dozen cookies.

M, E, P, N, W Free
Brett Derek's Lasagna Recipe

Ingredients/Preparation

3 c Italian-style tomato sauce
3 lb firm tofu, blended thoroughly

Mix with tofu

½ c fresh lemon juice
4 tsp honey or sugar
6 tbsp oil
4 tsp basil
1 tsp garlic powder or minced garlic
2 tsp salt (optional)
2 medium eggplants
or
5 Japanese eggplants (sweeter)

Cut into ¼-inch-thick slices and soak in salt water for 5 minutes; rinse the salt off. Dredge the eggplant slices in the following rice flour mixture.

Mix together

1¼ c rice flour
½ c corn meal
1 tsp oregano
2 medium cloves garlic, crushed
Dash of pepper
½ tsp salt (optional)

Oven-brown eggplant: lay on lightly oiled cookie sheet and bake at 350° until brown. Turn.

In a 9 in × 13 in pan, layer:

1 c sauce
1 layer of eggplant
1 thick layer of tofu mix
1 layer of eggplant
2 c sauce
Remainder of tofu

Bake at 350° for 35 minutes. Remove, set, cut, and serve.

M, E, P, S, N, W Free
Pumpkin Bread

½ c milk-free margarine
1 c sugar
1 tsp vanilla
½ tsp cinnamon
½ tsp nutmeg
¼ tsp ginger
1¾ c barley flour
1 tsp baking soda
½ tsp salt
1 c canned pumpkin
¼ c water
2 tsp baking powder
4 tbsp water
½ c raisins (optional)

Cream margarine, then blend in sugar and vanilla and set aside. Sift together cinnamon, nutmeg, ginger, flour, baking soda, and salt. Blend pumpkin with ¼ c water. Add baking powder dissolved in 4 tbsp of water to sugar/margarine mixture. Add dry ingredients alternately with pumpkin and water mixture. Blend well. Add raisins if desired. Pour into 9 in × 5 in × 3 in greased loaf pan. Bake at 350° for 1 hour.

M, E, P, S, N, W Free
Banana Muffins

2 mashed bananas
⅓ c sugar
¼ c corn oil
2 tsp baking powder
½ tsp vanilla
1¼ c rice flour
½ tsp baking soda

Preheat oven to 325°. Grease muffin tins.

Mix the bananas, sugar, and oil together well. Add the baking powder, vanilla, rice flour, and baking soda to the banana mixture. Mix well. Pour into the muffin tins and bake for 25 minutes until done.

M, E, P, S, N, W Free
Oatmeal Cake

1 c milk-free margarine
1 c brown sugar
4 c quick oats
1 tbsp baking powder
1 tbsp vanilla
½ c hot water

Mix all ingredients together. Flatten into ungreased 9 in × 13 in pan and let stand for 10 minutes.

Bake at 350° for 1 hour. Cut into squares and cool.

M, E, P, S, N, W Free
Brett's Gingerbread Men

¼ c milk-free margarine
½ c sugar
½ c molasses
1¼ c rye flour
1¼ c corn starch
1 tsp baking soda
¼ tsp ground cloves
¾ tsp ground cinnamon
¼ tsp ginger
½ tsp salt
½ c hot water

Preheat oven to 350°. Grease cookie sheets.

Cream margarine. Add sugar and then molasses. Sift dry ingredients and add alternately with water. If dough is too gummy, add rye flour.

Roll dough to ¼ inch thickness on floured board. Cut out and decorate. Bake for 8 minutes.

M, E, P, S, N, W Free
Oatmeal Cookies

1 c milk-free margarine
1 c brown sugar
1 c granulated sugar
4 c quick oats
½ tbsp baking powder
½ tbsp baking soda
1 tbsp vanilla
¼ c hot water
1 tsp cinnamon
1 c raisins (optional)

Preheat oven to 350°. Mix all ingredients together. Drop on to ungreased cookie sheet. Bake for 25–30 minutes, until golden brown.

Cool, then remove from baking sheet. The cookies must be cool before they can be removed or they tend to crumble.

M, E, P, S, N, W Free
Puffed Rice Treats

½ stick milk-free margarine
40 regular size marshmallows
5 c puffed rice cereal
Milk-free margarine (to grease pan)

Grease an 8 in × 8 in × 2 in baking dish. Melt the margarine in a large pot over low heat. Add marshmallows and stir until completely melted. Remove from heat and add puffed rice. Stir until mixture is well coated. Pour into baking dish, flatten with a spoon. Let cool before cutting into bars.

M, E, P, S, N, W Free
Playdough

1 c corn starch
1 lb baking soda
1¼ c water and food coloring, a little oil

Cook until mealy. Put on a plate. Cover with a damp cloth. Allow to cool. Knead.

Note: Doesn't keep very well, but is fun for someone who can't use wheat-based dough.

M, E, P, S, N Free
Home-Style Pancakes

2 c flour
4 tsp baking powder
½ tsp salt
2 tbsp sugar
2 c water
3 tbsp oil
¼ tsp vanilla extract

Sift dry ingredients together. Add remaining ingredients and beat together.

Pour the batter to form circles about 4 inches in diameter onto a hot, lightly greased griddle or heavy skillet. Cook for 2–3 minutes or until pancakes have a bubbly surface and slightly dry edges. Turn pancakes; cook for 2–3 minutes more or until golden brown.

Suggestions: For a special treat, pour this batter onto a hot griddle and form into a teddy bear, Mickey Mouse, or bunny shape. Add banana slices, blueberries, or other fruit to batter for variety.

Note: This batter can be used to make waffles.

M, E, P, S, N Free
English Muffin Bread

6 c flour
2 pkg active dry yeast
1 tbsp sugar
2 tsp salt
¼ tsp baking soda
2½ c water
Cornmeal

Grease two 8½ × 4½ inch pans and sprinkle with cornmeal. Combine 3 c flour, yeast, sugar, salt, and baking soda; set aside. Heat water until very warm (120°–130°). Add to dry mixtures; beat well. Stir in rest of flour to make a stiff batter. Divide between two loaf pans. Sprinkle tops with cornmeal. Cover and let rise in warm place for 45 minutes.

Preheat oven to 400°. Bake for 25 minutes. Remove from pans immediately and cool on wire racks. Slice and toast bread. This bread freezes well.

M, E, P, S, N Free
Blueberry Muffins

½ c milk-free, soy-free margarine at room temperature
1 c plus 2 tbsp sugar
3 tbsp water
3 tbsp oil
2 tsp baking powder, mixed together
1 tsp vanilla extract
2 tsp baking powder
¼ tsp salt
2 c flour
½ cup water
2½ c blueberries
1 tbsp sugar mixed with ¼ tsp ground nutmeg

Preheat oven to 375°. Line 12-muffin tin with paper liners. In a medium bowl, beat margarine until creamy. Beat in the sugar until pale and fluffy. Beat in water, oil, and baking powder. Add vanilla, remaining 2 tsp baking powder, and salt.

Fold in with a spatula half the flour and half the water. Add remaining flour and water. Fold in blueberries. Scoop batter into muffin cups. Sprinkle with nutmeg sugar. Bake 25–30 minutes or until golden brown. Let muffins cool slightly before serving.

M, E, W, P, S, N, G Free
Corn Muffins

⅓ c shortening
¼ c sugar
1 c Cream of Rice cereal
1 tbsp baking powder
⅔ c warm water
¼ tsp salt
1 tsp vanilla extract
1 tsp grated lemon rind
⅔ c cornmeal

Preheat oven to 375°. Line muffin tins with paper liners. Cream shortening and sugar. Mix rice cereal and baking powder in warm water. Combine with sugar and shortening mixture. Mix in remaining ingredients. Spoon into muffin cups (small muffins have a better texture). Bake 25 minutes. Makes 8 muffins.

Notes: These muffins hold together better if you let them cool a few hours or overnight.

M, E, P, S, N Free
Sweet Potato Muffins

1 c flour, sifted
1 tsp baking powder
¼ tsp baking soda
½ tsp salt
½ tsp ground cinnamon
½ tsp ground nutmeg (optional)
¼ c sugar
¼ c water
½ c cooked mashed sweet potatoes (about 1 large potato)
1½ tbsp water, 1½ tbsp oil, and 1 tsp baking powder, mixed together
2 tbsp milk-free, soy-free margarine, melted

Preheat oven to 350°. Line muffin tins with paper liners. In medium bowl, sift together flour, baking powder, baking soda, salt, cinnamon, and nutmeg if desired. Set aside. Combine sugar, water, sweet potatoes, water, oil, baking powder mixture, and margarine in mixing bowl. Add to flour mixture, stir until well moistened. Fill prepared muffin tins ⅔ full. Bake 25 minutes.

M, E, P, S, N Free
Zucchini Bread

3 c flour
½ tsp baking powder
1 tsp salt
2 tsp cinnamon
1 tsp baking soda
2 c grated zucchini (about 3 medium-size zucchinis)
4½ tbsp water, 4½ tbsp oil, and 3 tsp baking powder, mixed together
1 cup oil
3 tsp vanilla extract
2 c sugar

Preheat oven to 350°. Sift together first five ingredients. Add the remaining ingredients and mix well. Pour into loaf pans. Bake 55 minutes. Makes 3 loaves.

Note: This recipe freezes well.

M, E, P, S, N Free
Doughnut Holes

½ c plus 2 tbsp milk-free, soy-free margarine, softened
1 c sugar
3 tbsp water, 3 tbsp oil, and 2 tsp baking powder, mixed together
3 c flour
4½ tsp baking powder
½ tsp salt
½ tsp nutmeg
1 c apple juice (or water)

Preheat oven to 350°. Line mini-size muffin tins with paper liners. Blend margarine with sugar. Add the water, oil, and baking powder mixture. Mix well and then set aside. Sift together flour, baking powder, salt, and nutmeg. Add to the margarine and sugar mixture. Blend in the apple juice and mix together thoroughly. Fill muffin tins ⅔ full. Bake 15 minutes or until doughnut holes are golden brown.

Suggestions: Combine ½ c sugar with ½ tsp cinnamon; set aside. Melt 6 tbsp milk-free, soy-free margarine. While doughnuts are still warm, roll them in the margarine, and then roll them in cinnamon sugar.

M, E, W, P, S, N Free
Potato Stuffing

6 medium potatoes
⅔ c finely chopped onion
6 tbsp finely chopped fresh parsley
4 tbsp milk-free, soy-free margarine
Salt
Pepper

Peel and boil the potatoes in salted water. Drain, dice, and set aside. In a large frying pan, gently fry the onion and parsley in margarine. Add potatoes. Stir to coat the potatoes evenly. Season to taste. Place potato stuffing in poultry and roast.

M, E, P, S, N Free
Snacking Cake

1½ c flour
1 tsp baking soda
1 tsp cinnamon
½ tsp nutmeg
½ tsp salt
3 tbsp oil, 3 tbsp water, and 2 tsp baking powder, mixed together
½ c oil
½ c brown sugar, firmly packed
½ c white sugar
1½ c finely grated carrots (4 large)
1 (8 oz) can crushed pineapple, packed in its own juice, undrained

Preheat oven to 350°. Grease a 9-inch square or 7 in × 11 in pan.

In a large bowl, mix together flour, baking soda, cinnamon, nutmeg, and salt. Set aside. In another bowl, combine oil, water, and baking powder mixture. Add sugars. Stir well and then set aside. In a third bowl, combine the carrots and pineapple with its juice. Set aside.

Stir the oil, water, and baking powder mixture into the dry ingredients. Stir in the carrot-pineapple mixture. Spoon batter into the prepared pan. Bake 30–40 minutes or until a cake tester inserted in the center comes out clean.

Suggestions: This cake is delicious! Top with confectioner's sugar poured over a doily to dress it up for dessert or a party.

M, E, P, S, N Free
Cinnamon Crunch Cookies

1⅓ c flour
1 tsp cream of tartar
½ tsp baking soda
⅛ tsp salt
½ c milk-free, soy-free margarine, softened
¾ c sugar
½ tsp vanilla extract
1½ tbsp water, 1½ tbsp oil, and 1 tsp baking powder, mixed together
2 tsp ground cinnamon mixed with ¼ c sugar

Preheat oven to 400°. Grease cookie sheets. Stir together flour, cream of tartar, baking soda, and salt; set aside. In mixer bowl, combine margarine and sugar; beat until fluffy. Blend in vanilla. Beat in baking powder mixture. Gradually add to flour mixture, beating until just combined.

Drop by rounded teaspoons into the cinnamon sugar mixture. Roll cookies to coat well, shaping them into balls as you roll. Arrange balls about 1 ½ inches apart on greased baking sheets. Bake until edges are golden brown (8–10 minutes). Transfer to wire racks to cool. Makes about 3 dozen cookies.

Note: This cookie mixture can go from freezer to oven.

M, E, W, P, N Free
Coffee Can Vanilla Ice Cream

1 1-lb coffee can and lid, emptied and cleaned
1 3-lb coffee can and lid, emptied and cleaned
1 c milk-free, nondairy creamer
1 c soy milk
½ c sugar
½ tsp vanilla extract
1 c rock salt, divided
Crushed ice

Put all ingredients except rock salt and ice in the smaller can. Cover with lid. Place smaller can inside the 3-lb can. Pack crushed ice around outside of small can. Pour at least ¾ c rock salt evenly over ice. Cover the can and tape lid securely.

Roll back and forth on a table for 15 minutes. Open outer can and remove inner can. Remove lid. Scrape the ice cream off the sides of the can and stir the mixture to an even consistency. Replace lid. Drain ice water from larger can.

Insert smaller can and pack with more ice and salt. Roll back and forth for 15 minutes or until can frosts over. Stir and serve. Immediately freeze unused ice cream.

Suggestions: Fruit, crushed cookies, or a bit of coconut milk can be added for variety.

E, W, P, S, N Free
Coconut Rice Pudding

6 c coconut water
1 c uncooked medium-grain rice
½ c sugar
¼ tsp salt
2 tsp vanilla extract

Combine coconut water, rice, sugar, and salt in medium saucepan. Over medium heat, stir frequently until bubbles form around the edge. Reduce heat to low. Cover and simmer about 1 hour, or until rice is tender. Stir occasionally. Stir in vanilla extract. Cover and refrigerate until well chilled, about 3 hours.

Note: Excess coconut water can be kept in the refrigerator for later use or can be used as a refreshing coconut drink.

Coconut Water

1 (15-oz) can Coco Lopez cream of coconut
5 cans water

Before opening Coco Lopez, shake can well. Pour contents into a large pitcher. Add water and stir well.

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34

Hidden and Cross-Reacting Food Allergens

Scott H. Sicherer

Introduction

The two general topics covered in this chapter fall under the clinical rubric of multiple food hypersensitivity. The physician is often challenged by the patient who experiences reactions to multiple foods that are sometimes phylogenetically related and sometimes apparently unrelated. Table 34–1 lists several of the considerations for evaluating multiple food hypersensitivity. For the two general categories presented here, hidden food allergens may lead to the false assumption of multiple food hypersensitivities, because one or more previously identified food allergens are responsible for reactions to seemingly diverse food products through exposure in an unexpected manner. Alternatively, cross-reactivity may account for reactions to a variety of related foods of plant or animal origin based on immune reactions toward homologous proteins shared among them. Topics concerning the specific food proteins that frequently account for cross-reactions, oral allergy syndrome (OAS), diagnostic methods, and management of food allergy will not be emphasized here; rather, this chapter will introduce concepts and provide specific details to enhance the evaluation of patients with possible multiple food allergy, with a focus on hidden and cross-reacting food allergens.

Hidden Food Allergens

For the purpose of this chapter, the term "hidden food allergens" will refer to the variety of unexpected ways in which an individual may be exposed to food allergens (1–3). Of course, the degree of "unexpectedness" varies according to the knowledge one has about the topics presented. Also, a "hidden" food allergen may be a hidden ingredient to the consumer that is not necessarily a hidden ingredient in the mind of a manufacturer or chef who provided the food. For example, the use of peanut flour to thicken tomato sauce or chili underscores the importance of maintaining a clear line of communication when an allergic individual is depending on food provided by a restaurant or other commercial source without ingredient labels. Food proteins can turn up in many unexpected ways. For example, a teacher may use egg white to make finger paints smoother. Table 34–2 lists ways in which exposure may occur within the context of hidden food allergens.

Commercial Food Products: Manufacturing and Labeling Issues

Consumers with a known food allergy depend on accurate food label ingredient lists to determine the safety of their food. These consumers' safety is founded in both their ability to decipher the statements on the label and the accuracy of that label. Errors on both fronts can occur. Sometimes mistakes are apparent from simple misunderstandings: egg substitutes may appeal to an egg-allergic consumer who assumes the product is egg-free and not realize that egg is clearly labeled as an ingredient. In other cases, the consumer simply cannot trust the label because ingredient labels may not accurately reflect the presence of allergens. In January 2001, the US Food and Drug Administration (FDA) reported an investigation of 85 selected food companies in Minnesota and Wisconsin (4). The investigation was, in part, in response to a significant increase in the number of

Table 34–1.
Considerations in Evaluation of Patients with Apparent Multiple Food Allergies

Type	*Cause*	*Example*
True reactions to multiple food types		
True MFA	True allergic reactions to multiple, diverse food allergens. Usually in highly atopic patients.	Reactions to egg, milk, wheat and soy in one child
Intolerance	Non-immune mediated conditions causing adverse reactions when various foods are ingested.	Intolerance of fat resulting in GI upset to fatty meats; lactase deficiency resulting in symptoms from milk; fructose/sorbitol intolerance resulting in "acidic" diarrhea from multiple fruits.
Cross-reactivity	Homologous proteins among foods and between foods and environmental allergens	Pollen food allergy syndrome; latex-fruit syndrome; panallergens in related foods
False assumption of MFA		
Multiple positive SPT/RAST	Multiple tests for IgE antibody are positive and reactions are assumed to be related without further evaluation (history, oral challenge)	Atopic individual inappropriately tested to a wide battery of allergens has numerous positive tests and told to avoid all of the foods.
Hidden ingredients	Reactions to apparently diverse products because of exposure to a hidden/unexpected source of one or a few previously identified allergens	Milk-allergic child reacts to soy desserts and canned tuna because they contain casein.
Unproven tests	Use of unproven/experimental tests that identify multiple problematic foods for potentially vague symptoms	IgG antibody tests identify 43 foods purported to cause weakness in an elderly patient.
Psychological	Previous food-allergy related traumatic event generalizes to increasing numbers of reactions that are based on psychological triggers.	A severe peanut allergic patient develops paleness and syncope when exposed to a products that she thought contained peanut, but did not.
Misperception	Chronic complaints are attributed to adverse reactions to a variety of foods without a pathophysiological explanation.	Patient with perception that his headaches are triggered by orange foods (carrot, sweet potato, squash, orange soda).

MFA, multiple food allergies.

recalls of products for undeclared allergens. The firms investigated were small, medium, and large bakeries, candy manufacturers, and ice cream manufacturers. They were reviewed for their approach to food allergens. Assays were conducted to determine the presence of peanut and egg in finished products. The study found that 25% of products contained undeclared allergenic ingredients, often from cross-contamination, and that 47% of the firms did not check their products to ensure that the labels were accurate. The medical literature contains reports of clinical reactions to foods with allergen contamination not declared on the ingredient label for several allergens including egg, milk, and peanut (2, 5–8), despite the potential for minor ingredients to cause severe reactions that has been known for decades (3, 9).

Table 34–2.
Modes of Exposure to Hidden or Unexpected Food Allergens

Mode of exposure	*Examples*
Hidden ingredient in manufactured product	Undeclared ingredient, contaminant, ambiguous label, non-standard terminology
Non-food item	Pet food, shampoo, ointment, cosmetics, bath products
Medications	Egg, soy, and milk (often in clinically irrelevant concentrations) in a variety of medications (carriers)
Cross-contact	Shared equipment in restaurant/bakery causes contamination
Non-food allergen found in food	Dust mite contamination of grains
Unexpected exposure route	Skin contact from residual food on table or chair; inhalation of fumes during cooking

Governmental oversight of manufactured products varies worldwide (10, 11). In the US, regulations pertaining to the declaration of food ingredients and the impact on the declaration of allergens are evolving. For example, in 1993 the Code of Federal Regulations (CFR) 102.22 required food source identification for hydrolyzed proteins (e.g., hydrolyzed wheat protein). Some regulations have caused confusion. For example, collective terms such as flavors, colors, and spices may be used without denoting the source(s), and allergenic ingredients could be included among them. Examples include milk as a "natural flavor" and garlic as a "spice." The declaration of incidental additives found in "insignificant" quantities has also been an exemption until recently when allergens have

not been considered to qualify under this regulation. Precautionary labels such as "may contain" or "manufactured on equipment with" are also a source of confusion that are under scrutiny and reevaluation. Lastly, manufacturers may use technical terms that are unfamiliar to consumers such as "casein," "caseinate," or "whey" that indicate the presence of cow's milk protein.

To learn more about allergy-related issues with commercial food products, we (12) analyzed unsolicited calls from consumers to the Food Allergy and Anaphylaxis Network (FAAN), a lay organization that provides educational support for families with food allergy. The calls evaluated were made to alert FAAN of allergic reactions from incorrectly or problematically labeled commercial products or to provide "Good Samaritan" notification of labeling issues. Two hundred six calls about commercial products were analyzed over a period of 24 months. Among 58 episodes of cross-contamination, 65% were brought to attention because of otherwise unexplained reactions to the product, and 35% were based on consumer-initiated calls to the manufacturer. The potential for error was confirmed by the company in 88% of these incidents (e.g., shared processing equipment). A high rate of calls (22%) were to alert FAAN that a new allergen was added to a commercial product. Because members of FAAN are provided with lists of words that signify common allergens (e.g., "casein" and "whey" indicate cow's milk protein, "natural flavors" may indicate a food allergen), problems caused by these ambiguous terms were probably underreported. Figure 34–1 shows the distribution of calls according to the various complaints/notifications.

We also undertook an investigation to determine the ability of families of food-allergic children to accurately read ingredient labels (13). Parents of children on food-allergen restricted diets attending our referral center were asked to review 23 food labels from widely available commercial products. For each label, they were to indicate whether the product was safe for their child and, if not, which food(s) restricted from their child's diet were in the product. In total, 14 labels indicated milk, seven soy, five peanuts, 10 wheat, and seven egg. Ninety-one participants with food allergies were distributed as follows: peanut, 82 children; milk, 60; egg, 45; soy, 27; and wheat, 16. Iden-

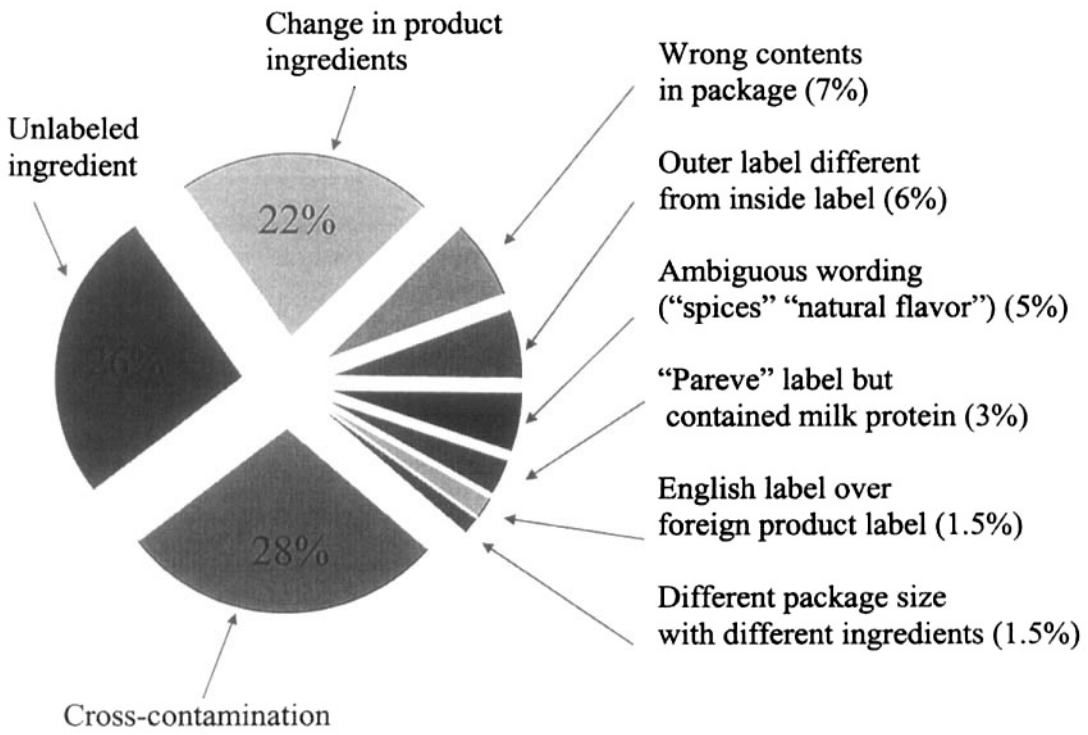

Figure 34–1. The distribution of labeling problems encountered with commercial products. Unlabeled ingredient: a visible ingredient was discovered in a product; change in product ingredient: a previously used product has a new allergenic ingredient; wrong contents: completely different product in package; outer label different: packages with smaller, individually labeled products inside.

tification of milk and soy was most problematic, with only 4 (7%) of 60 parents correctly identifying all 14 labels that indicated milk, and 6 (22%) of 27 correctly identifying soy protein in seven products. Peanut was correctly identified in five products by 44 (54%) of the 82 parents restricting peanut. Wheat (10 labels) and egg (seven labels) were correctly identified by most parents (14 of 16 and 42 of 45, respectively). Correct label identification was associated with prior instruction by a dietitian or membership in FAAN. Overall, however, most parents were unable to identify common allergenic food ingredients. The magnitude of the confusion related to label reading is further demonstrated by the fact that 48% of the participants indicated that they routinely need to contact manufacturers to determine if the food that they are avoiding is contained in particular products.

The previously recognized deficiencies in manufacturing and labeling have come under scrutiny by professional, public, governmental, and lay organizations that have made suggestions for improvements. The aims for improved manufacturing and labeling include the use of simple language such as "milk" in place of terms such as "casein"; that allergens be declared when present in spices or natural flavors; and that precautionary statements such as "may contain" not be used unless it is clear that there are no viable alternatives to prevent contamination, despite good manufacturing practices. Improvements in how allergens are indicated on food ingredient statements have also been suggested (e.g., "contains the following allergens," the use of bold fonts, etc). Changes in manufacturing practices have also been suggested that include regulations about review of labels, cleaning procedures, and use of reworked foods. Clearly, continued patient education on careful label reading is paramount to successful avoidance. The challenges are great, however, and the patient and clinician must keep an open mind when dealing with unexplained reactions to foods. For example, a milk-allergic child may react to seafood if it was treated with milk proteins on the dock to enhance a fresh scent, and it may be very difficult to trace this type of contamination.

Cross-Contact

Cross-contact or cross-contamination is an important issue in and out of commercial manufacturing. Small quantities of allergens can trigger reactions, including amounts that may be carried over in various ways from an "unsafe" food to one that is purportedly free of the allergen. Simple examples of this problem abound. In the home setting, a knife used to spread peanut butter could next contact and contaminate jelly. In restaurants, shared grills, pans, food processors, and other equipment used without thorough cleaning between preparation may be a source of cross-contact. Bakery goods pose similar problems as shared bowls, mixing equipment, and pans may allow for cross-contact. In ice cream shops, dipping scoops from one flavor to the next can cross-contaminate otherwise safe flavors. In the school setting, cross-contact has been identified as a possible source of inadvertent exposures to peanut and tree nut through shared utensils and cross-contact of foods (14). Products labeled "pareve" represent a problematic issue of cross-contamination combined with false assumptions by consumers is demonstrated by "pareve" labeled products (2, 12). Pareve is a religious term meaning non-dairy, but it does not ensure absence of milk proteins. These products may be used by unknowing milk-allergic consumers who consequently have reactions due to cross-contamination by cow's milk.

The author and colleagues (15) evaluated allergic reactions in peanut- and tree nut-allergic subjects that were associated with restaurants and food from establishments such as bakeries and ice cream shops. Of 5149 voluntary registrants in the US National Peanut and Tree Nut allergy registry, 14% indicated that they had experienced a reaction in these types of establishments. A review of 156 episodes among 129 randomly selected registrants revealed that 39% of reactions were due to peanut or tree nut not clearly identifiable to the patron (e.g., "hidden," in sauces, dressing, egg rolls, etc.). In 22% of cases, cross-contact was involved, primarily due to the use of shared cooking or serving supplies. Cross-contact in desserts, Asian cooking, and buffets was particularly problematic.

The lessons learned from the study of reactions in restaurants and food establishments highlight several important issues concerning allergen exposure in these settings and others. Ideally, procedures would be in place to benefit food-allergic patrons. Personnel would receive training about food allergy, the potential for trace protein contamination to trigger reactions, a variety of methods to avoid cross-contamination, and how to activate emergency assistance in the event of a reaction. A clear line of communication among the patron, server, and those preparing the foods must

be established and maintained. Menu items should include a description of the ingredients in the food. In addition, the restaurant personnel would be advised about the potential for cross-contamination (shared fryers, blenders, utensils, mixers, pans, and grills; contamination by garnishing bars, hands, and gloves) and methods to avoid this problem (use freshly cleaned, separate equipment; change gloves). For effective prevention of reactions due to cross-contact, education of the allergic individual about these issues is paramount.

Unexpected Sources of Food Proteins in Non-Food Items and Medications

Allergenic food proteins may be components of a variety of items not meant for ingestion by humans. Pet foods may contain many classically allergenic food proteins such as milk, peanut, soy, and seafood. Inadvertent ingestion by curious allergic children must be considered when these foods are left on the floor for household pets. A number of hair care products and topical skin care products contain food proteins (e.g., almond, soy). Reactions to these products applied topically are usually not severe. Nipple creams used in some countries may contain peanut protein and have been considered as a possible, but unlikely, cause of peanut sensitization in infants (16).

Patients with food allergies and their physicians must always consider that a drug (or vaccine) reaction may be induced by a food ingredient in the drug. Well-known examples of this phenomenon include egg protein (17, 18) in influenza and yellow fever vaccines, and gelatin (19) in a variety of other vaccines. Many other food-related ingredients used in medications have not been well studied in terms of their allergic potential. Pharmaceutical grade lactose is used in many medications, and egg or soy lecithin and soy oil are found in a variety of medications, but the clinical relevance to most individuals with these allergies remains unexplored.

Non-Food Allergens in Foods

Case reports exist of non-food allergen contamination of foods resulting in allergic reactions. For example, dust mites may contaminate flour mixtures and cause severe reactions when ingested by dust-mite allergic patients (20, 21). This appears to be a particular problem in tropical climates. The use of latex gloves by food handlers has resulted in unexpected reactions when these foods are ingested by latex-allergic individuals (22, 23). Indeed, latex allergens are detectable on food products following handling with powdered latex gloves (24). Insofar as parasites are not intentionally consumed, it is worthwhile to note that the nematode *Anisakis simplex* that infests some fish can induce allergic reactions. This appears to be a particular problem in Spain and other countries with a high fish consumption, and is associated with undercooking (25).

Non-Standard Exposure Routes to Food Allergens

Exceptional cases exist of systemic reactions to topical exposure to foods result in systemic reactions (26). More commonly, however, topical exposure leads primarily to isolated, local skin reactions. In such cases, residual food proteins on tables and chairs may induce rashes. Although not truly hidden or unexpected, school craft projects using peanut butter (e.g., peanut butter covered pine cone bird feeders) are commonly responsible for reactions despite school consciousness about avoiding peanut as an ingestant (14).

Airborne exposure to food allergens is not unexpected in a variety of industrial food processing settings (e.g., baker's asthma), but is a potential hidden source outside of these settings. There are several published case reports of acute allergic reactions to airborne food particles such as string bean (27), lentil (28), meats (29), and seafood (30–33) usually during cooking (rapidly boiling milk, frying eggs, steaming soups, sizzling fried seafood, etc). Peanut reactions to inhalation of peanut dust during commercial airline flights have been reported (34–38). The powdery material from roasted peanuts may become airborne in airliners (39) and induce reactions in that setting: a closed space where many bags are opened simultaneously. These reactions are generally isolated to the upper and sometimes lower respiratory tract.

Cross-Reacting Food Allergens

When an allergic response is established toward a particular protein, a homologous form of that protein in another substance may also trigger an allergic response (cross-reaction). Hence, true allergic reactions to multiple foods may follow initial sensitization caused by one food. The initial sensitization may occur by the oral or inhaled

route. In fact, as discussed in Chapter 6, the appropriate immunological response to ingested proteins is tolerance, and for most individuals food allergies do not occur, despite other atopic respiratory illnesses. However, immune tolerance to foods may be bypassed by initial sensitization to homologous proteins that contact the respiratory tree (e.g., pollen-allergy syndrome). In this way, IgE antibody toward the respiratory allergen can also induce disease when the homologous protein is ingested. As will be discussed below, the scenario of respiratory sensitization resulting in food allergy may apply to pollens, latex, and insect antigens that are airborne allergens with homologous proteins in foods. In addition to sensitization by the airborne route, typical sensitization to a particular food through the gastrointestinal (GI) tract can result in reactions to foods containing homologous proteins. Reactions in this setting are typically more severe because they involve proteins that are capable of sensitization by ingestion, and these are proteins that are typically more stable to digestion and able to enter the systemic circulation (40). In some cases, distantly related foods or environmental allergens contain common (conserved) homologous (pan)allergens. To complicate matters further, however, there may be homologous, allergenically important sequences (epitopes) shared among even more distantly related foods that may trigger reactions in some individuals (e.g., seed storage proteins in peanut, sesame, and tree nuts) (41).

Plant-derived proteins responsible for allergy include various families of pathogenesis-related proteins, protease and α-amylase inhibitors, peroxidases, profilins, seed storage proteins, thiol proteases, and lectins (42) and homologous animal proteins include muscle proteins, enzymes, and various serum proteins. Over 70% identity in primary sequence is generally needed for cross-reactivity (43). The biochemical attributes of these proteins will not be discussed here, rather the clinical relevance of potential cross-reactivity will be the focus.

To elicit a clinical response, the causal food protein must maintain the ability to present the epitope in an immunologically relevant form. That is, evidence of IgE binding to a potentially cross-reactive food protein (sensitization demonstrated by skin prick test [SPT] or radioallergosorbent test [RAST]) is not evidence of clinically relevant allergy to the food. In fact, it is quite common to find food-specific IgE antibody by SPTs or RASTs to foods related to the one causing the index reaction. For example, using RASTs, Barnett et al (44) screened sera from 40 peanut-allergic patients against 10 other legumes and demonstrated IgE binding to multiple legumes for 38% of patients. Similarly, Bernhisel-Broadbent and colleagues (45) studied 62 children with allergy to at least one legume and found that 79% had serologic evidence of IgE binding to more than one, and 37% bound all six legumes. The scenario is similar for tree nuts (46–48). In our studies of tree nut allergic children (46), 102 (92%) of 111 patients with peanut and/or tree nut allergy had IgE antibody to more than one tree nut. In all of these cases, however, it is much more common to find that the food to which there is cross-sensitization is actually tolerated when ingested (49). Factors that determine the clinical appearance of allergy in the face of sensitization are complex and relate to the host (immune response, target organ hyperreactivity) and the allergen (lability, digestibility) (40). Presumably, these factors also bear on the clinical relevance of potentially cross-reactive foods. The information that follows may be valuable in deciding on the best approach to diagnosis of potential allergy to cross-reactive foods (the utility of in vivo and in vitro tests).

Cross-Reactions Among Specific Foods or Food Families

Legumes

Despite the high rate of cross-sensitization to legumes (beans), clinical cross-reactions are uncommon. Peanut and soy are the most highly allergenic legumes that are dietary staples in North America, yet the rate of clinical cross-reactivity is low. Among 113 children with atopic dermatitis (AD) evaluated with double-blind, placebo-controlled oral food challenges (DBPCFC), only one (0.8%) had clinical allergy to both foods despite 19% reacting to peanut and 5% to soy (50). Bock and Atkins (51) studied 32 children with peanut allergy confirmed by DBPCFCs and found that 10 (31%) had a positive skin test to soy, but only one (3% of those with peanut allergy) had a clinical reaction to soy. In considering a wider variety of legumes, only 3 (1.8%) of 165 children with AD evaluated with DBPCFCs reacted to more than one legume despite 31 (19%) reacting to at least one (52). In recent reviews of children with peanut allergy where DBPCFCs were not routinely performed, higher estimates of co-reactions are re-

ported: 14 (14%) of 102 (46) and 34 (15%) of 223 children (53). Bernhisel-Broadbent and Sampson (45) specifically addressed the issue of legume cross-reactivity by performing open or DBPCFCs in 69 highly atopic children who had at least one positive skin test to a legume. Oral challenges to the five legumes (peanut, soybean, pea, lima bean, green bean) resulted in 43 reactions in 41 patients (59%). Only two (5%) of 41 with any one positive challenge reacted to more than one legume.

It may be the case that particular legumes are more likely than others to trigger reactions and also that the types of beans consumed in various cultures (e.g., lupine used whole or as flour in breads) also affects the rate of cross-reactions (54–56). For example, 11 (44%) of 24 French children with peanut allergy (56) had positive skin tests to lupine; of six subjects who underwent DBPCFCs, and two who had labial challenges to lupine, seven reacted. As a probable reflection of cultural and geographical influences on the diet, allergy to lentil is more common than to peanut in Spain (57). Furthermore, of 22 Spanish children with lentil allergy evaluated for reactions to other legumes (58), six had a history of reacting to chick pea, two to pea, and one to green bean. These findings raise suspicion for multiple legume allergy in those reacting to lentil, lupine, and chick pea, but more studies in a variety of geographic settings, utilizing blinded challenges to confirm reactivity, are needed to quantify the risks.

Clinically, multiple legume allergy is relatively uncommon, but positive skin tests to multiple legumes are common in an atopic patient with a reaction to one. Thus, it is not appropriate to assume that a particular patient has multiple legume allergy; rather, a more definitive evaluation should be undertaken to ensure that tolerated beans are available as personal preferences would indicate. Furthermore, tests for specific IgE antibody in this scenario may be helpful primarily when they are negative, because positive tests are common despite clinical tolerance. An individual with more than one legume allergy is at higher risk for even more legume reactions, and lentil, lupine and chick pea may be slightly more likely to be involved in this scenario than others (e.g., pea, string bean).

Tree Nuts

Clinical reactions to tree nuts can be severe (59) and potentially fatal, and can occur from a first apparent exposure to a nut in patients allergic to other nuts (60). Because of the frequency of severe reactions, there are no comprehensive studies on clinical cross-reactivity among tree nuts. Bock and Atkins (51) performed challenges to one or more nuts in 14 children and at least two reacted to multiple nuts (as many as five types). Ewan (59) reported allergy to multiple tree nuts in over one third of 34 patients evaluated for tree nut allergy. Similarly, our group noted that in 54 children with a tree nut allergy, reactions to more than one nut occurred in 20 (37%) (46). Some nut allergens may be homologous and cause reactions (e.g., in pistachio and cashew [61]), and others may be homologous but rarely elicit clinical cross-reactivity (e.g., proteins in coconut and walnut [62]).

Legume, Tree Nuts, and Seeds

Co-sensitization to allergenic foods such as peanut, tree nuts, and seeds (e.g., sesame, poppy, mustard) is common. In a study of 731 subjects in the UK, 449 (59%) sensitized to peanut were also sensitized to hazelnut and/or Brazil nut (47). Although clinically significant cross-reacting proteins have not yet been described, some amino acid sequences (epitopes) are highly homologous among some of the seed storage proteins that constitute the major allergens in these foods (41). Co-allergy to peanut and tree nut has been reported at 23%–50% in referral populations of atopic patients (46, 59, 63, 64). This observation provokes the question, is this high rate of co-reactivity due to homologous proteins or to expected allergies to intrinsically allergenic foods among highly atopic patients? Tools are available to answer this important question, and methodical searches are under way. Until more data are available, the clinician must consider the patient's age, history, and sensitization in considering categorical elimination of these allergenic foods (65). Reactions to seeds such as sesame, mustard, and poppy are increasingly reported (48, 66, 67), and cross-reactivity with foods (hazel, kiwi, other seeds) and pollens is potentially important.

The full clinical implications of possible cross-reactivity among peanuts, tree nuts, and seeds are not yet established. From a practical perspective, considering the potential severity of the allergy and issues with accurate identification of particular nuts in prepared foods, caution seems prudent and total elimination of the nut family (perhaps with the exception of previously tolerated nuts eaten in isolation) is often suggested (46, 68). These recommendations may be over-restrictive. No

consensus yet exists as to whether seeds are highly likely to elicit reactions among individuals with peanut/tree nut allergy, but based on the studies thus far, some caution is warranted.

Fish

The clinical studies concerning fish allergy mirror those of tree nut allergy, in that clinical reactions to multiple fish is a common phenomenon, high cross-sensitization rates are even more common, and the allergic reactions tend to be severe (69–71). A few studies have utilized DBPCFCs challenges to evaluate fish allergy. In 10 US children evaluated by DBPCFCs to four to six species of fish, and in whom reactions were confirmed to at least one species, three reacted to more than one type (69). Hansen and colleagues (72) evaluated eight adults with codfish allergy proven by DBPCFCs. Sensitization to plaice, herring, and mackerel was nearly 100%, and among patients exposed to each (six, five, and six patients, respectively) all had a history of clinical reactions. In a study of six adults from Denmark with a positive DBPCFC to at least one of three fish (catfish, codfish, snapper) and challenged to at least two types, four reacted to more than one species (70). Several studies that did not utilize DBPCFCs provide additional information that is in agreement with these formal studies. In 61 children with a history of fish allergy exposed to two to eight species, 34 (56%) reacted to all and 27 (44%) tolerated some types (71). In a study of 20 codfish-allergic Italian children (73), a high frequency of positive skin tests (from 5% to 100% for each of nine species tested) was documented. For those who ingested the fish to which antibody was detected, the clinical reaction rate per fish, based on history, was 25%–100% depending on the species. Some fish were more problematic than others in these cod-allergic children. Eel, bass, sole, and tuna most frequently provoked reactions, and salmon, sardine, and dogfish were least likely to induce symptoms. Regional exposure patterns are relevant. Pascual and colleagues (74) from Spain evaluated cross-reactivity among six regionally important species in 79 children with fish allergy where codfish is not a common food. Although all subjects had positive skin tests to multiple species, only 31 (39%) of 79 had clinical reactions, and hake and whiff had the highest, and albacore the lowest reaction rate. In contrast to the studies that indicate a high likelihood of multiple fish allergy, several reports demonstrate isolated allergy to a single species of fish (e.g., tropical sole [75], swordfish [76]). This apparently occurs because of immune responses toward species-specific allergens without IgE antibody to the common fish panallergens (e.g., Gad c 1). Formal studies of fish hypersensitivity have also indicated that fish proteins may be denatured when heated (canned) or lyophilized, which may explain a history of specific fish that appear to be tolerated in some forms, e.g., reactive to salmon but not reactive to canned salmon (77).

In summary, a fish-allergic patient is at high risk for reactions to other fish but may tolerate some fish species; therefore, if the patient wishes to try other fish, further evaluation with supervised oral challenges is warranted. The fact that fish allergy can be severe and that cooking/canning and other processing can alter allergenicity must be considered during these evaluations (77).

Shellfish

The clinical impression is that reactions to multiple crustaceans are fairly common, but few clinical studies have addressed this issue. The major shared allergenic protein is invertebrate tropomyosin found in crustaceans such as shrimp, crab, and lobster (78–80), and mollusks such as oyster, scallop, and squid (81). Not surprisingly, the rate of cross-sensitization is high. In 16 atopic, shrimp-allergic patients, > 80% had positive SPTs to crab, crayfish, and lobster (82). Unfortunately, formal clinical studies to determine the rate of clinical reactivity are lacking. In a study of 11 patients with immediate reactions to shrimp ingestion, the reaction rate to lobster, crab, and crayfish was 50%–100% per species (83). On the other hand, some individuals react not only to shrimp alone, but to certain species of shrimp (84).

Also poorly defined is the risk of mollusk allergy for crustacean- or mollusk-allergic individuals. Lehrer and McCants (85) studied serologies of six oyster-sensitive, seven oyster and crustacean, and 12 crustacean-sensitive patients. Most of the reactions to oyster were isolated to the GI tract and not associated with oyster-specific IgE antibody. However, among the 19 patients with sensitivity to crustacean, 9 (47%) had positive RASTs to oyster, indicating potential cross-reactivity. In another study that evaluated nine patients with shrimp anaphylaxis, binding to tropomyosin of 13 crustaceans and mollusks was universal (81). These studies only evaluated serologies so the rate of clinical reactivity is unclear, but apparently not great.

Invertebrate tropomyosin is also found in airborne insect allergens of cockroach and dust mite (81, 86, 87), which raises the possibility of sensitization by the respiratory route. A seafood restaurant worker developed IgE to tropomyosin and occupational asthma to both scallop (mollusk) and shrimp (crustacean) (88). In a report of wheezing induced by snail consumption in 28 patients, RAST inhibition studies indicated that house dust mite sensitization was the likely initial sensitizing event (87). Several reports link allergen immunotherapy with *Dermatophagoides pteronyssinus* to development of severe reactions to mollusks and crustaceans. Five of six patients from the Canary Islands with anaphylaxis to limpet, a mollusk, had received immunotherapy with dust mite (89). In a prospective study, two of 17 patients receiving dust mite immunotherapy developed cross-reactive IgE antibodies to tropomyosin and oral symptoms to shrimp (90).

Overall, crustacean species represent an increased risk of cross-reactivity with a potential for severe reactions and a potentially high rate of clinical symptoms. However, some individuals tolerate most types, so individualization, done cautiously, may be warranted. Allergy to mollusks is less well established and appears less common. Allergy to, and immunotherapy with, dust mite may be an additional risk factor, but determination of the precise risks requires further investigation.

Cereal Grains

Wheat, rye, barley, and oat share homologous proteins with grass pollens and with each other (91, 92), which may account for the high rate of cosensitization among these foods (91). Among children with at least one grain allergy undergoing DBPCFCs to multiple grains, 80% were tolerant of all other grains. Caution is warranted, but clinical reactivity to multiple grains appears uncommon and individualization is warranted for these common foods.

Avian and Mammalian Food Products

For avian foods such as chicken, sensitization has been described to α-livetin found in feathers, egg, and meat (93). Reactions to chicken meat is often based on reactivity to this protein (22%–32%) (93, 94). Chicken meat allergy is uncommon (95), but when it occurs in the absence of egg allergy, the risk of reaction to multiple species of avian meats (e.g., turkey, pheasant, quail) may be increased. This observation is probably because a meat-specific protein, rather than within species meat-egg-specific protein, is causally related to reactions (96, 97). Cross-reactive proteins among various avian eggs are also common (98), but the clinical implications have not been systematically studied. Conversely, allergy to one egg type may not guarantee reactions to others; reactions to duck and goose egg, but not to hen's egg, has been described (99).

Some patients with allergy to mammalian milks also react to mammalian meats. Homologous proteins may be responsible, or, more likely, identical proteins that are residual in meat and milk from the same animal. An oral challenge study showed that six (9.7%) of 62 cow's milk-allergic (CMA) children reacted to beef (100). Heating and other cooking processes can reduce the allergenicity of beef (101), so well-cooked beef is less likely to cause a problem for those with CMA. Reactions to multiple mammalian milks is more common than milk-meat reactions. In vitro studies showed extensive cross-reactivity among sheep, cow, ewe, buffalo, and goat milks (102), but not to camel's milk (103). Oral challenge studies of goat's milk showed this to be unsafe for patients with CMA; 24 (92%) of 26 CMA patients reacted to goat's milk (104). Mares' milk appears comparatively safe; only one (4%) of 25 children with CMA reacted to it (105). Unfortunately, most of the readily available animal milks are problematic for those with an allergy to any one of them.

In practical terms, most milk-allergic patients tolerate beef, cooking the meat well may improve tolerability, but some highly milk-allergic individuals do react. Overall, then, individualization is usually warranted. It may be less important to try to identify a mammalian milk for those with CMA, because cross-reactivity is very high and suitable alternatives (soy milk, rice milk) are available. Cross-sensitization is more common within than between avian and mammalian meats, but clinical correlation with sensitization is generally under 50%, so individualization is also usually warranted (106).

Fruit, Pollens, and Latex

Oral Allergy Syndrome

OAS (pollen-food allergy syndrome) is described elsewhere (Chapter 13) and the focus here will be on cross-reactions within families of fruits. Several studies have selected patients on the basis

of particular fruit allergies rather than pollen allergies, and evaluated them for reactions to related fruits. Rodriguez and colleagues (107) evaluated 34 adults in Madrid with reported allergy to foods in the Rosaceae family (peach, apple, apricot, almond, plum, pear, and strawberry). Twenty-eight (82%) had positive SPTs and/or RASTs to at least one of the foods, with a median of five positive foods per patient. Clinical reactivity determined by DBPCFCs was less than 10% for those positive to pear and up to 90% for peach (overall, 35% with a positive skin test reacted to a given food). Multiple fruit allergy was common in the 22 (46%) who reacted to at least one fruit. Peach was the dominant allergenic fruit; 46% reactive to peach reacted to another Rosaceae fruit. Pastorello and colleagues (108) studied patients selected for a history of reactions to peach confirmed through open oral food challenges; among 19 evaluated, 12 (63%) reacted to at least one other fruit among cherry, apricot, and plum. Of 19 patients with melon allergy confirmed by DBPCFC (of 54 patients suspected) 12 (94%) reacted to at least one of the following related fruits: watermelon, avocado, kiwi, chestnut, banana, and peach (109).

Severity of reactions to these foods is an important issue. Pollen-related fruit allergy is usually mild (OAS), yet in one study 8.7% experienced associated systemic symptoms outside of the GI tract (110), 3% at some time experience systemic symptoms without oral symptoms, and 1.7% experienced anaphylactic shock. It is becoming clear why some patients are more likely to experience severe reactions. When fruit allergy develops in the absence of pollen allergy, reactions are directed not only to Bet v 1 or profilins, but also to lipid transfer proteins (LTPs). Reactions involving fruits with homologous LTPs are more likely to be severe (111, 112). Fernández-Rivas and colleagues (113) compared patients with Rosaceae fruit allergy with (n = 22) and without (n = 11) pollenosis and found that systemic reactions occurred in 82% who did not have pollenosis, compared to 45% of those who did. Anaphylactic shock was also more common in the former (36% vs 9%, respectively). A similar theme was noted for hazelnut, where patients without pollenosis experienced severe reactions and had IgE binding to hazelnut proteins that were heat-stable (114). Asero (115) found that individuals with positive skin tests to commercial Rosaceae food extracts were more likely to experience systemic reactions than those positive only to fresh extracts, (64% vs 6%, $P < .001$). This observation is presumably explained by the likelihood that more stable allergens are present in the commercial extract compared to fresh fruit proteins, which include labile proteins that are more likely to induce only symptoms of oral allergy. The clinical lesson is that once a patient experiences more than oral symptoms to a fruit, a careful search by history and/or challenge may be warranted to prove the safety of related fruits. Furthermore, positive skin tests to commercial extracts and a lack of pollen allergy may indicate a higher risk of significant reactions.

Latex-Food Syndrome

Commonly reported latex cross-reactive foods include banana, avocado, kiwi, chestnut, potato, and papaya, and numerous latex allergens cross-react with food and pollen proteins (116, 117). In a study of 136 latex-allergic patients evaluated by RAST to 12 foods reported to be involved in latex-food reactions, 94 (69%) were positive to at least one food, and 67 (49%) were positive to more than one (118). Challenges were not performed, but 58 (42.5%) reported reactions to particular fruits. Of these 58 subjects, only 19 (33%) had a positive RAST to the implicated food. In another study of 47 latex-allergic patients, 100 (27%) of 376 food skin tests were positive, but only 27 (7.2%) were associated with clinical reactions (119). In evaluating the converse situation of fruit-allergic patients (excluded if there was a well-known risk factor for latex allergy) for sensitization to latex, 49 (86%) of 57 patients had latex-specific serum IgE antibody, and 6 (11%) experienced clinical reactions to latex (120).

Evaluation of natural rubber latex-food cross-reactivity is complicated by cross-reacting pollens, foods and co-allergy to various substances with potential allergenic relationships. There may be clinical value in differentiating individuals with isolated food, pollen, or latex sensitization (121). Levy and colleagues (122) evaluated adults with latex allergy with (N = 24) and without (N = 20) pollenosis, and a group without latex allergy and with pollenosis (N = 25) for allergies to 12 foods (by convincing history) classically associated with latex and pollen allergy. In those with latex allergy without pollenosis, reactions were reported to banana (four; 20%), avocado (four; 20%), kiwi (two; 10%), melon (one; 5%), and peach (one; 5%), whereas those with pollenosis were more likely to react to Rosaceae foods and celery. In the

pollen-allergic groups (n = 49), positive skin tests to the foods were found in 46% (268 of 588 tests), but for isolated latex allergy, only 24% (58 of 240 tests) were positive. The numbers of reactions among those with positive tests were generally less than 25%, except for reactions to banana, avocado, and kiwi, which approached 50% in those with latex allergy without pollenosis. Overall, caution is warranted and individualization is necessary, but for patients with allergy to latex, banana, avocado, or kiwi, it may be prudent to consider potential reactions to related foods.

Management of Cross-Reactivity

As outlined in each preceding section, there is a high likelihood of sensitization to foods that bear homologous allergens, but clinical reactivity correlates poorly. It is therefore necessary to consider several issues when evaluating a patient for the possibility of multiple food hypersensitivities on the basis of possible cross-reactions. Among these are a priori reasoning about likelihood of reactions (Fig. 34–2), severity of reactions, social and nutritional importance of the food, and the (poor) predictive value of tests for IgE antibody in this setting. However, for most foods and for most patients, multiple food allergies are relatively uncommon and the extra effort to prove which foods are or are not tolerated is worthwhile.

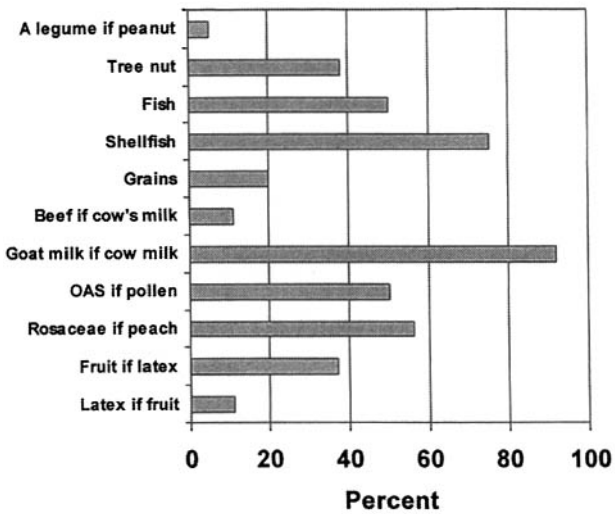

Figure 34–2. The approximate rate of clinical reactivity to at least one other related food. The probability of reacting to related foods varies and depends on numerous factors. Data reviewed in (49). OAS, oral allergy syndrome/pollen-food syndrome.

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Food Toxicology

Steve L. Taylor

Food toxicology can be defined as the science that establishes the basis for judgment about the safety of foodborne chemicals. The central axiom of toxicology, set forth by Paracelsus in the 1500s, states: "Everything is poison. Only the dose makes a thing not a poison." Thus, all chemicals in foods, whether natural or synthetic, inherent, adventitious, or added, are potentially toxic. The vast majority of foodborne chemicals are not hazardous because the amounts of each in the typical diet are not sufficient to cause injury. The degree of risk posed by exposure to any specific food-borne chemical is determined by the dose, duration, and frequency of exposure (and especially in the case of allergies, the degree of sensitivity of the individual). The age-old wisdom about the benefits of eating moderate amounts of a varied diet protects most consumers from harm. Food-borne chemicals that are considered to be toxicants are those of which the dose, duration, and frequency of exposure can sometimes be sufficient to elicit adverse reactions. Unusual diets can sometimes result in intoxication from chemicals that would normally be considered safe and desirable. For example, the intake of large amounts of vitamin A was hazardous to polar explorers who consumed large amounts of polar bear liver (1).

Acute adverse reactions to foods can occur through mechanisms such as infections (viral, bacterial, parasitic), intoxication, and allergies and intolerances. Food allergies are the major focus of this book. Other medical conditions, including some food intoxication, can cause symptoms that resemble food allergies. These other conditions must be considered and eliminated in diagnosing food allergy.

Food intoxication encompasses all food-associated illnesses that are caused by chemicals in food, although food-borne chemicals vary greatly in toxicity. All consumers are susceptible to most food intoxication. Food allergy can be viewed as a category of food intoxication that affects only certain individuals in the population. Other categories of food intoxication, such as metabolic food disorders, also affect only certain individuals in the population. This chapter will focus on some types of acute food-borne intoxication, including the most common metabolic food disorders. Some of the examples have certain manifestations in common with food allergies and intolerance, and are thus of some importance in the differential diagnosis of food allergies.

Intoxication Caused by Synthetic Chemicals in Foods

Most of the synthetic chemicals in foods including food additives, agricultural chemical residues, and chemicals migrating from packaging materials have been rigorously tested for toxicity. These synthetic chemicals are safe under normal circumstances of exposure, although adverse reactions can occur from misuse, either intentional or accidental. In most situations, the concentrations of chemicals in these categories are well below any levels that might be associated with adverse reactions. The focus here will be on a few food additives, agricultural chemical residues, packaging migrants, and other man-made chemicals that can occur in foods at concentrations sufficient to cause concern.

Other Food Additives

These examples were chosen because some of the manifestations are similar to symptoms that can occur during IgE-mediated allergic reactions.

Niacin

Excessive consumption of niacin (nicotinic acid), which is part of the B vitamin complex, can cause an acute onset of flushing, pruritis, rash, and burning or warmth in the skin, especially on the face and upper trunk (2). Gastrointestinal (GI) discomfort is noted by some patients (3). Outbreaks have occurred from the excessive enrichment of flour used to make pumpernickel bagels (3) or corn meal (4) as the result of inaccurate or inadequate labeling of food ingredient containers. Such episodes are rare because the amount of niacin required to elicit such symptoms is at least 50 times the recommended dietary allowance (3, 4). The symptoms of niacin intoxication are self-limited and without sequelae.

Sorbitol and Other Polyhydric Alcohols

Sugar alcohols, such as sorbitol, are widely used sweeteners in dietetic food products. They are especially common in candy and chewing gum because they are non-cariogenic. Diarrhea can result from excessive consumption of sugar alcohols (5). Sorbitol and the other sugar alcohols are not as easily absorbed as sugar. Because of their slow absorption, these sweeteners can cause an osmotic-type diarrhea if excessive amounts are ingested. Consumers have been reported as ingesting more than 20 g of these sweeteners per day, although infants are more susceptible to the osmotic effects than adults (5). In one representative case, the ingestion of 12 pieces of hard candy over a short period of time provided 36 g of sorbitol and resulted in diarrhea (6). The illness is self-limited.

Toxic Oil Poisoning

In 1981 and 1982, an epidemic occurred in Spain linked to the ingestion of unlabeled, illegally marketed cooking oils (7, 8). A total of 19,828 cases and 315 deaths were recorded in this epidemic (9). The illicit cooking oil contained oils from both plant and animal sources but some of the oils were denatured and intended for industrial rather than food uses. The causative toxin in the oils remains unknown, although fatty acid anilides resulting from the denaturation process are suspected to be at least partially responsible (9, 10).

The clinical manifestations of this illness involved multiple organ systems (9, 10). In the first few days after ingestion of the oil, patients experienced fever, chills, headache, tachycardia, cough, chest pain, and pruritis. Physical examinations revealed various skin exanthema, splenomegaly, and generalized adenopathy. Pulmonary infiltrates were noted in 84% of patients, probably as the result of increased capillary permeability. The intermediate phase of the illness tended to begin in the second week and persist through the eighth week post-ingestion. GI symptoms, primarily abdominal pain, nausea, and diarrhea, predominated. Clinical examination revealed marked eosinophilia in 42% of patients, high IgE levels, thrombocytopenia, abnormal coagulation patterns, and evidence of hepatic dysfunction with abnormal enzymes. Some patients became jaundiced, and many had hepatomegaly. The late phase of the illness developed in 23% of cases and began after 2 months of illness. This phase was characterized initially by neuromuscular and joint involvement. Later, patients developed vasculitis and a scleroderma-like syndrome. Patients complained of intense muscular pain, edema, and progressive muscular weakness. Muscular atrophy was apparent in some patients. Neurological involvement included depressed deep tendon reflexes, anesthesia, and dysesthesia. Respiratory problems due to neuromuscular weakness developed and progressed to pulmonary hypertension and thromboembolic phenomena. The scleroderma-like symptoms included Raynaud's phenomenon, sicca syndrome, dysphagia, and contractures due to thickening collagen in the skin. Vascular lesions were noted in all organs apparently resulting from endothelial proliferation and thrombosis. All patients in the late group had antinuclear antibody and many had antibodies against smooth muscle and skeletal muscle (11). The pathological and clinical features are consistent with an autoimmune mechanism for this illness. Since the precise causative agent and its mechanism have not been delineated, a recurrence is not impossible (9). Also, the toxin, if present in small amounts in other foods, may be producing or aggravating other clinical conditions (9).

Agricultural Chemicals

A wide diversity of chemicals are used in modern agricultural practices. Residues of these

chemicals can occur in raw and processed foods, although federal regulatory agencies evaluate the safety of such chemicals and regulate and monitor their use on food products (5). The major categories of agricultural chemicals include insecticides, herbicides, fungicides, fertilizers, and veterinary drugs including antibiotics.

Insecticides

Insecticides are added to foods to control the extent of insect contamination. The major categories of insecticides include organochlorine compounds (e.g., dichlorodiphenyltrichloroethane [DDT], chlordane, and others of which many are now banned), organophosphate compounds (e.g., parathion and malathion), carbamate compounds (e.g., carbaryl and aldicarb), botanical compounds (e.g., nicotine and pyrethrum), and inorganic compounds (e.g., arsenicals).

The exceedingly low residue levels of insecticides found in most foods are not particularly hazardous, especially on an acute basis. Large doses of insecticides can be toxic to humans. For example, the organophosphates and carbamates are cholinesterase inhibitors and act as neurotoxins by blocking synaptic nerve transmission. Several reasons exist for the low degree of hazard posed by insecticide residues in foods. 1) The level of exposure is very low; 2) Some insecticides are not very toxic to humans; 3) Some insecticides decompose rapidly in the environment; and 4) Many different insecticides are used, which limits exposure to any one particular insecticide.

No food poisoning incidents have been attributed in the literature to the proper use of insecticides on foods. However, problems have occasionally arisen from the inappropriate use of certain insecticides (12). An outbreak of aldicarb intoxication from watermelons occurred on the West Coast in 1985 (13). Aldicarb use on watermelons is illegal because excessive levels of aldicarb become concentrated in the edible portion of the melon. In this episode, several farmers used aldicarb illegally, resulting in consumer illnesses and the recall and destruction of thousands of watermelons. A total of 1373 illness reports were received in this outbreak, with 78% classified as probable or possible aldicarb poisoning cases (13, 14). This episode is the largest known outbreak of pesticide poisoning in North America (13, 14). Aldicarb has also been involved in several food poisoning outbreaks associated with ingestion of hydroponically grown cucumbers (14, 15). The symptoms of aldicarb intoxication include nausea, vomiting, diarrhea, and mild neurological manifestations such as dizziness, headache, blurred vision, and loss of balance (13–15). Many other episodes of pesticide intoxication have resulted from the misuse of pesticides, including contamination of foods during storage and transport, the use of pesticides in food preparation due to their mistaken identity as common food ingredients such as sugar and salt, and their misuse in agricultural practice as in the examples noted above (12).

Herbicides

Herbicides are applied to control the growth of weeds. Among the more important herbicides are chlorophenoxy compounds (e.g., 2,4-dichlorophenoacetic acid [2,4-D]), dinitrophenols (e.g., dinitroorthocresol), bipyridyl compounds (e.g., paraquat), substituted ureas (e.g., monuron), carbamates (e.g., propham), and triazines (e.g., simazine). Generally, herbicide residues in foods are not a hazard to consumers. No food poisoning incidents have been reported from the proper use of herbicides on food crops. The lack of hazard from herbicide residues is associated with the normally low level of exposure, their low degree of toxicity to humans and selective toxicity toward plants, and the use of many different herbicides which limits exposure to any particular herbicide.

Because most herbicides are selectively toxic to plants, they pose little hazard to humans in the amounts normally used for weed control. The bipyridyl compounds are an exception. These nonselective herbicides are toxic to humans and tend to exert their effects on the lung (16). However, no food poisoning incidents have ever been attributed to inappropriate use of the bipyridyl compounds.

Fungicides

Fungicides are used to prevent the growth of molds on food crops. Important fungicides include captan, folpet, dithiocarbamates, pentachlorophenol, and the mercurials. The hazards from food-borne fungicides are miniscule because normal exposure is quite low, most fungicides do not accumulate in the environment, and fungicides are typically not very toxic.

Exceptions are the mercurial compounds and hexachlorobenzene. The mercurials are often used to treat seed grains to prevent mold growth during storage. These seed grains are usually colored

pink and are clearly intended for planting rather than consumption. However, on several occasions, consumers have eaten these treated seed grains and developed mercury poisoning (12). Although some severe episodes have resulted in deaths, mild cases of mercury intoxication can be manifested in GI symptoms such as abdominal cramps, nausea, vomiting, and diarrhea and dermal symptoms such as acrodynia and itching (12). Hexachlorobenzene caused one of the most massive outbreaks of pesticide poisoning in recorded history affecting over 3000 individuals in Turkey from 1955 through 1959 (17). Hexachlorobenzene was used to treat seed grain that was consumed rather than planted. The symptoms were severe, with a 10% mortality rate, porphyria cutanea tarda, ulcerated skin lesions, alopecia, porphyrinuria, hepatomegaly, and thyroid enlargement (17).

Fertilizers

The commonly used fertilizers are combinations of nitrogen and phosphorus compounds. Nitrogen fertilizers are oxidized to nitrate and nitrite in the soil. Both nitrate and nitrite are hazardous to humans if ingested in large amounts. Infants are particularly susceptible to nitrate and nitrite intoxication. Some plants, such as spinach, can accumulate nitrate to hazardous levels if allowed to grow on overly fertilized fields. Because nitrite is more toxic than nitrate, the situation can be worsened if nitrate-reducing bacteria are allowed to proliferate on these foods.

Acute nitrite intoxications have occurred. In low doses, the symptoms include flushing of the face and extremities, GI discomfort, and headache; in larger doses, cyanosis, methemoglobinemia, nausea, vomiting, abdominal pain, collapse, and death can occur (18). The lethal dose of nitrite is estimated at about 1 g in adults (18). Several intoxications have occurred from ingestion of over-fertilized spinach (19). The problem arises from consumption of nitrate-rich, unprocessed spinach in which nitrate has been converted to nitrite before ingestion, probably by bacterial action (18). Improper storage of carrot juice caused proliferation of nitrate-reducing bacteria that resulted in the accumulation of hazardous levels of nitrite in the product (20).

Veterinary Drugs and Antibiotics

Food-producing animals are often treated with a variety of veterinary drugs, especially antibiotics. Residues in foods are typically quite low. Acute food poisoning incidents have not occurred as a result of properly used veterinary drugs and antibiotics. Penicillin is probably one of the major concerns because of the potential for allergic reactions to penicillin residues. However, the likelihood of allergic reactions to the very low levels of penicillin residues found in foods is quite remote (21).

Chemicals Migrating from Packaging Materials and Containers

Chemicals migrating from packaging materials into foods and beverages do not represent a significant source of chemical exposure. A variety of chemicals, including plastics monomers, plasticizers, stabilizers, printing inks, and others, do migrate at extremely low levels into foods. These chemicals do not often create any hazards for consumers. Lead, copper, and tin are perhaps the main concerns associated with packaging materials (5, 22). Storage of acidic foods in inappropriate containers can result in the leaching of toxic heavy metals such as zinc. Contact of acidic beverages with copper can also release potentially hazardous levels of copper into the beverage. Cadmium is occasionally implicated in heavy metal intoxication associated with foods (5).

Lead

Lead (Pb) exposure from foods has always been a comparatively moderate contributor to overall environmental lead exposure. The migration of Pb from Pb-soldered cans was previously a source of some concern (22). However, Pb-soldered cans have been successfully phased out of use in the US. The main issue with Pb contamination remains the occasional use of Pb-based glazes on pottery or paint on glassware that may come in contact with acidic foods or beverages. Pb is a well-known toxicant that can affect the nervous system, kidney, and bone.

Tin

Tin plate is commonly used in the construction of metal cans for foods. The inner surfaces of these cans are lined with a lacquer material when cans are used for acidic foods or beverages. Acute tin intoxication has occurred from the inappropriate use of unlined cans for tomato juice or fruit

cocktail (5). Because tin is poorly absorbed, the primary symptoms are bloating, nausea, abdominal cramps, vomiting, diarrhea, and headache occurring 30 minutes to 2 hours after consumption of the acidic product (22).

Copper

Copper poisoning, characterized primarily by nausea and vomiting, most commonly occurs from faulty check valves in soft drink vending machines (5). The check valves prevent contact between the acidic, carbonated beverage and the copper tubing that delivers the water or ice in the machine. Several outbreaks of copper poisoning have resulted from such occurrences (23).

Zinc

Zinc intoxication typically results from the unwise storage of acidic foods or beverages in galvanized containers (5, 24). Zinc is a potent emetic. The symptoms of zinc intoxication include irritation of the mouth, throat, and abdomen; nausea and vomiting; dizziness; and collapse.

Industrial Chemicals

Industrial and/or environmental pollutants often migrate into foods in small amounts. On rare occasions, hazardous levels of such chemicals enter the food supply, often with devastating consequences.

Polychlorinated Biphenyls (PCBs) and Polybrominated Biphenyls (PBBs): The contamination of foods with PCBs and PBBs has occurred on several occasions (5). PCBs and PBBs are quite persistent in the environment and are considered to be toxic pollutants from industrial practices. PBBs are commonly used as fire retardants, while PCBs are frequently used in transformer fluid. PCBs and PBBs are not worrisome acute toxicants in foods. However, they are lipid-soluble, so the chronic effects of exposure to these contaminants in foods are of concern. The most infamous incident involved the accidental contamination of dairy feed in Michigan with PBBs. This incident resulted in the destruction of many cows and their milk. Leaking transformers have contributed to the contamination of feeds with PCBs which led to the destruction of chickens, eggs, and egg-containing food products.

Mercury: Minamata disease, due to mercury intoxication, is a classic example of the contamination of foods by industrial pollutants (22). An industrial firm located on the shores of Minamata Bay in Japan dumped mercury-containing wastes into the bay where bacteria converted the inorganic mercury into highly toxic methylmercury. Fish in the bay became contaminated with the methylmercury. Over 1200 cases of mercury intoxication occurred among consumers of Minamata Bay fish (25). The symptoms included tremors and other neurotoxic effects and kidney failure.

Intoxications Caused by Naturally Occurring Chemicals in Foods

The naturally occurring chemicals in foods are less frequently tested for their potential toxic effects than are synthetic chemicals. Although the vast majority of naturally occurring chemicals in foods are safe under the normal circumstances of exposure, some potentially hazardous situations do exist. Those naturally occurring chemicals with significant pharmacological activity including the vasoactive amines, methylxanthines, ethanol, and myristicin are covered elsewhere. However, naturally occurring chemicals in foods can elicit a wide variety of adverse reactions including both acute and chronic intoxication. Naturally occurring toxicants could be defined as those naturally occurring chemicals in foods that might be hazardous under typical circumstances of exposure. Naturally occurring chemicals in foods are more likely to be hazardous under typical circumstances of exposure than are synthetic chemicals. Although chronic illnesses, such as cancer, are undeniably important, this section will focus exclusively on acute intoxication caused by natural, food-borne toxicants.

Naturally Occurring Contaminants

Naturally occurring contaminants can be produced in foods as the result of contamination by bacteria, molds, algae, and insects. The chemicals produced from these biological sources can remain in foods even after the living organism has been removed or destroyed. Naturally occurring contaminants are not always present in foods and can be avoided, if contamination is prevented. Such contaminants represent the most important and potentially hazardous chemicals of natural origin existing in foods. The bacterial and insect

toxins will not be discussed in detail. The bacterial toxins cause very familiar diseases such as staphylococcal food poisoning and botulism (26, 27). The insect toxins have not been studied to any extent, and their impact on human health is uncertain.

Toxicants produced by algae that bioaccumulate in seafoods are among the most common causes of food-borne illness of chemical etiology (28). These algal toxicants are involved with several of the seafood poisonings including ciguatera poisoning and paralytic shellfish poisoning. Mycotoxins produced by food-borne molds are a source of considerable toxicological concern and occur at low levels rather frequently in certain stored foods. Several of the mycotoxins will be discussed in detail because they are confirmed to be involved in acute food-borne illness. A bigger concern with the mycotoxins is their potential involvement with chronic toxicity. The chronic toxicity of mycotoxins will not be discussed here because it is unlikely to be relevant to the investigation of allergic reactions.

Ciguatera Poisoning

Ciguatera poisoning results from the ingestion of fish that have fed on toxic dinoflagellate algae. Ciguatera poisoning is the most common cause of acute food-borne disease of chemical etiology reported to the Centers for Disease Control (CDC). This food-borne illness is common throughout the Caribbean, South Pacific, and Indian Ocean areas, but is now encountered around the world due to the improved distribution of fish (29, 30). In the US, the illness occurs most frequently in Florida, Hawaii, and the Virgin Islands (30–32). The fish most commonly implicated in cases of ciguatera poisoning are large tropical and semi-tropical reef fishes such as grouper, barracuda, sea bass, Spanish mackerel, snappers, and sea perches, although as many as 400 different fish species have been implicated in this illness (33, 34). Curiously, although most cases involve tropical or semi-tropical fishes, one outbreak involved farm-raised salmon (35).

Tropical and semi-tropical reef fishes acquire the toxic agent(s) by feeding on smaller fishes that acquire the toxin from the poisonous planktonic algae (33). Several species of dinoflagellate algae appear able to produce toxins of the type associated with ciguatera poisoning (30, 33); *Gambierdiscus toxicus* is one of the most prominent (30, 33). Several toxins may be involved in ciguatera poisoning (33). The major toxins, known as ciguatoxins, are lipid-soluble, heat-stable, polyether compounds with an approximate molecular weight of 1.11 kilodaltons (kDa) (33, 36). Ciguatoxin has ionophoric properties, which selectively opens voltage-sensitive sodium channels of the neuromuscular junction (33). Maitotoxin also appears to be responsible to a lesser extent for ciguatera poisoning (33). Maitotoxin is a water-soluble compound of 3.42 kDa that activates both voltage-sensitive and receptor-operated calcium channels in the plasma membranes of cells (33). The toxins accumulate in the liver and viscera of the fish, but enough can enter the muscle tissues to result in ciguatera poisoning among humans ingesting these fish (33). Larger fish pose a greater risk than smaller fish (33). The toxins are heat-stable and are unaffected by processing or cooking practices (33).

The symptoms of ciguatera poisoning tend to be variable, perhaps confirming the role of several different dinoflagellate algae and several different toxins in this syndrome (33, 34). GI and neurological manifestations are the predominant symptoms (30, 33, 34), although in some cases the GI symptoms predominate, and in others the neurological symptoms predominate (30). The GI symptoms include nausea, vomiting, diarrhea, and abdominal cramps. Neurological symptoms include dysesthesia, paresthesia especially in the perioral region and extremities, pruritis, vertigo, muscle weakness, malaise, headache, and myalgia. A peculiar reversal of hot and cold sensations occurs in about 65% of all patients (30). In severe cases, the neurological manifestations can progress to delirium, pruritis, dyspnea, prostration, bradycardia, and coma (30). Many patients recover within a few days or weeks, although treatment is difficult and deaths from cardiovascular collapse have been encountered in about 0.1% of cases (34).

Paralytic Shellfish Poisoning

Paralytic shellfish poisoning results from the ingestion of molluscan shellfish such as clams, mussels, cockles, and scallops that have become poisonous by feeding on toxic dinoflagellate algae (37). Paralytic shellfish poisoning occurs worldwide but is most commonly encountered along the Pacific and North Atlantic coasts of North America, the coastal areas of Japan, and the coasts of Chile and Argentina (33). Several species of toxic dinoflagellate algae have been implicated in paralytic shellfish poisoning; *Alexandrium catanella*

(formerly *Gonyaulax catanella*) and *A. tamarensis* are the two most common ones (29). "Blooms" of toxic dinoflagellates are sporadic, so most shellfish will be hazardous only during the times of the blooms (34, 38). Although most shellfish species clear the toxins from their systems within a few weeks of the end of the dinoflagellate bloom, a few species, such as the Alaskan butter clam, seem to retain the toxin for long periods (39). The toxins involved in paralytic shellfish poisoning are known as saxitoxins (36, 38). Saxitoxins are neurotoxins that bind to and block the sodium channels in nerve membranes (33). The saxitoxins are heat-stable so processing and cooking have no affect on the toxicity of the shellfish (39).

Through the blocking of nerve transmission, the saxitoxins are very potent neurotoxins. The symptoms of paralytic shellfish poisoning include a tingling sensation and numbness of the lips, tongue, and fingertips followed by numbness in the legs, arms, and neck, ataxia, giddiness, staggering, drowsiness, incoherent speech progressing to aphasia, rash, fever, and respiratory and muscular paralysis (29, 33, 38). Death from respiratory failure occurs frequently, usually within 2–12 hours depending on the dose ingested. No antidotes are known, although prognosis is good if the victim survives the first 24 hours of the illness (29, 33).

Amnesic Shellfish Poisoning

Amnesic shellfish poisoning was first recognized following an outbreak in Canada in late 1987 (40). Amnesic shellfish poisoning was associated with the ingestion of mussels from Prince Edward Island that resulted in over 100 cases and at least 4 deaths (40, 41). The source of the toxin was a planktonic algae, *Nitzschia pungens,* which was blooming in an isolated area of Prince Edward Island at the time of the outbreak (42). The toxin was identified as domoic acid, a neuroexcitatory amino acid (41). Amnesic shellfish poisoning is characterized by GI symptoms and unusual neurological abnormalities (41). The GI symptoms, which occurred within the first 24 hours, were vomiting, abdominal cramps, and diarrhea. The neurological symptoms, which had onset within 48 hours, were severe incapacitating headaches, confusion, loss of short-term memory, and, in a few cases, seizures and coma. Severely affected patients who did not die experienced prolonged neurologic sequelae, including memory deficits and motor or sensorimotor neuronopathy or axonopathy (41).

Diarrheic Shellfish Poisoning

Diarrheic shellfish poisoning is primarily associated with the ingestion of clams that have become toxic through the ingestion of toxic dinoflagellate algae of the genera *Dinophysis* and *Prorocentrum* (33). No confirmed outbreaks have occurred in North America but have in Japan and Europe (34). The toxins responsible for diarrheic shellfish poisoning are polyether compounds: okadaic acid and its derivatives, the dinophysistoxins (33). The symptoms include diarrhea, nausea, vomiting, and abdominal cramps (34).

Puffer Fish Poisoning

Puffer fish poisoning occurs primarily in Japan and China, the only parts of the world where puffer fish are frequently consumed. About 30 species of puffer fish are found worldwide, but most species are not toxic (39). The most hazardous puffer fish belong to the genus *Fugu,* which are considered delicacies in Japan and China. The toxin in puffer fish is a potent neurotoxin called tetrodotoxin (38). For many years, the toxin was thought to be produced by the fish, but evidence now exists that marine bacteria may be the original source of the toxin (37). Tetrodotoxin is heat-stable and, like saxitoxin, acts by blocking the sodium channels in nerve cell membranes (34). The symptoms of tetrodotoxin poisoning usually begin with a tingling sensation of the fingers, toes, lips, and tongue, followed by nausea, vomiting, diarrhea, and epigastric pain (33, 34, 43). Twitching, tremors, ataxia, paralysis, and death often ensue (43). The fatality rate is about 60% in untreated cases (43). Most of the tetrodotoxin accumulates in the liver, viscera, and roe of the puffer fish (34). Careful cleaning of the fish before ingestion of the edible muscle is required to safeguard against tetrodotoxin intoxication (34).

Mycotoxins

Mycotoxins are produced by a wide variety of molds which can grow and produce toxins on a wide variety of foods (44). Most of the known mycotoxins are recognized because of their toxicity to domestic animals fed moldy feed grains. However, a few mycotoxins are noteworthy because they are known hazards for humans.

Ergotism

Ergotism was the first recognized mycotoxin-associated illness (44). The responsible mold is *Claviceps purpurea,* which can infect the grains of rye, wheat, barley, and oats. The last recorded outbreak occurred in Europe in 1951. Ergotism is caused by a group of toxins known as the ergot alkaloids, and is manifested in two forms: gangrenous ergotism and convulsive ergotism. Gangrenous ergotism, also known as Saint Anthony's fire, is characterized by a burning sensation in the feet and hands followed by progressive restriction of blood flow to the hands and feet resulting ultimately in gangrene and loss of limbs. Convulsive ergotism is characterized by hallucinations progressing to convulsive seizures and sometimes death. Modern agricultural practices and grain milling procedures have virtually eliminated ergotism as a concern.

Alimentary Toxic Aleukia

Alimentary toxic aleukia (ALA) was observed in Russia during World War II and was associated with the consumption of over-wintered millet that contained trichothecene mycotoxins (44). Trichothecenes are a group of mycotoxins produced by molds of the genus, *Fusarium.* ALA occurs in four stages. In the first stage, affected individuals experience burning sensations in the mouth, throat, and esophagus, followed 1–3 days later by diarrhea, nausea, and vomiting. The GI symptoms cease after about 9 days. The second stage of ALA begins during the second week and lasts through the second month. This stage involves bone marrow destruction, leukemia, agranulocytosis, anemia, and loss of platelets. Small hemorrhages begin to appear at the end of this stage. The third stage of ALA lasts for 5–20 days and involves total loss of bone marrow with necrotic angina, sepsis, total agranulocytosis, moderate fever, larger hemorrhages on the skin, and the appearance of necrotic skin lesions. Bronchial pneumonia usually develops along with abscesses and hemorrhages in the lungs. The fourth stage of ALA is death, which occurred in about 80% of cases within 3 months of the onset of symptoms. Because of the circumstances at the time of this outbreak, identification of the exact species of *Fusarium* and the trichothecenes responsible for ALA were not accomplished. The level of contamination of the millet with trichothecenes was not determined.

Fusarium molds are common on grain crops worldwide. Trichothecene mycotoxins continue to occur at low levels in many cereal foods. However, no acute illnesses in humans including ALA have been attributed to trichothecene intoxication since the original outbreak. The effects of ingestion of low levels of toxic trichothecenes on humans remain uncertain.

Naturally Occurring Constituents

Many fungi, some plants, and a few animals contain hazardous levels of various naturally occurring toxicants. Such fungi, plants, and animals should not be eaten, but when accidentally or intentionally consumed result in food-borne illness. Furthermore, many plants and animals contain levels of naturally occurring toxicants that are probably not hazardous to humans ingesting typical amounts of these foods. The ingestion of abnormally large quantities of such foods and their naturally occurring toxicants is potentially hazardous. Some naturally occurring toxicants are inactivated or removed during processing or preparation of foods prior to consumption. Failure to adhere to such processing and preparation practices can result in food-borne illness.

Poisonous Animals

Very few animal species are poisonous, although several species of poisonous fish and other marine animals are known to exist (43, 45). Puffer fish is the best known example, although the toxin in puffer fish may actually derive from bacteria (37).

Animal tissues and products also contain very few naturally occurring toxicants that could cause adverse reactions if ingested in abnormally large quantities. The best example is vitamin A (1). The ingestion of polar bear livers and fish livers in large quantities can lead to vitamin A intoxication (1). Cases of vitamin A intoxication have occurred in infants when fed diets rich in vitamin A (e.g., chicken livers and fortified milk and carotenoids, e.g., pureed carrots, while also being administered daily vitamin supplements) (46).

Poisonous Plants

Many poisonous plants exist in nature (47). Classic examples include water hemlock and nightshade, which were used in centuries past to

poison one's enemies. Although consumers purchasing foods from commercial sources can usually avoid the ingestion of poisonous plants, intoxication occurs each year in individuals who have harvested their own foods in the wild (48). An elderly couple succumbed after mistaking foxglove for comfrey while harvesting herbs for tea; foxglove contains digitalis (49). In another example, a team member in a desert survival course died after eating a salad prepared in part from a *Datura* species, jimsonweed (48). Jimsonweed contains tropane alkaloids, including atropine. While atropine is a useful pharmaceutical agent, ingesting it from natural sources in uncontrolled doses can be fatal because it has potent anticholinergic properties. Individuals ingesting jimsonweed and other plants containing tropane alkaloids suffer neurotoxic effects. Many more such examples could be provided.

More rarely, intoxication from poisonous plants occurs with products purchased from commercial sources (50). In one well-investigated outbreak, a commercial herbal tea was contaminated with *Senecio longilobis,* a well-known poisonous plant (50). The herbal tea, called gordolobo yerba, was sold to the Mexican-American population in Arizona, and promoted as a cure for colic, viral infections, and nasal congestion in infants. Several infants died from the ingestion of this contaminated herbal tea. *Senecio* and many other plants contain a group of chemicals known as pyrrolizidine alkaloids, which can cause both acute and chronic symptoms. Chronic low doses cause liver cancer and cirrhosis (39). The acute symptoms associated with the contaminated herbal tea included ascites, hepatomegaly, veno-occlusive liver disease, abdominal pain, nausea, vomiting, headache, and diarrhea (50). Death resulted from liver failure.

Occasionally, intoxication from poisonous plants occurs from the intentional addition of such materials to foods. The intentional addition of marijuana to bakery items is the most common example.

Many plant-derived foods contain naturally occurring toxicants at doses that are not hazardous, at least on an acute basis, unless large quantities of the food are eaten. Examples include solanine and chaconine in potatoes, oxalates in spinach and rhubarb, furan compounds in mold-damaged sweet potatoes, and cyanogenic glycosides in lima beans, cassava, and many fruit pits (51).

The cyanogenic glycosides (51) can release cyanide from enzymatic action occurring during the storage and processing of the foods, or on contact with stomach acid. Commercial varieties of lima beans contain minimal amounts of these cyanogenic glycosides having a hydrogen cyanide (HCN) yield of 10 mg per 100 g of lima beans (wet weight). The lethal oral dose of cyanide for humans is 0.5 mg/kg, so a 70 kg adult would need to ingest 35 mg of cyanide, an amount that would require the ingestion of at least 350 g, or nearly a pound, of lima beans. Such levels of consumption are unlikely, and human illnesses from cyanide intoxication from lima bean ingestion have not been reported. Wild varieties of lima beans contain much higher levels of the cyanogenic glycosides (up to 300 mg HCN/100 g) and are likely hazardous to consume. Cyanide intoxication has occurred in Africa and South America due to the consumption of cassava, which is sometimes ingested in large quantities because of a lack of other foods (39, 51). Cyanide intoxication has also occurred from the ingestion of fruit pits (39), especially by the grinding of pits with the fruit in food processors during the preparation of jams and wines. The symptoms of cyanide intoxication include a rapid onset of peripheral numbness and dizziness, mental confusion, stupor, cyanosis, twitching, convulsions, coma, and death (39).

Many toxic constituents of plants are inactivated or removed during processing and preparation. For example, raw soybeans contain trypsin inhibitors, lectins, amylase inhibitors, saponins, and various antivitamins (39). Fortunately, these toxicants are inactivated during the heating and fermentation processes used with soybeans. Failure to remove or inactivate these toxicants can result in food-borne illness. For example, raw kidney beans contain lectins, which are typically inactivated during cooking. In the United Kingdom, immigrants who did not appreciate the importance of thorough cooking of kidney beans have ingested undercooked kidney beans, leading to the onset of nausea, vomiting, abdominal pain, and bloody diarrhea from the lectins (39).

Poisonous Mushrooms

Many species of mushrooms are poisonous. The harvesting of mushrooms in the wild can be a hazardous practice. Intoxications occur each year in the US from the ingestion of poisonous mushrooms (22), which contain a variety of naturally occurring toxicants classified into Groups I-VI (39, 52).

The Group I toxins are the most hazardous and include amatoxin and phallotoxin. Amatoxin

is produced by *Amanita phalloides,* the death cap mushroom. Amatoxin poisoning occurs in three stages. The first stage involves abdominal pain, nausea, vomiting, diarrhea, and hyperglycemia beginning 6–24 hours after ingestion of the mushrooms. A short period of remission then occurs. The third and often fatal stage involves severe liver and kidney dysfunction, hypoglycemia, convulsions, coma, and death. Death resulting from hypoglycemic shock occurs 4–7 days after the onset of symptoms.

The Group II toxins are hydrazines; gyromitrin is the best known example. Gyromitrin is produced by *Gyromitra esculenta* or false morel mushrooms. The symptoms elicited by ingestion of these mushrooms include a bloated feeling, nausea, vomiting, watery or bloody diarrhea, abdominal pain, muscle cramps, faintness, and ataxia occurring with a 6–12 hour onset time.

The Group III toxins are characterized by muscarine and affect the autonomic nervous system. Muscarine is found in fly agaric (*Amanita muscaria*) sometimes in association with the Group I toxins. Symptoms include perspiration, salivation, lacrimation with blurred vision, abdominal cramps, watery diarrhea, constriction of the pupils, hypotension, and a slowed pulse occurring rapidly after ingestion of the poisonous mushrooms.

The Group IV toxins cause symptoms only when ingested with alcoholic beverages. Coprine, a Group IV toxin produced by *Coprinus atramentarius,* is the best example. Symptoms include flushing of the neck and face, distension of the veins in the neck, swelling and tingling of the hands, metallic taste, tachycardia, and hypotension progressing to nausea and vomiting. Symptoms begin within 30 minutes of ingestion of the mushrooms and can last up to 5 days.

The Groups V and VI toxins act primarily on the central nervous system, causing hallucinations. The Group V toxins include ibotenic acid and muscimol and cause dizziness, drowsiness followed by hyperkinetic activity, confusion, delirium, incoordination, staggering, muscular spasms, partial amnesia, a coma-like sleep, and hallucinations beginning 30 minutes to 2 hours after ingestion. Fly agaric is a good source of the Group V toxins.

The Group VI toxins include psilocybin and psilocin. The symptoms of the Group VI toxins include pleasant or aggressive mood, anxiety, unmotivated laughter and hilarity, compulsive movements, muscle weakness, drowsiness, hallucinations, and sleep. The Group VI toxins are found in Mexican mushrooms, *Psilocybe mexicana.* Symptoms usually begin 30–60 minutes after ingestion of the mushrooms, and recovery is often spontaneous in 5–10 hours. When the dose of the Group VI toxins is high, prolonged and severe sequelae, even death, can occur.

Metabolic Food Disorders

Like food allergies, metabolic food disorders affect only certain individuals in the population. These individuals display increased sensitivity to certain chemicals in foods because they lack an enzyme necessary to metabolize that particular chemical or because they have a genetic abnormality that makes them especially susceptible to the toxic effects of a particular food-borne chemical. The best examples of metabolic food disorders are lactose intolerance and favism.

Lactose Intolerance

Lactose intolerance is associated with an inherited deficiency in the amount of the enzyme β-galactosidase in the small intestine (53, 54). β-Galactosidase is needed for hydrolysis of lactose, a milk disaccharide, into its constituent monosaccharides glucose and galactose. Whereas glucose and galactose can be absorbed and used for metabolic energy, lactose cannot be absorbed without prior hydrolysis. If the activity of β-galactosidase is insufficient, the lactose from milk or dairy products will be incompletely hydrolyzed. Undigested lactose passes into the colon, where bacteria convert it to CO_2, H_2, and H_2O. The symptoms associated with lactose intolerance are abdominal cramps, flatulence, and frothy diarrhea.

Almost all individuals are born with sufficient levels of β-galactosidase. However, with increasing age, the levels of enzyme activity diminish. At some point, the levels of β-galactosidase activity may be insufficient to handle the load of lactose ingested in the diet. Symptoms of lactose intolerance can begin to appear in the early teen years and often worsen with age. Many lactose-intolerant individuals can tolerate some lactose in their diets, often as much as the amount found in an 8-oz glass of milk (55).

Lactose intolerance is an inherited trait. It affects only about 6%–12% of all whites, but ultimately affects 60%–90% of some ethnic groups including blacks, Native Americans, Hispanics, Asians, Jews, and Arabs (53, 54).

Lactose intolerance is treated with dairy product avoidance diets, although some dairy products can usually be ingested without harm. Lactose-intolerant individuals can often safely consume yogurt if the yogurt contains live bacterial cultures with β-galactosidase activity (54). Lactose-hydrolyzed milk is also available in many markets.

Favism

Favism is caused by ingestion of fava beans or inhalation of pollen from the *Vicia faba* plant by individuals with a deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PDH) in their erythrocytes (56). Erythrocyte G6PDH deficiency is the most common enzyme deficiency in the world, affecting perhaps 100 million individuals; it is most prevalent among Kurds, Iraqis, Iranians, Sardinians, Cypriot Greeks, American blacks, and some African populations (56). This deficiency is virtually unknown among northern Europeans, North American Indians, and Eskimos (56). G6PDH is a critical enzyme that is essential for the maintenance of adequate levels of the reduced form of glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH) in erythrocytes. GSH and NADPH protect the erythrocyte membrane from oxidation. Fava beans contain two potent, naturally occurring oxidants, vicine and convicine. These oxidants can damage the erythrocyte membranes in G6PDH-deficient individuals, but not normal persons. Exposure to fava beans in sensitive individuals results in acute hemolytic anemia (56). The typical symptoms are pallor, fatigue, dyspnea, nausea, abdominal and/or back pain, fever, and chills. In a few severe cases, hemoglobinuria, jaundice, and renal failure may occur. Favism is not a common malady in the US, because fava beans are rarely ingested here. Favism occurs primarily in the Mediterranean area, the Middle East, China, and Bulgaria, where the genetic trait is fairly prevalent and fava beans are more frequently consumed.

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36

Seafood Toxins

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Introduction

Fish and shellfish are nutritious foods that are desirable components of a healthy diet. However, fish, shrimp, lobster, crabs, crayfish, mussels, and clams are listed among the most frequent causes of food allergy (1–3). The differential diagnosis of seafood allergy is extensive. It includes true hypersensitivity reactions to non-seafood components, such as peanut or tree nuts, foods that may cross-react with seafood allergens or food contaminants such as antibiotic residues contained in seafood, adverse reactions to food additives such as sulfites, monosodium glutamate (MSG), and tartrazine, as well as seafood-associated poisoning (Table 36–1). Seafood poisoning, including reactions to natural toxins, frequently masquerade as allergic reactions on presentation to emergency departments and urgent care clinics, and are often misdiagnosed (4–8). Bacteria and bacterial toxins may cause gastrointestinal (GI) and systemic symptoms that can also be confused with food allergy. In the US, fish poisoning, principally scombroid and ciguatera fish poisoning (Table 36–2), was responsible for 4.2% of all confirmed food-borne disease outbreaks listed by the Centers for Disease Control (CDC) from 1988 through 1999 (9, 10) (Table 36–3). This was significantly smaller than the 17.8% reported for the period 1978–1987 (11). National surveillance data on seafood-related poisoning is based on outbreaks of acute food-borne disease reported by state health departments to the CDC. From 1978 through 1999 there were 362 outbreaks with 1798 cases of scombroid poisoning, and 309 outbreaks with 1295 cases of ciguatera poisoning. Twenty-four outbreaks of paralytic shellfish poisoning (PSP) involving 219 people were reported that included two large California outbreaks in 1980. There was one case each of puffer fish poisoning, neurotoxic shellfish poisoning (NSP), and amnesic shellfish poisoning (ASP) during this period (9, 10, 12, 13). However, these figures are likely to under-represent the true incidence of seafood poisoning, because some cases remain undiagnosed and many are not reported to health authorities.

Based on the presence or absence of the toxin at the time of capture, fish poisoning can be classified into two categories. In ciguatera and puffer fish poisoning the toxin is present in the live fish, whereas in scombroid it is produced in the flesh only after capture, by contaminating bacteria that spoil improperly refrigerated fish. Puffer fish poisoning is associated with a high rate of mortality, as opposed to scombroid and ciguatera, which are self-limiting in the vast majority of cases. Most shellfish-associated illness is infectious; it can be bacterial or viral, with the Norwalk virus likely to account for most cases of gastroenteritis. Ingestion of contaminated shellfish results in a wide variety of symptoms, depending on the toxins present, their concentrations in the shellfish, and the amount of contaminated shellfish consumed. Five types of shellfish poisoning have been identified: paralytic, neurotoxic, diarrheic, amnesic and azaspiracid. Toxins responsible for the clinical manifestations are generally produced by microscopic marine algae in the warmer summer months and are then concentrated in filter-feeding bivalve mollusks such as clams and mussels, and their toxins are retained and concentrated over time. Of the estimated 4000 species of marine algae worldwide, fewer than 80 (2%) produce toxins (14).

Table 36–1.
Differential Diagnosis of Seafood-Associated Poisoning

A. Common Seafood Poisons
  1. Fish poisoning
    a. Ciguatera
    b. Scombroid
    c. Tetrodon poisoning
  2. Shellfish poisoning
    a. Paralytic
    b. Neurotoxic
    c. Amnesic
    d. Diarrheic

B. Less Common Seafood Poisoning
  1. Fish
    a. Clupeotoxin
    b. Elasmobranch
  2. Mollusks
    a. Red whelks

C. Infections and Bacterial Intoxications
  1. Bacterial toxins
    a. *Clostridium botulinum*
    b. *Staphylococcus aureus*
  2. Bacterial infections
    a. *Vibrio cholerae*
    b. *Vibrio parahemolyticus*
    c. *Vibrio vulnificus*
  3. Viral infections
    a. Norwalk and Norwalk-like enteric viruses

Only about 30 dinoflagellate and a few diatom species are known to cause human illness, and fewer still are potentially lethal (15). Anthropogenic eutrophication has been incriminated in the increasing frequency of harmful algal blooms and production of biotoxins by marine dinoflagellates (16). However, the incidence of shellfish poisoning has been declining, most likely because of careful monitoring, beach closures, and improved public awareness. Treatment of shellfish poisoning is primarily supportive. It is recommended that the public avoid collecting shellfish from areas where red tides are known to occur, and refrain from consuming suspect shellfish that should be submitted to health authorities for investigation (17).

Seafood poisoning is largely a regional problem, and cases are usually concentrated in endemic areas. However, scombroid and poisoning associated with imported seafood are an exception, since they occur sporadically and do not follow geographic patterns. At present well over half the seafood supply in the US is imported and, because reef fish are increasingly exported from tropical areas, seafood poisoning has become a more widespread problem. Most current health risks associated with seafood contamination originate in the environment and should be dealt with by control of harvest or at the point of capture by application of principles of hazard analysis and critical control point (HACCP). Some seafood poisoning, although not currently a problem in the US, could become one as international tourism in-

Table 36–2.
Epidemiology of Seafood Poisoning in the US from 1978 Through 1998*

Year	*Scombroid*	*Ciguatera*	*PSP**	*NSP**	*AFP**	*PFP**
1978	7 (30)	19 (56)	4 (10)	0	0	0
1979	14 (134)	21 (91)	1 (3)	0	0	0
1980	28 (151)	15 (52)	5 (116)	0	0	0
1981	9 (93)	30 (219)	0	0	0	0
1982	18 (58)	8 (37)	1 (5)	0	0	0
1983	13 (271)	13 (43)	0	0	0	0
1984	12 (53)	18 (78)	0	0	0	0
1985	14 (56)	26 (104)	2 (3)	0	0	0
1986	20 (60)	18 (70)	0	0	0	0
1987	22 (98)	11 (35)	0	0	0	0
1988	16 (65)	4 (8)	1 (6)	0	0	0
1989	17 (80)	19 (66)	0	0	0	0
1990	11 (194)	11 (44)	2 (24)	0	0	0
1991	17 (40)	7 (50)	1 (6)	0	1 (29)	0
1992	15 (135)	1 (8)	0	0	0	0
1993	5 (21)	13 (44)	0	0	0	0
1994	21 (83)	11 (54)	3 (29)	0	0	0
1995	16 (91)	10 (27)	1 (7)	0	0	0
1996	19 (55)	9 (32)	0	1 (3)	0	1 (3)
1997	22 (92)	18 (65)	2 (4)	0	0	0
1998	27 (126)	16 (71)	1 (6)	0	0	0
1999	19 (59)**	11 (41)	0**	0	0	0
Total	**362 (1798)**	**309 (1295)**	**24 (219)**	**1 (3)**	**1 (29)**	**1 (3)**

*Number of outbreaks and cases (in parenthesis) reported to the Center for Disease Control and Prevention (CDC).
**In 1999 eleven additional suspected scombroid outbreaks involving 36 cases and one suspected PSP outbreak involving 3 cases were reported to the CDC.
Compiled from (9–11).

Table 36–3.
Number of Outbreaks and Cases Associated with Seafood Toxins and Their Relative Contribution to the Food-Borne Diseases (1988–1999)

Year	*No (Seafood toxins)*		*% (Seafood toxins)*		*Food-borne diseases*	
	Outbreaks	*Cases*	*Outbreaks*	*Cases*	*Outbreaks*	*Cases*
1988	21	79	4.7	0.5	451	15,732
1989	36	144	7.1	0.91	505	15,867
1990	24	262	4.5	1.36	533	19,232
1991	26	125	4.9	0.83	531	15,052
1992	16	143	3.9	1.29	411	11,083
1993	18	65	3.5	0.46	514	14,080
1994	35	166	5.1	0.98	690	16,995
1995	27	125	4.2	0.93	645	13,497
1996	30	93	5.0	0.6	602	15,421
1997	42	161	5.2	0.86	806	18,802
1998	44	203	3.3	0.76	1314	26,719
1999	30	100	2.2	0.4	1344	25,286
Total	**349**	**1666**	**4.2**	**0.8**	**8346**	**207,766**

Calculated from (9, 10).

creases and seafood from different regions of the world becomes available. Thus, knowledge about these clinical syndromes is helpful.

Some marine toxins are allelopathic and function in nature as an adaptive mechanism to inhibit the growth of other microalgae. Animals may have evolved to acquire toxicity by sequestration of toxic compounds in their food source, which provides protection from predators that have learned to avoid them. Recently, two new classes of marine toxins that cause human disease were discovered, azaspiracid and spirolides. The sources of these toxins have also been identified in phytoplanktons that have widespread presence in Atlantic waters. The occurrence of these toxin classes in seafood presents new challenges to the seafood industry and regulatory agencies. In addition, aquaculture is gaining an increasing importance in production of seafood, which introduces new challenges to healthcare and practicing physician. Use of algicides, antibiotics, and antiparasitic medications that leave detectable residues in farm-raised seafood is a potential human health hazard. Genetic engineering and neoantigens incorporated into seafood or introduced into other food with a marine origin can present alternative sources of antigen that could potentially lead to allergic sensitization.

In this chapter, special emphasis is placed on important aspects of the clinical picture, the marine species most commonly involved, and their general geographic distribution—information that we hope will be helpful in recognizing these reactions, making the correct diagnosis, and differentiating such reactions from seafood allergy. Current knowledge of mechanisms of toxicity and methods of detection and quantification of various seafood toxins are reviewed, and general treatment and preventive measures are discussed.

Common Intoxications Associated with Fish (Table 36–4)

Scombroid (Histamine Poisoning)

A constellation of GI, neurological, cardiovascular, and cutaneous symptoms such as nausea, vomiting, diarrhea, abdominal cramping, throbbing headache, palpitations, flushing, tingling, burning, itching, hypotension, urticaria, and angioedema characterize scombroid. In severe cases and in persons with asthma, bronchospasm may develop. The most frequent symptoms are tingling and burning sensations around the mouth, GI complaints, and a skin rash. Patients sometimes describe a peppery or bitter taste to the fish, but often the fish tastes completely normal. In general, the onset of symptoms is rapid, usually within 10–30 minutes of ingesting the fish. Physical signs may include a diffuse blanching erythema, tachycardia, wheezing, and hypotension or hypertension. Immediate reactions may be indistinguishable from anaphylaxis, and scombroid is often misdiagnosed as an allergic reaction (4–8). Scombroid intoxication is believed to result from ingestion of fish containing high levels of free histamine. Histamine is resistant to heat, so cooking the fish, and even high temperatures used in the canning process, will not

Table 36–4.
Summary of Common Toxic Syndromes Associated with Naturally Occurring Toxins in Seafood

Type of Poisoning	*Type of Toxin*	*Source*	*Symptom Onset*	*Clinical Syndrome*
Scombroid	Histamine	Tuna, mahi mahi, bonita, marlin, bluefish, wahoo, mackerel, and salmon	Minutes to 4 hours	Severe headache, dizziness, nausea, vomiting, flushed skin, urticaria, and wheezing
Ciguatera	Ciguatoxins, maitotoxin	Coral reef fish: amberjack, snappers, grouper, goat fish, barracuda, sea bass, surgeon fish, ulua, and papio	1–4 hours	Abdominal pain, diarrhea, vomiting, paresthesias, cold-to-hot sensory reversal, weakness and myalgias
Puffer fish	Tetrodotoxin	Ocean sunfishes, porcupine fishes, and puffer fish (fugu)	10–45 minutes	Paresthesias, headache, vomiting, diaphoresis, and respiratory paralysis
Paralytic shellfish	Saxitoxins	Mussels, clams, and oysters	5–30 minutes	Vomiting, diarrhea, facial paresthesias, and respiratory paralysis
Neurotoxic shellfish	Brevetoxins	Mussels and clams	30 minutes to 3 hours	Diarrhea, vomiting, abdominal pain, myalgias, paresthesias, and ataxia
Amnesic shellfish	Domoic acid	Mussels, clams, crabs, and anchovies	15 minutes to 38 hours	Vomiting, diarrhea, headache, myoclonus, loss of short-term memory, seizures, coma, and hemiparesis
Diarrheic shellfish	Okadaic acid, dinophysistoxins, pectenotoxins, yessotoxin	Mussels, clams, and scallops	30 minutes to 6 hours	Diarrhea, nausea, vomiting, and abdominal pain

prevent scombroid poisoning (18). Because the symptoms are usually self-limited and resolve in the vast majority of cases within 4–10 hours without any sequelae, there is usually no need for specific treatment. However, H1 and H2 antihistamines ameliorate the symptoms in severe cases (19). The mildness and transient nature of scombroid contribute to underreporting of the disease.

Initially, the disease was associated with consumption of scombroid fish. Scombroid means fish like mackerel (*Scomber* spp). Fish belonging to the Scombroidea family that are found in temperate and tropical waters include tuna, mackerel, bonito, and saury. More recently, other nonscombroid species have been identified as causing this intoxication, including mahi mahi, bluefish, jack, mackerel, amberjack, herring, sardine, and anchovy. Some of these species constitute highly commercialized marine products and have been among the most valuable resources of the canning industry (20). In the US between 1978 and 1999, scombroid poisoning caused by mahi mahi, tuna, and bluefish accounted for the majority of the cases reported to CDC (9–11).

The histamine is not present when the fish are caught, but is produced later during spoilage by decarboxylation of free histidine, which is naturally present at high levels in species of fish implicated in scombroid (21). The production of histamine is due to the action of histidine decarboxylase, an enzyme produced by bacteria growing on the fish. The enteric bacteria *Morganella morganii, Klebsiella pneumoniae,* and *Hafnia alvei* are most frequently implicated. These organisms are not natural flora of living fish, and contamination probably occurs during catching and handling (22). This reaction occurs optimally between 68°F (20°C) and 86°F (30°C), and is prevented by refrigeration or chemical decontamination. Experimental studies have shown that histamine formation is negligible in fish stored at 32°F (0°C) (23).

Even though histamine levels may not be correlated with any obvious signs of decomposition, histamine content may be used as an index of spoilage in certain fish. Fresh fish normally contain histamine levels of less than 10 parts per million (ppm) or 1 mg/100 g of fish flesh (1 ppm = 1 μg/g). Laboratory confirmation of scombroid is based on demonstrating elevated histamine levels greater than 50 ppm in the muscle tissue of incriminated fish using an enzyme-linked immunosorbent assay (ELISA) (24, 25).

Although histamine was first suggested as the causative toxin more than 50 years ago, it was not until 1991 that urinary excretion of histamine, in quantities far exceeding those required to produce toxicity, was documented in vivo in humans with the clinical syndrome (26). Subsequently, eleva-

ted plasma histamine levels were also demonstrated in scombroid (27). Various hypotheses have been put forward to explain why histamine consumed in spoiled fish is more toxic than pure histamine taken orally. One theory postulates a role for other heat-stable substances produced in fish by putrefactive bacteria that inhibit the metabolism of histamine by intestinal flora and permit absorption of a more substantial portion of the ingested histamine. Another hypothesis suggests that urocanic acid, another imidazole compound derived from histidine in spoiling fish, may induce mast cell (MC) degranulation, and endogenous histamine release may augment the exogenous histamine consumed in spoiled fish (28). There is still controversy about the exact mechanism, and none has proved totally satisfactory.

Scombroid is preventable by proper handling and prompt refrigeration of fish at the time of capture, and during subsequent storage, processing, and distribution, until the fish is preserved or cooked. Fish should be chilled rapidly to temperatures below 50°F (10°C) within 4 hours of capture, and stored at 32°F–39°F (0°C–4°C), to keep bacterial numbers and histamine levels low. Despite the huge expansion in trade in recent years, great progress has been made in ensuring the quality and safety of fish products. This is largely the result of the introduction of international standards of food hygiene and the application of risk analysis and HACCP principles (28).

Ciguatera

Ciguatera fish poisoning is a clinical syndrome that presents, after consumption of ciguatoxic fish, with characteristic GI, neurological, and, occasionally, cardiovascular symptoms (29). The onset of the symptoms ranges between 10 minutes and 12 hours after ingestion of contaminated fish. Nausea, vomiting, watery diarrhea, and abdominal pain usually develop within 3–6 hours and typically last 12–24 hours. Neurologic symptoms tend to be the most distinctive and enduring. They include paresthesias that initially involve the lips, tongue, and throat, which later may extend to the extremities, hypoesthesia, dysesthesia, pruritus, generalized weakness, anxiety, and reversal of temperature sensation (30). These paresthesias do not follow dermatomal patterns (31, 32). Neurologic symptoms are often aggravated by alcohol consumption, stress, and physical activity (33). Other less common symptoms include diaphoresis, chills, dizziness, headache, blurred vision, prostration, myalgias, dry mouth, taste disturbances or a metallic taste, and pain or a loose sensation in the teeth. Weakness may last for 1–7 days. Mean duration of acute illness is typically 8.5 days, although it is not unusual for neurologic symptoms such as paresthesias or temperature reversal to periodically re-occur for a month or longer. Diminished or increased reflexes and dilated pupils may also be noted, which usually resolve in 2–3 days. Cardiovascular symptoms are found in 10%–15% of cases, most commonly in individuals previously exposed to the toxin. When present, bradycardia or hypotension may require urgent management (34). In cases of severe intoxication, seizures, coma, and respiratory paralysis may occur, which can be fatal in the absence of adequate life support (31, 33). Ciguatera fish poisoning is thus usually a self-limiting disease, but symptoms may be extremely debilitating, resulting in extended periods of disability.

Current estimates place the annual number of ciguatera cases at 50,000 worldwide (35). This poisoning spans the globe and generally is observed in warm waters between latitudes within 35° of the equator (36). It is the most common type of fish poisoning in the Caribbean (37). In the US during the period from 1978 through 1999, 309 outbreaks of ciguatera involving 1295 persons were reported to CDC. No ciguatera-related deaths were reported (9, 10, 13). Of the 216 outbreaks between 1983 and 1999, 173 were reported from Hawaii and 28 from Florida (10, 38). In most cases, outbreaks in other states have been related to travel to the endemic areas or from eating fish caught in endemic ciguatera areas. And there is concern that many cases are not recognized by mainland US physicians (38, 39). Despite its exceedingly low incidence outside endemic areas, as the domestic fish industry expands its sources of supply, the diagnosis of this "tropical" disease must also be considered in areas where coral reef fish are not native.

Ciguatoxins (CTX), the toxins responsible for ciguatera, are produced by *Gambierdiscus toxicus,* a marine dinoflagellate that belongs to the family of benthic macroalgae. They usually grow attached to dead coral and are ingested by small herbivores off the reef (29). They are lipid-soluble polyether toxins that, when ingested by certain subtropical and tropical fin fish, can accumulate in their tissues. Biotransformation of CTX in the fish increases their polarity and thus their toxicity. The toxins and their metabolites are concentrated when carnivorous reef fish (e.g., barracuda, grouper, and amberjacks) prey on smaller herbivorous fish; the

toxic effect is amplified in large predatory fish, which become the most toxic to humans at the end of the food chain (37). Factors influencing the concentration of CTX that accumulate in fish include the rate of dietary intake, the efficiency of assimilation, the degree and nature of any toxin biotransformation, the rate of depuration, and the rate of growth of fish (40). More than 400 species of fish can be vectors of CTX, but generally only a relatively small number of species of reef fish belonging to the family Carrangidae are regularly incriminated in ciguatera. The fishes most commonly implicated include amberjack, snapper, grouper, barracuda, and goatfish. The toxin may be most concentrated in the head, the liver, intestines, testes, ovaries, and roe. CTX activate voltage-dependent sodium (Na^+) channels, causing cell membrane excitability and instability (41). In vitro studies suggest that the CTX causes a nerve conduction block after initial neural stimulation (42).

Maitotoxin (MTX) is a water-soluble polyether phytotoxin also produced by *G. toxicus* that is distinct from CTX. MTX induces severe pathological changes involving the stomach, heart, and lymphoid tissues in experiments with mice and rats (43). It also displays hemolytic and ichthyotoxic activities. MTX-induced hemolysis depends on calmodulin and phospholipase A2 activity. Its toxicity to fish depends on pH and Ca^{2+} concentration (44). It is a potent activator of a voltage-independent, nonselective cationic channel that causes an elevation of the intracellular Ca^{2+} concentration, which is ultimately responsible for its toxicity (45).

Ciguatera often affects only a discrete region of a reef, with flare-ups of ciguatera being both temporally and spatially unpredictable (30). Although low levels of *G. toxicus* are found throughout tropical and subtropical waters, the presence of blooms is unpredictable and patchy. Only certain genetic strains produce CTX, and environmental triggers for increasing toxin production are unknown (46). However, there is concern as to whether disruptions in the reef ecosystem may shift the balance toward a higher rate of toxin producing *G. toxicus and* an increased incidence of ciguatera poisoning (47).

CTX are heat stable, so they are not inactivated by either cooking or freezing. They are not affected by gastric acid and are harmless to the fish itself. Because they are odorless, colorless, and tasteless, ciguateric fish look, taste, and smell normal, and detection of toxins in fish remains a problem. A radioimmunoassay and subsequently a stick-enzyme immunoassay and a solid-phase immunobead assay were developed in Hawaii that detect even negligible amounts of toxins in suspect fish flesh (48–50). The stick-enzyme immunoassay has been improved and has become a simple, rapid, sensitive, and specific test for CTX (51). Recently a test (Cigua-Check, by Oceanit Test Systems) based on this detection method has become available for use by sports fishermen that could screen fish for CTX. However, its cost, and the lack of awareness of the kit, remain an obstacle to its use. Because there is no generally approved assay for the presence of CTX in humans who have consumed suspected contaminated fish, the diagnosis must be based on clinical findings and by the detection of toxin in samples of fish. Thus, any uneaten portions of fish should be saved in a freezer and submitted to state or local public health officials when suspected cases are reported, to assist with the investigation and control of a possible outbreak (52).

There is no immunity and no known antidote for CTX poisoning. Treatment is primarily supportive and for relief of symptoms. However, intravenous mannitol may be effective early in the course of illness in reducing the associated neurological and muscular symptoms (53–55). These promising results with mannitol were not confirmed in a randomized, placebo-controlled trial; thus it cannot be endorsed as a general therapeutic recommendation (56). To prevent ciguatera, persons living in or traveling to areas where ciguatera toxin is endemic should follow these general precautions (52):

1) Avoid consuming large, predatory reef fish, especially barracuda and amberjack;
2) Avoid eating the head, viscera, or roe of any reef fish;
3) Avoid eating fish caught at sites with known ciguatera toxins.

Puffer Fish (Tetrodon Poisoning)

Symptoms begin with paresthesias 10–45 minutes after ingestion, usually a stinging of the lips, tongue, and inner surface of the mouth. Common symptoms include headache lightheadedness, dizziness, vomiting, diaphoresis, pallor, weakness, malaise, and feelings of doom (56). Some patients may experience a floating sensation, salivation, muscle twitching, and pleuritic chest pain. Depending on the amount of tetrodotoxin (TTX) in-

gested, the patient may experience ataxia, dysphagia, aphonia, and convulsions. Severe poisoning is indicated by hypotension, bradycardia, depressed corneal reflexes, and fixed dilated pupils. An ascending paralysis develops, and death can occur within 6–24 hours secondary to respiratory muscle paralysis (11). Petechial hemorrhage, blistering and desquamation, and hematemesis have also been reported. Prognosis is good if the patient survives the first 24 hours (56).

Diagnosis is based on clinical symptoms and a history of recent consumption of suspect fish. Treatment is supportive, including active airway management, and ventilatory and circulatory support as needed. To minimize the amount of toxin absorbed, gastric lavage and activated charcoal may be beneficial soon after the ingestion. There is no specific antitoxin for human use; however, 4-aminopyridine was recently shown to effectively reverse neuromuscular blockade and cardiorespiratory depression in a guinea pig model of TTX poisoning (57).

Puffer fish poisoning is rare in the US. Since 1951, only approximately 10 cases have been reported, including three fatalities (58, 59). It is more common in Japan, where 20–100 fatal cases occur each year (11). The mortality rate is high and approaches 60% (32).

Puffer fish poisoning results from ingestion of the flesh of certain species of fish belonging to the order Tetraodontidae that includes ocean sunfishes, porcupine fishes, and fugu, which are among the most poisonous of all marine life (60, 61). These fish get their name because they characteristically inflate to several times normal size by swallowing air or water when threatened. The liver, gonads, intestines, and skin of these fish contain TTX but the flesh is edible if cleaned and prepared properly, and it is considered a delicacy by some persons in Japan who may pay the equivalent of $400 for one meal. Rigid public health standards including training and certification of fugu chefs has decreased the incidence of puffer fish poisoning but it has not eliminated the risk altogether. All puffer species in US waters, including *Sphoeroides maculates, Sphoeroides annulatus,* and *Arothron hispidus* have been implicated in fatalities and it is prudent to consider them potentially toxic. However, the US Food and Drug Administration (FDA) has generally permitted fugu to be imported and served in Japanese restaurants by certified fugu chefs on special occasions. A cooperative agreement with the Japanese Ministry of Health and Welfare ensures that fugu is properly processed and certified safe for consumption by the government of Japan before export (59).

TTX is a heat-stable alkaloid that blocks Na^+ conductance and neuronal transmission in skeletal muscle. The natural source of TTX was identified in marine *Vibrio* species that are part of puffer fish microflora rather than the fish itself, which merely accumulates the toxin in its tissue (62–64). It is interesting that TTX is also present in other species, such as blue-ringed octopus and some newts and toads (65, 66). TTX concentration in puffer fish fluctuates drastically with its reproductive cycle, reaching a peak around the spawning season, and it is considerably higher in the female than the male (56). For the very toxic fish, consumption of as little as 10 g of the toxic tissue may be fatal, and 1–4 mg of TTX constitutes a lethal dose for humans (11).

Common Intoxications Associated with Shellfish

Paralytic Shellfish Poisoning

PSP, which is caused by saxitoxins (STX), is the best known of the shellfish poisonings and causes the most severe symptoms. It is a serious illness in which neurological symptoms predominate. The first and most consistent symptom is numbness and tingling or burning of the lips, tongue, and throat that begin within 30 minutes of ingestion. Paresthesias spread to the face and neck and often to the fingertips and toes. They usually precede muscular weakness that affects the upper and lower limbs, and in more severe cases are followed by dysphonia, dysphagia, and ataxia. Finally, PSP may result in paralysis within 2–12 hours that may persist for as long as 72 hours. A sensation of floating in air, dizziness, weakness, drowsiness, headache, salivation, intense thirst, and tightness in the throat are commonly described. Diaphoresis, nausea, vomiting, diarrhea, tachycardia, and temporary blindness may also occur. Reflexes may be normal or absent, and most patients remain calm and conscious throughout. Death can result from paralysis of the respiratory muscles within 2–24 hours, depending on the dose. Prognosis is good for individuals surviving past 12 hours. The duration of the illness may be from a few hours to a few days but occasionally muscular weakness can persist for weeks following recovery (17).

Diagnosis is based on characteristic symptoms and on a history of recent ingestion of shellfish. There is no specific laboratory diagnostic test for a patient with PSP. However, examination of water samples for toxic algae and laboratory tests on the suspect food can provide supportive evidence.

Treatment is symptomatic. Gastric emptying has been advocated by some authors as an early treatment, and activated charcoal has generally been recommended to help block further absorption of the toxins. Airway management and ventilatory support is the mainstay of treatment and can be life-saving. However, larger doses of poison may result in death despite this treatment. Fluid therapy facilitates renal excretion of the toxin, and intravenous administration of sodium bicarbonate may be beneficial to correct possible acidosis. Since the half-life of elimination of the toxin from the body is about 90 minutes, 9 hours should be adequate in most cases for physiological reduction of toxin concentration to relatively harmless levels. There is no immunity to PSP, and the second attack may be more severe than the first. No effective antidote is available, but experimental results with 4-aminopyridine are promising in a guinea pig model of PSP (57).

In the US, PSP is a problem primarily in the New England states and in Alaska, California, and Washington. Most disease incidents involve mussels and clams gathered and eaten by recreational collectors, often from closed areas, reflecting the effectiveness of current testing and control measures for commercially produced shellfish. The CDC listed 24 outbreaks involving 219 people, with four fatal cases, during 1978–1999, suggesting a mortality rate of $< 2\%$ (9–13). The case fatality rate has been quoted at about 8.5% (67), but at present it is probably less than 1% in developed countries (68). Although PSP is an extremely dangerous disease that can cause death, there is reason to believe that mild cases due to consumption of marginally toxic clams by recreational diggers are never reported to health authorities, or are misdiagnosed.

The first case of PSP was described in 1793 as poisoning by mussels in explorers of the coastline of British Columbia, Canada (69). The dinoflagellate *Alexandrium catenella* (then called *Gonyaulax catenella*) was identified as the actual cause about 1927 (70). Bivalve mollusks, such as mussels, clams, and oysters, assimilate and temporarily store STX produced by dinoflagellates; thus they function merely as vectors for the toxin. The primary sources of STX include three morphologically distinct genera of saltwater dinoflagellates, *Alexandrium* spp (previously *Gonyaulax*), *Pyrodinium* spp, and *Gymnodinium* spp (71); and four species of freshwater blue-green algae, *Aphanizomenon flos-aquae, Anabaena circinalis, Lyngbya wollei,* and *Cylindrospermopsis raciborskii* (17). The STX are water-soluble alkaloids that consist of various sulfonated and hydroxylated derivatives and that contain the basic structure of a tetrahydropurine skeleton and two guanidinium groups. They are among the most potent neurotoxins known. More than 20 STX analogs have been described. The positively charged guanidinium group of the toxins binds specifically to a negatively charged site of the Na^+ channel on the extracellular side of the plasma membrane of nerve and muscle cells, thus blocking the flow of Na^+ through the channel. As Na^+ entry through the nerve cell membrane is essential for impulse transmission, blockage interferes with signal transmission and results in paralysis (71). Most shellfish contain a mixture of several STX, depending on the species of algae, geographic area and type of marine animal involved. Biotransformation of the toxin results in generation of forms with greater toxicity. The higher the net charge, the greater the toxicity. The potency of STX is expressed in mouse units per milligram (MU/mg). One MU is the amount of toxin required to kill a mouse weighing 20 g in 15 minutes after intraperitoneal injection, and is equivalent to 0.18 μg of STX. Toxicity of the STX is generally expressed in terms of STX equivalents (STX eq) per 100 g of shellfish meat. There is great variation in individual susceptibility, and children are thought to be more susceptible. As little as 120–180 μg of STX can induce moderate symptoms in adults, and fatalities have been associated with levels of 0.3–12 mg (17). Although normal steaming or boiling will not inactivate the toxins, exposure of toxic shellfish to high temperatures (e.g., in the sterilization step of the canning process) substantially reduces STX concentrations, although the effectiveness of canning as a means of reducing STX levels below the statutory limit depends on the initial toxicity and must be used with caution (72).

The mouse bioassay is the classic method for analysis of STX. It is a standardized procedure in which mice are injected with toxin extracts, and their responses compared with those from known amounts of toxin. It is insensitive, with a detection limit of only 40 μg STX eq/100 g shellfish meat (73). High-performance liquid chromatography (HPLC) is quite rapid and has been considered as a possible replacement for the mouse bioassay.

HPLC detection limits are generally an order of magnitude lower than that of the mouse bioassay (74). A direct enzyme immunoassay has been available for determination of STX in shellfish that correlates closely with mouse bioassay (75). A lateral flow immunochromatographic (LFI) assay has been developed, the MIST Alert dipstick test for PSP. In toxic shellfish it can detect STX > 80 μg/100 g, with 100% sensitivity, and detects 95% of samples in the range 32–80 μg/100 g. It has a false positive rate of 15% at < 32 μg/100 g, which is below detection limit of the mouse bioassay (76). MIST Alert was also evaluated for rapid identification of PSP toxins in the water column and benthos. PSP toxins are detected at 100 cells per sample with no false negative responses. It appears to be an effective tool for broad scale monitoring of algal toxins in coastal waters and has the potential to replace existing surveillance techniques (77). STX is a potent inhibitor of the membrane depolarizing effects of the Na^+ channel activator veratridine. On the basis of this property, a membrane potential assay using mouse brain synaptoneurosomes has been developed. PSP toxins contained in shellfish extracts can be detected by inhibition of veratridine-induced depolarization using the fluorescent probe rhodamine 6G (78).

In the US, the important toxigenic dinoflagellates causing PSP are *Alexandrium catenella* and *A. tamarense;* the first being most dominant on the West Coast and responsible for PSP outbreaks in the Pacific; and the second on the East Coast and associated with New England outbreaks (79). When the dinoflagellates proliferate, or "bloom," they often give the water a red or reddish-brown discoloration, hence the name "red tide." Outbreaks of PSP tend to cluster from shortly before and up to several weeks after the appearance of red tide (80). Some *Alexandrium* species do not produce toxins, and not all red tides are caused by toxic algae (81). Conversely, shellfish may also become toxic in the absence of red tide (31). Anthropogenic eutrophication has been incriminated in a higher frequency of red tides or harmful algal blooms and increased production of biotoxins by marine dinoflagellates (16, 82).

STX persist in shellfish for varying periods, depending on the shellfish and the tissue involved (79). Mussels become highly toxic within a few hours to a few days of the onset of a red tide, but lose their toxin rapidly. Clams and oysters generally do not become as toxic as mussels. They require more time to accumulate high levels of toxins and longer to cleanse themselves. The Alaska butter clam, once contaminated, may never be safe to consume, as it retains PSPs for years (70). Sea scallops can take up large amounts of STX, even in the absence of algal blooms, but generally do not pose a threat because their adductor muscle, the only part of the scallop that is usually consumed, does not accumulate toxins. Gastropods also accumulate significant amounts of STX, and in Spain, levels as high as 44 ppm have been recorded in meat of abalone. Even though paralytic shellfish toxins have been found in the viscera of rock lobsters and crabs, STX do not generally accumulate in significant amounts in muscle tissue. Similarly, these toxins can accumulate up to 50 ppm in intestine, liver, and gills of Atlantic mackerel, but not to any extent in their muscles. Therefore crustaceans and finfish do not present a threat of PSP unless consumed whole, or unless livers are consumed (17).

Shellfish containing STX cannot be detoxified by depuration, and the toxins, as noted, can persist within shellfish at dangerous levels for weeks or months after the algae are no longer present in the growing waters. The most effective way of protecting consumers is thus to establish and maintain comprehensive monitoring programs for toxic algal blooms and toxins in shellfish in all growing areas. Seafood containing STX looks and tastes normal, and cooking or steaming only partially destroys the toxins (67). When toxic algal species are present in significant numbers, seafood products must be tested for toxicity and withheld from marketing if necessary. The FDA "alert level" for STX is 0.8 ppm (80 μg/100 g) in shellfish meat. Commercial shellfish harvesting in the US is suspended if higher concentrations are detected in routine monitoring programs. Toxin levels can exceed 10 mg/100 g of mussels (83). As illness has been reported to occur in adults at a total oral dose of only 120 μg, and death at 300 μg, this maximum permitted level is not particularly conservative. The best way to prevent PSP is thus to adhere to the public health agency guidelines on harvesting, processing, and consumption of shellfish. To further minimize the risk of PSP, the public should avoid collecting shellfish from areas of known red tides, and refrain from consuming suspect shellfish. In addition, since these toxins are water soluble, they can dissolve and concentrate in the broth, which should be discarded after cooking or steaming (17).

Neurotoxic Shellfish Poisoning

NSP is characterized by both GI and neurological symptoms. The illness resembles a mild case of ciguatera or PSP but without a paralytic component. The onset is rapid and symptoms occur within 3 hours of ingestion of contaminated shellfish. Symptoms include numbness of lips, tongue, and throat; paresthesias, which are initially circumoral, spread to other parts of the body. Reversal of hot and cold temperature sensation, muscular aches, dizziness, nausea, vomiting, diarrhea, abdominal pain, and headache can also occur. Less commonly, victims may experience a feeling of inebriation, burning pain in the rectum, dysphagia, ataxia, tremor, decreased reflexes, mydriasis, and bradycardia. The intoxication is usually self-limited and resolves spontaneously within a few hours. Treatment is supportive, and most patients recover with no aftereffects within a few days. No fatalities have been reported. There are no known antidotes for the toxin (84). From 1978 through 1998, the CDC listed only a single small outbreak involving three members of a family that consumed toxic small clams harvested from Sarasota Bay, Florida in June 1996 (10). The diagnosis was confirmed by detection of the causative toxins, brevotoxins (BTX) in the urine of the patients and in extracts of shellfish collected from the same location by radioimmunoassay (RIA) and by receptor binding assay (RBA) (85). Recently a competitive ELISA was developed that detects BTX in body fluids such as urine and serum, seawater, and shellfish extract with a detection limit of 2.5 μg/100 g of shellfish meat. This appears to be a useful tool for monitoring shellfish and seawater, and for diagnostic investigations (86).

Interestingly, unlike other shellfish toxins, the BTX can aerosolize by surf and wave action along the beach during red tides. Irritant toxin aerosols produce a syndrome characterized by conjunctival irritation, sneezing, and rhinorrhea that resembles an allergic response. Also, they can trigger shortness of breath, nonproductive cough, and wheezing due to bronchospasm in individuals with underlying asthma or chronic obstructive pulmonary disease that can precipitate severe respiratory distress. The syndrome is self-limited and treatment of the bronchospastic episodes due exposure to aerosolized toxins is symptomatic. In vitro data indicate that BTX produce contraction of human lower airway smooth muscle via stimulation of cholinergic nerve fibers through activation of Na^+ channels (87). In 1987 during a red tide off the coast of North Carolina, Tester and Fowler (88) reported 48 individuals that experienced upper and/or lower respiratory symptoms.

Karenia brevis (formerly *Gymnodinium breve*) is the dinoflagellate that synthesizes BTX, a group of related heat-stable toxins that are responsible for clinical manifestations of NSP. BTX are lipid-soluble polyether toxins of unique structure and pharmacological function. They are active in vivo in the nanomolar to picomolar concentration range. Their excitatory effect is mediated by the enhancement of cellular Na^+ influx through the voltage-sensitive Na^+ channel (89). Filter feeding bivalve mollusks, such as oysters, clams, and mussels that consume *K. brevis,* concentrate the toxins in various organs and become toxic to humans but remain unaffected. NSP in the US is generally associated with the consumption of shellfish harvested along the coast of the Gulf of Mexico from Florida to Texas, and, sporadically, along the southern Atlantic coast. This is identical with the geographic distribution of *K. brevis* blooms, also known as red tides, that occur in many different areas within the Gulf of Mexico, where it results in massive fish kills. The earliest record of fish kills, later attributed to a *K. brevis* bloom, was in 1844 off the west coast of Florida. Such red tides continue to occur, and may be carried north in the Gulf Stream, occasionally affecting the coastline of adjacent states. They also occur throughout the world, including New Zealand and Japan (83, 90).

K. brevis blooms appear to be initiated on the continental shelf or at the shelf edge, usually more than 40 miles offshore, rather than near the shore where they produce the most deleterious effects. Bloom initiation is characteristically associated with intrusion of deeper, offshore waters onto the shelf. Once dense blooms move inshore, they cannot be sustained without maintaining a minimum nutrient level. Thus, human inputs of nutrients could be responsible for extending the duration and impact of red tides when blooms enter the near-shore waters (91). These blooms on the southwest Florida shelf served as a source for cells that inoculated the Florida east coast and North Carolina in 1987–1988 (92). The concern has again been raised that human activity may thus increase the frequency of harmful algal blooms and disseminate *K. brevis* and other toxic phytoplanktons to nonindigenous waters (93, 94).

K. brevis is well adapted and is able to outcompete or otherwise exclude other phytoplankton species. Low concentrations (< 1000 cells/L)

of the organism occur in offshore waters throughout the year and can be detected microscopically. Typically in late summer and fall when nutrients are abundant, and physical, chemical, and biological conditions are favorable, *K. brevis* can grow rapidly, gradually building high densities that reach bloom concentrations in 2–8 weeks (1×10^5 to 25×10^5 cells/L).

During severe blooms fish die rapidly from the neurotoxic effects, so do not survive to accumulate high toxin concentrations in their tissue. However, fish exposed to sublethal concentrations may accumulate these toxins. Such bioaccumulation in fish eaten by marine mammals such as dolphins and manatees results in their demise due to BTX exposure and may also affect human health.

Chlorophyll in *K. brevis* results in discoloration of surface water at 10–100 mg/m^3 and is a good surrogate for biomass. It can be detected by satellite color sensors at densities three orders of magnitude less than when water discoloration is visible to human eye, at about 10^6 cells/L. However, it cannot detect deep patches or distinguish *K. brevis* from other algae, which limit the utility of this technology as an early warning system for a ban on shellfish harvest and beach closure. Local authorities then routinely close shellfish harvesting to industries and the public. The basis for closure is the occurrence of more than 5000 *K. brevis* cells per liter of seawater, and reopening of harvest depends on demonstrated absence of BTX in shellfish meat (95, 96). The FDA has established a guidance level for BTX at 0.8 ppm (80 μg/100 g) BTX-2 equivalent (20 MU/100 g) in shellfish meat, and shellfish harvesting is banned if higher concentrations are detected in monitored areas (83). The small number of cases of NSP testifies to the effectiveness of the surveillance and closure systems operated by the states.

Amnesic Shellfish Poisoning

ASP presents initially with vomiting, diarrhea, and abdominal cramps within 24 hours of ingestion of contaminated shellfish. In some cases, varying degrees of neurologic dysfunction ensues within 48 hours, including confusion, loss of memory, and disorientation. Other neurologic symptoms are headache, hyporeflexia, hemiparesis, ophthalmoplegia, and abnormalities of arousal ranging from agitation to coma, seizures, and myoclonus especially affecting the face. The acute symptoms are milder compared with PSP. Loss of short-term memory is unique among the marine poisonings, hence the name amnesic shellfish poisoning (97). This is the most persistent symptom and can be permanent.

The syndrome was first described in a series of outbreaks in persons that had eaten mussels cultivated in the river estuaries of Prince Edward Island in Canada from November through December 1987 (98). In this cohort, acute symptoms were vomiting (76%), abdominal cramps (50%), diarrhea (4%), severe headache (43%), and loss of short-term memory (25%). GI symptoms were present in all but seven of the 107 cases. Onset of symptoms after mussel ingestion ranged from 15 minutes to 38 hours, with a median of 5.5 hours. Nineteen patients (18%) were hospitalized, of whom 12 required intensive care because of seizures, coma, profuse respiratory secretions, or unstable blood pressure. Severity of the disease and permanent neurologic sequelae, especially cognitive dysfunction, are associated with age over 60 years, male sex, pre-existing illnesses, and the amount of mussels consumed. Three elderly patients died directly and one died indirectly from the intoxication. Neuropathologic studies in these four fatal cases showed neuronal necrosis in the hippocampus and amygdala (99). Teitelbaum et al (99) reviewed clinical records of 14 more severely affected patients that displayed neurologic manifestations. All 14 patients reported confusion and disorientation within 1.5–48 hours after ingestion and exhibited a variety of neurologic abnormalities including coma (nine), mutism (11), seizures (eight), purposeless chewing and grimacing (six), and uncontrolled crying or aggressiveness (six). In neuropsychological testing performed in those 14 patients several months after the acute episode, 12 had severe anterograde-memory deficits, with relative preservation of other cognitive functions. Eleven of the 14 individuals had clinical and electromyographic evidence of pure motor or sensory motor neuronopathy or axonopathy. The maximal neurologic deficits were seen 4 hours post ingestion in the least affected patients and 72 hours in those most affected, with maximal improvement 24 hours to 12 weeks post ingestion. Acute coma was associated with the slowest recovery. Seizures ceased by 4 months but were frequent up to 8 weeks (100). Relative preservation of intellect and higher cortical function appears to distinguish ASP from Alzheimer's disease, and the absence of confabulation with well-preserved frontal lobe function differentiates it from Korsakoff's syndrome. Diagnosis is based on a recent

history of shellfish ingestion and is made on clinical grounds. It is confirmed by demonstration of domoic acid (DA) in shellfish samples. At this point, the treatment of ASP is symptomatic and supportive. Seizures respond well to parenteral benzodiazepines and phenobarbital. There is no antidote, and immunity does not develop.

In mussels left uneaten by the patients, and in mussels harvested later from the same estuaries, the toxic agent was isolated and identified as DA. Its concentration ranged from 31 to 128 mg/100 g of mussel meat, which suggested an ingestion of 60–290 mg of DA per patient (97).

The source of DA at Prince Edward Island in 1987 was subsequently identified as the phytoplanktonic diatom *Pseudo-nitzschia multiseries,* formerly known as *Nitzschia pungens* (100). ASP is the only shellfish poisoning caused by diatoms. DA is a potent neurotoxin that accumulates in mussels and clams that feed on toxic plankton during their bloom. On the Pacific coast, DA is produced by *P. multiseries* and *P. australis* that bloom in late summer and fall. DA is water-soluble and heat-stable and similar in structure and function to another excitatory neurotoxin known as kainic acid (KA), which is found in Japanese seaweed, *Digenea simplex.* DA and KA both appear to produce neurotoxic effects by activating the glutamate receptors (101). These receptors are ligand-gated, voltage-dependent calcium (Ca^{2+}) channels that are activated by glutamic acid, mediating a fast excitatory synaptic transmission in the mammalian central nervous system (CNS). Persistent activation of KA receptors results in elevated levels of intracellular Ca^{2+} that causes neurotoxicity with subsequent lesions in areas of the brain where glutaminergic pathways are heavily concentrated (102). The observations that the glutamate receptors are present within the cardiac conduction system, intramural ganglia, and cardiac nerve fibers could explain some of the clinical manifestations such as the arrhythmia described with DA intoxication in humans. Hence individuals with premorbid cardiac conditions may be at higher risk of the toxic effects of these excitatory compounds (103). In animals, DA is three times as potent as KA and 30–100 more potent than glutamic acid (104).

DA poisoning first became a noticeable problem in the West Coast of the US in September 1991 when it was reported that brown pelicans had died after eating anchovies in Monterey Bay off the coast of California. It was subsequently found that the death of these pelicans was due to the bloom of *P. multiseries* that produced high levels of DA (105). Since this time and until December of 1998, 29 cases of ASP have been reported to CDC, all of which occurred in November 1991 and were caused by razor clams harvested in Washington (10). No fatalities have occurred in the US, but the mortality rate was 3.7% in the 1987 Canadian outbreak.

Traditionally, mouse bioassay has been used for detection of DA, which is the same assay as for PSP; however, the relative potency of DA appears to be less than for STX. There are several newer methods used to detect DA in seawater and shellfish, such as HPLC, immunoassay, and an RBA. Also, two indirect competitive enzyme immunoassays (EIA) for measurement of DA in shellfish and seawater have been developed. One utilizes polyclonal ovine antibodies and the other is based on monoclonal murine antibodies. They have a working range of 0.15–15 μg/L and 0.15–10 μg/L, respectively and a quantification limit of less than 4 μg/100g of shellfish flesh (106, 107). There is also an RBA that measures the competitive displacement of radiolabeled KA bound to a cloned glutamate receptor (GluR6) by DA in a sample. A comparison of the latter two methods showed that the RBA has a larger working range whereas EIA is more sensitive. The respective detection limits and working ranges are 3.1 μg/L and 5–100 μg/L for the RBA, and 0.01 μg/L and 0.15–15 μg/L for the EIA. RBA and EIA yield statistically equivalent results for detection of DA in seawater (108). The MIST Alert dipstick test for ASP, an LFI assay, is the newest assay and has a detection limit of approximately 8–12 μg/g DA in shellfish extracts, which is about half the regulatory limit, and has a sensitivity approaching 100% (76).

In Canada, to prevent future outbreaks of ASP, sacks of mussels are now labeled with respect to time and place of harvesting. In addition, water columns and shellfish are monitored for the presence of *Pseudo-nitzschia* and DA, respectively. Since an estimated concentration of 20 mg/100 g wet weight DA has affected some consumers, applying a safety factor of 1/10, Canadian surveillance authorities have set 2 mg/100 g as the threshold level above which shellfish commercial operations are suspended.

On the Pacific coast, DA poisoning has also been a serious problem affecting razor clams and Dungeness crabs in Washington, and oysters, bay and razor clams, and mussels in Oregon. Authorities in Washington, Oregon, and California now randomly analyze samples of commercially harvested or cultivated shellfish for DA. The FDA has adopted the level of 20 ppm (2 mg/100 g) for DA, except in the viscera of Dungeness crab, in which

30 ppm is permitted; when higher levels are detected in their tissues, closure of beds is enforced (83, 109). States in the northeastern US are also monitoring shellfish for DA, which is present at low levels that do not necessitate quarantine.

Diarrheic Shellfish Poisoning (DSP)

DSP is the mildest and most benign of the toxic shellfish poisonings. Clinical features are generally limited to the GI tract and include diarrhea (92%), nausea (80%), vomiting (79%), abdominal pain and cramps (53%). Chills, fever or headache may also be present in up to 10% of cases (110). The symptoms usually manifest in a period ranging from 30 minutes to 6 hours after ingestion of contaminated shellfish and persist on average for 36 hours. No known fatalities have occurred, and total recovery is expected within three days. Due to the transient nature of the illness and its spontaneous resolution, patients often do not seek medical attention. However, the duration of symptoms may be shortened with charcoal, which reduces the bioavailability of the toxins, and its repeated administration interrupts their enterohepatic recirculation. Treatment, if required, is limited to alleviation of symptoms.

DSP is associated with the consumption of mussels, scallops, clams, and oysters contaminated with biotoxins produced by toxic marine dinoflagellates during their blooms in the summer. *Dinophysis* and *Prorocentrum* species have been identified as the source of DSP toxins, which are heat-stable and not denatured by normal cooking. Although to date no DSP outbreaks have been documented in the US, toxin-producing *Dinophysis* species are present in US waters, and in 1990 caused an outbreak in eastern Canada. The disease occurs worldwide in temperate waters. It is common in Japan, where over 200 cases are reported annually, and it has become a public health problem in Europe. Sporadic outbreaks have also been documented in Southeast Asia, Chile, Australia, New Zealand and eastern Canada (83).

At least 10 different toxins have been isolated from dinoflagellates and shellfish in association with DSP. The major toxins are high molecular weight polyethers including okadaic acid (OA), dinophysistoxins (DTX), and several of their metabolites, as well as pectenotoxins (PTX) and yessotoxin (YTX) (111). OA is most commonly encountered in Europe where *Dinophysis acuminata* is the usual agent, whereas in Japan mixtures of OA, DTX, and PTX are detected, usually involving *D. fortii* (112). OA is a highly selective inhibitor of protein phosphatases that causes dramatic increases in phosphorylation of numerous proteins and can act as a potent tumor promoter. It induces diarrhea by increasing paracellular permeability of intestinal epithelial lining without inducing cytotoxicity (113). DTX are structurally related to OA and cause, in laboratory experiments, highly similar intestinal lesions that appear within 5 minutes of dosing and resolve completely within 2 days (114). PTX, although nondiarrheagenic, are potently cytotoxic and are tumor promoters in animals (115). YTX is only a weak cytotoxin, and is not orally lethal to mice. It does not cause accumulation of intestinal fluid or inhibit protein phosphatases, and has no diarrheagenic or hemolytic effects, suggesting that it should not be classed as a DSP toxin (116). The DSP toxins, particularly OA and some DTX, are potent microalgal inhibitors. They are probably an evolutionary adaptive mechanism and are produced by toxic dinoflagellates to create a survival advantage against other competing microalgae (117).

Mouse bioassay is the standard method for DSP surveillance; however, it is nonspecific and lacks sensitivity. HPLC is an alternative technique and has a low detection limit of 26 μg/100 g of shellfish for both OA and DTX, but it is cumbersome and requires extensive calibration (118). Most of the recent developments in rapid screening methods for OA detection are based either on the use of specific antibodies, or on its ability to inhibit protein phosphatases coupled with use of fluorescence substrates. The fluorimetric assay achieves a detection limit of 1 μg /100 g OA in mussel tissue, which is well below 20 μg/100 g that has been established by FDA as the toxicity threshold level (119). Commercially available EIA kits detect OA and some of its metabolites, but not all DSP toxins. Thus EIA kits underestimate total toxin present in crude toxic shellfish (120, 121). Both the HPLC and protein phosphatase inhibition (PPI) assays correlate well with each other and with the standard mouse bioassay. Although EIA does not accurately and consistently detect low DSP toxin concentrations, it offers advantages of rapidity and ease of use and may become useful as a screening tool (118).

At present, for the US consumer, the risk of DSP is limited to imported products and should be controllable by import regulations that permit import of shellfish only from countries that test it for the presence of toxins. Nevertheless, because

Dinophysis does occur in US coastal waters, regulatory agencies in the US should be alert to the possibility of an outbreak (122).

Less Common Seafood Poisonings

Clupeotoxism

Clupeotoxism is a highly fatal form of human intoxication due to ingestion of Clupeidae (herring-like fish). It is a rare poisoning, occurring in the tropics only during the warm summer months. Like ciguatera, it occurs sporadically and over an extensive area of the tropical Pacific and Indian Ocean coasts. A few cases have been reported from Madagascar related to eating sardines, including one fatality in 1994. Symptoms include nausea, vomiting, diarrhea, abdominal pain, headache, dry mouth, sweats, chills, lightheadedness, and paresthesias, and in one report one of the two patients with clupeotoxism died (123). Physical findings include tachycardia, hypotension, tachynea, and cyanosis. Treatment is supportive and no specific antidote is available. The causative toxin was identified as palytoxin, one of the most potent phycotoxins known (124). It is tasteless and odorless and not inactivated by cooking. Recently, palytoxin was reported to induce rhabdomyolysis in individuals who had eaten parrot fish in Japan. Patients presented with weakness and myalgia within 5 hours of ingestion, and recovered without long term complications. In one case, myocardial damage was present as indicated by electrocardiographic changes and elevation of cardiac enzymes (125, 126).

The benthic dinoflagellate *Ostreopsis siamensis* has been identified as the source of palytoxin (124). *O. siamensis* is found in African, Caribbean, and Indo-Pacific coastal waters. Plankton feeders such as herring, pilchard, tarpon, and anchovies that ingest it can become toxic. In most cases the fish have been captured close to shore indicating that they obtained the toxin from benthic algae in the bottom sediments.

Palytoxin alters the function of excitable cells and acts as a hemolysin. The mechanism of toxicity of palytoxin is not completely elucidated. However, it appears to activate a family of low-conductance cationic channels in excitable cells, which results in reduction of the membrane potential. This depolarization then triggers secondary activation of voltage-dependent Ca^{2+} channels and results in neurotransmitter release by nerve terminals, and contractions of striated and smooth muscle cells (127).

Elasmobranch Poisoning

Elasmobranch poisoning is caused by the ingestion of contaminated meat or liver from several species of sharks, most notably the Greenland sleeper shark (128). The disease is characterized by GI and neurological symptoms including nausea, vomiting, diarrhea, abdominal pain, headache, and perioral paresthesia. Malaise, weakness, muscle cramps, ataxia, and visual disturbances may also develop. Severe progressive respiratory distress, hyporeflexia and coma usually precede death. The onset of symptoms is 30 minutes to 5 hours following ingestion. The shark and its meat do not display any unusual characteristics. The toxicity is not affected by cooking. Trimethylamine has been proposed as the cause of this poisoning.

In November 1993, an outbreak involving 188 people who ate the meat from a single shark was reported from Madagascar. The patients presented within 5–10 hours of ingestion with neurological symptoms. Ataxia was almost universally present and of moderate to severe intensity. GI symptoms, including diarrhea and vomiting, were rare. The attack rate approached 100% and the overall case mortality was close to 30%. Carchatoxin-A and -B, two novel lipid soluble toxins, were isolated from the shark's liver, which were distinct from other known marine toxins (129).

Red Whelk Poisoning

In red whelk poisoning, symptoms develop within 30–120 minutes that include headache, dizziness, blurred vision or diplopia, paresthesias, dry mouth, muscular twitching or cramps, ataxia, weakness, and collapse. Nausea, vomiting, and diarrhea may also be present in some patients. The red whelk (*Neptunea antiqua*) is a gastropod species common in Japan and Northern European waters and is distinguished from the edible whelk (*Buccinam undatum*) by its larger size and smooth shell that has a distinctive pale orange coloration. It contains a heat-stable toxin, tetramine, present in the salivary gland, that produces symptoms due to its curare-like effect. Because symptoms resolve rapidly and recovery is complete within 24 hours, few people are likely to seek medical attention. Thus, it is rarely reported. It is notably more common in Japan (130, 131).

Newly Discovered Marine Biotoxins

Azaspiracid

Azaspiracid (AZA) is a structurally novel phycotoxin that contains a unique spiro ring assembly. It has been found to be responsible for outbreaks of diarrheic food poisoning associated with consumption of contaminated shellfish in Europe. The first outbreak was reported in November 1995 in the Netherlands, following the consumption of mussels harvested on the west coast of Ireland. It was initially mistaken for DSP, but subsequently proven to be azaspiracid shellfish poisioning (AZP). Since then, outbreaks have been reported in France, Italy, Ireland, Norway, and the UK (132). The onset is 12–24 hours after consumption of mussels, and the symptoms of the illness include severe diarrhea, vomiting, nausea, abdominal cramps, headaches, and chills, which resolve in 2–5 days (133). Although other shellfish species have not reportedly caused AZP, in several instances toxin levels in oysters have been comparable to the levels found in mussels from the same cultivation area (134).

As many as eight forms of AZA exist, and some evidence suggests that these toxins may be susceptible to heat but are not affected by freezing. The causative organism is *Protoperidinium crassipes,* a dinoflagellate found in North Atlantic waters. Although AZA was previously classified as a DSP toxin, it has been reclassified into a new poisoning category known as azaspiracid poisoning (135). AZA has a number of unique properties that set it apart from the "classic" DSP toxins OA, DTX, and YTX. In animal experiments, AZA administered orally induces pronounced neurotoxic effects and causes necrosis in the lamina propria of the small intestine, liver, and lymphoid tissues in the Peyer's patches, spleen, and thymus, whereas toxic effects of OA are limited to the GI mucosa (136).

In mice, AZA leads to progressive paralysis and is rapidly fatal within 5–60 minutes, whereas OA and DTX cause convulsions and prostration and ultimately death over a longer period of time. OA, DTX, and YTX are known to be located exclusively within the hepatopancreas (HP) of the shellfish, while AZA may initially concentrate in HP but eventually distributes throughout the body and migrates also into the flesh. Since depuration occurs in the HP first, mussels contaminated with AZA may take longer to depuriate. In addition, the current surveillance method that assesses shellfish toxicity on the basis of toxin concentration in HP will underestimate AZA hazard and may allow toxic shellfish to be harvested. A mouse bioassay is being developed, but until it is ready for use, an interim threshold concentration of 10 μg/100 g of whole shellfish has been proposed as a regulatory standard in Europe to prevent further AZP outbreaks. This level, based on available data, may be revised. A liquid chromatography/mass spectrometry (LC/MS) method for determination of AZA has been developed that has a detection limit of 50 pg AZA, and is far more sensitive than the mouse bioassay (137).

Spirolides

Spirolides are a novel family of lipophilic shellfish toxins isolated from the marine dinoflagellate *Alexandrium ostenfeldii.* They consist of a spiro-linked tricyclic ether ring system and an unusual seven-membered spiro-linked cyclic iminium moiety (138). To date, no human disease has been associated with spirolides. However, on the basis of their toxicity profile, they may be viewed as a potential cause of seafood poisoning. They were discovered in 1991 during routine biotoxin monitoring of shellfish in eastern Canada. Their distinct toxicologic and chemical properties differentiate them from other known lipophilic shellfish toxins. Spirolides are macrocyclic imines that were initially labeled as fast-acting neurotoxins, since in mouse bioassay they result in neurological symptoms and lead to death in 3–20 minutes. They appear to activate muscarinic receptors in the brain and particularly affect the brain stem. Rapid lethal action in rodents is probably due to compromise of cardiorespiratory centers in the brain stem (139). LC/MS is a highly sensitive analytical assay that can detect spirolides in concentrations as low as 2 μg/L, and is the method currently used for surveillance of biotoxins in Canada (140).

Potential Allergens Associated with Seafood

Residues of Bioactive Substances from Aquaculture

Aquaculture is an important source of food worldwide and now contributes up to 15% of the US seafood supply (141). Traditionally, the environmental safety risks of seafood products have been subdivided into natural hazards, such as biotoxins, and anthropogenic contaminants, such

as synthetic chemicals. In aquaculture, the latter hazard becomes more prominent as more synthetic products are used in the seafood industry. The use and misuse of antibiotics to control diseases in aquacultured species is widespread and worldwide, and will probably increase in the future. Similarly, the improper or illegal use of chemicals to control pond pests and algae can also result in human health hazards. On the other hand, natural products that are not present in aquatic environments can become health hazards when misused or abused. For instance, raw chicken manure as pond fertilizer may result in the transmission of *Salmonella* from manure to the cultured product (142).

Chemicals commonly used in aquaculture might be considered a potential threat to human health, including drugs and biologics, pesticides, disinfectants, and water-treatment products. FDA oversees the use of drugs in aquaculture and has approved oxytetracycline, sulfadimethoxine/ormetoprim, formalin, and tricaine for use in various aquatic species (143). However, many more drugs are believed to be used in an off-label fashion in aquaculture. The FDA Office of Seafood began a monitoring program for animal drug residues in farmed seafood in 1991 and has detected a few violations: residues of chloramphenicol in shrimp and oxolinic acid in salmon, for example (144).

Oxytetracycline is a prototype antibiotic that has been approved by the FDA for use in fish farming to control certain diseases in salmonids and catfish, but it is likely to be used in other fish species without formal FDA approval (145). The normal method of administration of oxytetracycline to the fish is to mix the drug into the feed. As a consequence, the concentration of the drug in feed and the composition of feed can influence the disposition of the drug itself (146). Oxytetracycline is depleted over a period following the completion of the treatment, and detectable residues in the fish could be transferred to humans if the fish is marketed during that period (147). In addition, illegal use of other antibiotics as, for example, chloramphenicol in shrimp culture, may similarly result in significant levels in the harvested product. Likewise, other bioactive substances, such as antiparasitic agents and algicides can accumulate in aquaculture products; for instance, mebendazole and its metabolites have been shown to leave detectable residues in cultured eel (148).

Allergic reactions have been reported following the ingestion of penicillin-containing milk in a few previously sensitized patients; however, residues in other foods, including seafood, have not yet been reported to cause allergic reactions. Primary sensitization of humans to antimicrobials through the consumption of drug residues in foods has never been clearly documented, and evidence suggests that the residue levels in food are too low to cause sensitization. Drug toxicity, other than allergic reactions, appears not to result from residues of antimicrobial drugs in food (149, 150). Although the available data suggest that these cases are exceedingly rare, they illustrate the continuing need to control antibiotic residues (150).

Genetically Engineered Neo-Antigens

Food biotechnology, the use of recombinant-DNA and cell-fusion techniques to confer selected characteristics on plants and animals used for food, can be used to increase agricultural productivity. The transfer of genes from microbes, plants, or animals into foods raises issues about the unintended consequences of such manipulations. Allergenicity could be one such consequence, because recombinant genes encode proteins that potentially could be allergenic (151). Although several bioengineered products have been introduced into the human diet since 1990, they have not resulted in any reported and confirmed case of food allergy.

An allergen from a food known to be allergenic can be transferred into another food by genetic engineering (152). Thus, before marketing, products of food biotechnology should be subject to a careful and complete safety assessment that includes evaluation of the potential allergenicity of the novel proteins (153).

Polar fish produce antifreeze proteins (AFPs), which, at low concentrations, decrease the freezing point of solutions and inhibit ice crystal growth. Transgenic expression of AFP in plants can prevent frost damage to crops and improve the quality of frozen fruits and vegetables. Genes encoding fish AFPs have been successfully expressed in tobacco and tomato plants (154); an AFP gene transferred from winter flounder to Atlantic salmon results in functionally effective levels of AFP (155). Fish AFPs are not known fish allergens, but they may or may not acquire allergenic properties when expressed in a different host. Using the same technique, other genes can be transferred from marine species to other animals or plants that could create neo-antigens and result in aller-

gic sensitization. In addition, fish can be recipients of transgenes that enhance disease resistance, increase growth rate and size, improve food conversion ratios, or benefit consumers by enhancing nutritional value or palatability. Transgenic expression of growth hormone has been achieved in commercially farmed fish, such as tilapia, catfish, trout, and salmon (156–158). Limited data are available on safety of biotechnology products in aquaculture. However, no post-marketing rise in incidence of seafood allergy has been reported, and in a published trial no adverse effects were detected in healthy subjects after the consumption of growth hormone-transgenic tilapia (159).

Poisoning by Bacterial Toxins

Botulism

Food-borne botulism is acquired from ingestion of food contaminated with preformed toxin that is produced by *Clostridium botulinum,* a sporulating, anaerobic, gram-positive bacillus. Symptoms are symmetric, descending, flaccid paralysis of motor and autonomic nerves, usually beginning with the cranial nerves, which may cause death by respiratory failure. Onset is abrupt and usually occurs 12–36 hours after ingestion of toxin. The first manifestations are often dry mouth, diplopia, blurred vision, blepharoptosis, and photophobia due to loss of papillary light reflex, but may be preceded by GI symptoms such as nausea, vomiting, abdominal pain, and diarrhea. Other common symptoms are generalized weakness, dysphagia, dysarthria, nasal voice, and constipation due to paralytic ileus. Sensory disturbances, fever, and tachycardia are typically absent. The diagnosis of botulism is based on compatible clinical findings and history of exposure to suspect foods, and is confirmed by detection of toxin in serum, stool, or gastric contents of the patient or in leftover fish. Differential diagnosis includes the Guillain-Barré syndrome, myasthenia gravis, basilar meningitis, and stroke (160).

C. botulinum produces seven types of toxins; types A, B, and E are usually involved in human poisoning. Botulism from seafood products is most frequently caused by type E toxin, which is the predominant type in Alaska and the Great Lakes area. Type E spores have been demonstrated in lakeshore mud, coastal sands, and sea bottom silt in northern latitudes that can contaminate the intestinal tracts of fish. Outbreaks of botulism have been reported after eating unviscerated, salted, air-dried whitefish and mullet, known as kapchunka and faseikh, respectively (161, 162). In Alaska, cases have been linked to Alaska native foods, such as marinated raw fish aged in plastic bags, seal meat stored in oil, and smoked salmon wrapped in seal skins (163).

The spores are highly heat-resistant and may not be inactivated by boiling for several hours. However, commercial canning procedures that use moist heat at temperatures above 250°F (121°C) will kill the spores. Although the majority of reported cases of botulism have been associated with the consumption of inadequately processed home-canned food, about 10% of outbreaks have resulted from contamination of commercially canned fish. In these cases, post-processing contaminants from faulty cans, or inadequate heating were responsible for the outbreaks (160, 164). Toxins, on the other hand, are readily destroyed by heat and are inactivated by boiling for 10 minutes, or by heating at 176°F (80°C) for 30 minutes. They are, however, resistant to digestive enzymes and are readily absorbed into circulation from the GI tract. The toxins are zinc metalloproteinases that cleave specific components of the synaptic membrane docking and fusion complex, preventing the release of acetylcholine at the neuromuscular junction and autonomic synapses (165).

Treatment includes close medical supervision, supportive care, and early use of trivalent equine antitoxin (types A, B, and E) and GI decontamination. The source of an outbreak must be determined to prevent further cases. Only prompt recognition, therapy, and epidemiologic investigation can reduce the death toll from botulism (166). In Alaska, where approximately 27% of US food-borne botulism cases occur, early diagnosis and antitoxin treatment have contributed to the decline of the case-fatality rate from about 31% during 1950–1959 to no deaths since 1994 (167).

Staphylococcal Food Poisoning

Acute gastroenteritis is caused by ingestion of food contaminated with pre-formed staphylococcal enterotoxin. The onset is abrupt and ranges 2–8 hours post ingestion. Symptoms start with severe nausea and vomiting in most cases. Other symptoms include abdominal cramping, diarrhea, and occasionally headache and fever. The attack is brief, often lasting only 3–6 hours. Recovery is usually complete, but in severe cases it may lead

to dehydration, prostration, and shock. Diagnosis is based on clinical findings confirmed by demonstration of coagulase-positive staphylococci in the suspected food or vomitus. Treatment is symptomatic (168). The disease is caused by the enterotoxins produced by *Staphylococcus aureus,* rather than the organism per se, which multiply at a temperature range of 39°F–115°F (4°C–46°C). Fish, along with cream pastries, milk, processed meat, and mayonnaise provide excellent media for bacterial growth, and if contaminated and allowed to remain at room temperature, these organisms can rapidly multiply and produce toxins. Currently nine enterotoxins have been identified. They are resistant to heat and are destroyed only by prolonged boiling (169).

Bacterial and Viral Infections

Vibrio Species

Nine marine *Vibrio* bacterial species have been associated with food-borne disease in humans. *Vibrio* species are not detected by standard methods of monitoring coastal waters for bacterial contamination, and standard commercial decontamination techniques do not rid shellfish of them.

Vibrio cholerae infection is most prevalent during the summer months. It is characterized by abrupt onset, watery diarrhea and vomiting (32). It is endemic in the coastal waters of the Indian Ocean. The largest outbreak in the US involved 18 persons. It was reported in 1986 in Louisiana, and was associated with undercooked crabs, which are the most important vehicle for *V. cholerae* infection in the US. Shrimp and oysters can also transmit the disease. A persistent reservoir along the Gulf Coast may continue to cause sporadic cases (170).

V. parahaemolyticus is found in coastal waters throughout the world. This agent is the leading cause of acute diarrheal disease in Japan, presumably because of the frequency of ingestion of raw seafood. In the US, it has been related to inadequately cooked seafood, usually shrimp, and was recently reported to be associated with crayfish consumption (171). *V. parahaemolyticus* damages the intestinal mucosa and the stool may be bloody. Diarrhea develops 12–48 hours after ingestion of contaminated food and is associated with abdominal cramping. Chills and fever are observed in more than half the cases. Between 1973 and 1999, 43 outbreaks of *V. parahaemolyticus* infections were reported to the CDC that involved fewer than 1000 individuals. Most of these outbreaks occurred during the warmer months and were attributed to seafood, particularly shellfish. Of patients with acute *V. parahaemolyticus* gastroenteritis, 88% reported having eaten raw oysters during the week before their illness occurred. The median attack rate among persons who consumed the implicated seafood was 56% (172).

Although quite rare, infection of immunocompromised persons with *V. vulnificus* can be associated with high mortality (50%). This species appears to be part of the normal bacterial flora of estuaries along the US Gulf, Atlantic, and Pacific coasts. The septicemia induced by *V. vulnificus* is associated with eating raw oysters. Of patients with primary septicemia, which accounts for about half of the cases, 96% consumed raw oysters and 61% died, usually in association with underlying liver disease. Oysters harvested in the Gulf of Mexico that were grown in water temperature exceeding 72°F (22°C) closely correlated with the infection (173).

Norwalk Virus

In the US, about 55% of the reported shellfish-related incidents are registered as unknown etiology, but are believed to be due mainly to Norwalk, Norwalk-like, or human enteric virus infections, with a smaller proportion caused by *Vibrio* bacteria (174). The first documented shellfish-associated gastroenteritis involving Norwalk virus was in Australia in 1979, with more than 2000 cases (175). Since then, many outbreaks of Norwalk, or Norwalk-like viral gastroenteritis have been reported in the US. Incubation periods were generally 24–48 hours long. The most common symptoms were nausea (100%), vomiting (83%), diarrhea (50%), and abdominal cramps (176, 177). The diagnosis is clinical, with typically unrevealing bacterial studies on stool and shellfish specimens. Exposure is confirmed by demonstration of seroconversion and the formation of IgM antibody to Norwalk virus. In addition, Norwalk virus has been identified by RIA in clam and oyster specimens. Reported incidents have increased in the last decade.

Shellfish-borne disease occurs mostly from mollusks consumed raw or lightly heated. In a confirmed outbreak of Norwalk virus gastroenteritis, 83% of persons who ate raw oysters became ill. The outbreak was caused by contamination of oysters in the oyster bed by stool from ill harvesters who rou-

tinely disposed of their sewage overboard (178). Steaming clams to open the shells takes about 1 minute, but to inactivate viruses it takes 4–6 minutes (179). These organisms do not multiply once released into the marine environment but remain infectious in presence of organic material in the water and temperatures below 50°F (10°C) (180).

Finally, marine organisms such as oysters may concentrate microorganisms including hepatitis A (181). Contamination occurs by consuming shellfish grown in sewage-polluted waters or contaminated waters used in irrigation, as well as through infection of foods by food handlers.

Conclusions

This chapter presents the more common clinical syndromes produced by the ingestion of natural seafood toxins. For the practicing allergist, knowledge of this wide array of toxic syndromes is important for the proper differential diagnosis of seafood allergy. A careful history and physical exam are essential to establish the diagnosis of seafood poisoning on clinical grounds, which may often be confirmed by detection of toxins either in remnants of the seafood or in specimens collected from the patient. The history should include the type and severity of the symptoms, time of the last reaction, frequency of reactions, whether others became ill, previous history of food allergy, types of marine species ingested and where they were captured, and the quantity of food consumed and the way in which it was prepared. Whether the food was eaten at a restaurant, the patient was traveling, alcohol was consumed or medications taken by the patient should also be recorded.

Presence of similar symptoms in other individuals who shared the seafood meal and the "endemic" nature of the syndrome are paramount in alerting the physician to possible seafood poisoning. The absence of prior reactions to the same seafood and its subsequent tolerance without symptoms point away from an allergic etiology and should be considered as corroborative evidence in support of a toxic syndrome. Since histamine mediates the symptoms of both scombroid and type I hypersensitivity reactions, clinical manifestations of scombroid may be virtually indistinguishable from seafood allergy. History of a "peppery" taste to the food, and the type of fish consumed, as well as suspected temperature abuse, are helpful in reaching the proper diagnosis.

Neurologic symptoms associated with an allergic reaction are the result of hypoperfusion of the CNS and correlate with the severity of cardiovascular involvement and hypotension in anaphylaxis. This may help the physician to distinguish ciguatera, PSP, NSP, and ASP where neurologic impairment is commonly present in the absence of hypotension. In ciguatera, knowledge of the type of fish, which is either imported from or consumed in endemic areas, such as Caribbean, Hawaii, and Pacific Islands, provides clinical information to differentiate it from seafood allergy. Likewise, in puffer fish poisoning, consumption of fugu, a delicacy of Japanese cuisine, and in shellfish poisoning the location where seafood was caught, for instance Pacific Coast in cases of PSP and ASP, are crucial pieces of information. The seasonal association with algal blooms and presence of high levels of biotoxins or toxic algae that are reported by authorities surveying coastal waters should increase the index of suspicion for physicians practicing in endemic areas. In the majority of these toxic syndromes, the causative toxin does not alter the taste and appearance of the seafood and is not inactivated by normal cooking.

The treatment is supportive, with active early respiratory support, especially in cases where neurological involvement could lead to respiratory paralysis. Upper respiratory reactions in individuals with no history of atopy and exacerbation of chest symptoms in asthmatics may be caused by aerosolized NSP toxins. These irritation reactions that are usually associated with a red tide and have occurred on the Atlantic Coast, should not be mistaken for allergic respiratory symptoms.

Viruses, bacteria, and bacterial toxins may cause GI and systemic symptoms that can be confused with food allergy. Raw or lightly steamed shellfish and raw fish, e.g., sushi are potential sources of infection with hepatitis A and Norwalk viruses, and *Vibrio* spp. Botulism is a hazard associated with consumption of home-canned, vacuum-packed smoked, or unviscerated salt-dried fish. If alternative diagnoses cannot be ruled out and seafood allergy remains a likely diagnosis, SPT and oral food challenge are diagnostic procedures of choice.

Most current health risks associated with seafood contamination originate in the environment and should be dealt with by control of harvest or at the point of capture. The most effective way of protecting consumers is to establish and maintain comprehensive monitoring programs for toxic algae and toxins in shellfish in all growing areas. Developing a better understanding of factors that pro-

mote harmful algal blooms and lead to production of toxins by marine algae is crucial to controlling human health and deleterious environmental effects. Further research is needed in most areas of seafood poisoning. Easy, accurate, and cost effective methods for detection of toxins in seafood, monitoring shellfish for viral and bacterial contamination, and surveillance of coastal waters for harmful marine algae and their toxins are needed. Knowledge gained from research on the mechanism of action of marine toxins should lead to more specific treatment modalities that would limit the morbidity and mortality of seafood intoxication. The following general preventive measures could greatly reduce the incidence of poisoning outbreaks that are associated with seafood:

1. Avoid eating raw seafood.
2. Avoid eating lightly steamed and undercooked shellfish.
3. Adhere to the public health agency guidelines on harvesting, processing, and consumption of shellfish and avoid shellfish from areas of frequent red tides.
4. Promptly refrigerate catch of sport fishermen.
5. Avoid eating large, predatory reef fish usually implicated in ciguatera poisoning, especially barracuda, amberjack, and snapper.
6. Avoid reef fish caught in ciguatera-endemic areas, especially the head, viscera, and roe.
7. Promptly report the suspected outbreaks of seafood poisoning to local health departments.
8. Submit leftover seafood or uncooked portions of the fish or shellfish to local health departments for analysis to establish nature and amount of contaminating toxin.

Finally, the informed physician is of great help in public health prevention through public education and involvement with the local and public agencies that deal with these health issues.

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Neurologic Reactions to Foods and Food Additives

Richard W. Weber

The impact of foods or food additives on neurological functioning has received varying attention, ranging from case reports to double-blind placebo controlled (DBPC) challenges. Signs and symptoms range from those that are purely subjective to those that may be validated by objective findings. Syndromes such as food-induced migraine and epilepsy will be addressed in this chapter.

Migraine Headache

Migraine headache is a common affliction, occurring in 5%–30% of the general population, with a familial predisposition in 60%–80% of cases; and affecting females three times more often than males. A 1992 survey of 20,468 individuals revealed that 5.7% of males and 17.6% of females suffered one or more migraines per year, with the prevalence highest between 35–45 years of age (1). It was projected that moderate to severe disability from migraine affects 8.7 million women and 2.6 million men of the US population. In 1962, the Ad Hoc Committee on the Classification of Headache defined migraine as "recurrent attacks of headache, widely varied in intensity, frequency, and duration. The attacks are commonly unilateral in onset; are usually associated with anorexia and, sometimes, with nausea and vomiting; in some are preceded by, or associated with, conspicuous sensory, motor, and mood disturbances; and are often familial" (2). Migraine may be divided into several clinical syndromes. "Classic migraine" presents with a prodromal "aura," frequently visual in nature, which precedes onset of the headache by 5–30 minutes. The visual disturbance is typically that of "scintillating scotomata," multicolored sawtoothed arcs, which may move across the visual field. "Common migraine" lacks a prodrome before the headache. "Complicated migraine" indicates the association of more significant neurologic dysfunction such as hemiplegia; symptoms may persist beyond the duration of the headache, but usually resolve.

Precipitating factors of migraine are varied, and include stress, bright lights or loud sounds, physical exertion, fasting, and foods. Menses or oral contraception use may precipitate headaches, but migraine frequently improves during pregnancy. No definitive laboratory tests exist to confirm the diagnosis. Electroencephalogram (EEG) abnormalities have been noted but are minimal and are more common in childhood migraine. The diagnosis of migraine is based primarily on history. Other medical conditions that may mimic migraine must be excluded: aneurysm, temporal arteritis, carcinoid tumor, pheochromocytoma, brain tumor, arteriovenous malformation, glaucoma, mastocytosis, or carotid or vertebrobasilar vascular insufficiency.

Theories of Migraine Etiology

Despite its description centuries ago, there is still no consensus on the etiology of migraine. The frequently pulsatile nature of the headache suggests the vascular theory by which the aura was explained as an initial phase of regional intracerebral vasoconstriction followed by vasodilatation with inflammation. This theory was supported by

evidence of slowed intracerebral blood flow in patients with classic migraine, but patients with common migraine showed no such changes. However, a report of spontaneous migraine during a positron-emission tomography (PET) study in a patient with common migraine revealed bilateral cerebral hypoperfusion spreading anteriorly from the occipital lobes to the temporal and parietal lobes (3).

The neurogenic theory suggested that the basic defect was in neuronal response to certain neurotransmitters and that vascular changes were secondary to neuronal impulses and vasoactive properties of such neurotransmitters as substance P (4). Abnormal platelet serotonin (5-hydroxytryptamine [5-HT]) metabolism has been described in migraine patients, but it is unclear whether these are primary defects or epiphenomena from drug effects (4, 5). It has been difficult to reconcile these theories with the actions of agents that have been found empirically either to provoke or to relieve migraine.

Moskowitz and Macfarlane (6) emphasized that several levels of pathophysiologic triggering and potentiating factors may consolidate neurogenic and vasogenic elements in migraine headache. It has been proposed that ionic and metabolic cortical mechanisms release nociceptive substances that stimulate trigeminovascular sensory fibers. These impulses cause pain and release vasoactive neuropeptides such as substance P and neurokinin A, inducing vasodilatation and protein extravasation, causing further nociceptive substance release and sensory nerve ending sensitization. Receptors for 5-HT on sensory nerve endings and vascular smooth muscle are central to this cascade. The marked success of selective 5-HT_1 agonists in controlling migraine headaches underscores the importance of these receptors. The large number of dural mast cells (MCs) have also been implicated in this process (6). The complexity of initiating and potentiating elements in the migraine reaction may explain the great variety of therapeutic modalities.

Diet Manipulation in Migraine

Diets may play a role in migraine severity by limiting precursor availability for generation of vasoactive mediators or nociceptor transmitters. Carbohydrate-rich, protein-tryptophan-poor diets have been attempted to modify migraine headaches (7). The rationale is that if platelet serotonin is a precipitator of the vasoconstrictory phase of migraine, restricting dietary intake of serotonin and the serotonin precursor tryptophan may decrease levels within platelets and thereby alleviate migraine headaches. However, it has also been suggested that increased brain serotonin levels may improve migraine through the anti-nociceptive system. Insulin release induced by carbohydrate-rich meals would increase tryptophan availability to the brain, with subsequent increased serotonin synthesis. Hasselmark and coworkers (7) tried such a diet for 50 days (after a 30-day routine diet) in 10 migraineurs, seven of whom completed the study (four with classic and three with common migraine). Three of the four with classic migraine had a marked improvement in headache frequency, and none of the common migraineurs noted benefit, but there were no differences in platelet serotonin uptake. Benefit was postulated as due either to decreased ingestion of migraine-precipitating foods, or to increased brain serotonin levels.

Association of Food Allergy and Migraine

Allergy to food is self-reported more commonly in migraineurs than those with non-migrainous headache or without headache (8). Pinnas and Vanselow commented on the hundred year association between allergy and migraine (9). In 1885, Trousseau had included periodic headache in the allergic diathesis; Tileston in 1918 likened migraine to asthma, and the following year, Pagniez considered migraine as a manifestation of anaphylaxis (9). Several reports then hypothesized food allergy as the cause of migraine, but methodological weaknesses made these less than compelling. In 1921, Brown (10) linked attacks to such foods as milk, egg, fish, beef, pork, and chocolate. In 1927, Vaughan (11) reported that 10 of 33 migraine patients showed specific food triggers, identified by skin testing followed by elimination and rechallenge. With the exception of a solitary blinded challenge, these were open challenges. Shortly thereafter, Eyermann (12) reported that 69% of headache patients improved on an elimination diet. Forty-four subjects had headaches with suspected foods within 3–6 hours of ingestion. The diet was directed by skin test results, but of those who did not respond to the diet, 53% had positive tests, suggesting overinterpretation of the skin test responses. Additionally, many of the patients did not meet accepted criteria for migraine

headache. Balyeat and Rinkel (13) stated that of 202 consecutive migraine patients managed with food skin testing and elimination diets, 120 had 60% or greater improvement, with only 12% of the patients demonstrating little or no improvement. In 1932, DeGowin (14) reported on 60 migraine patients who had positive prick or intradermal skin tests to foods. Elimination diets in 42 patients brought about complete relief in 33% and partial relief in another 45%; incidence of headache on reintroduction of foods was not reported.

These early studies suggested that food allergy, diagnosed by positive immediate skin test, was a significant cause of migraine headache. However, they are flawed by being open studies and susceptible to expectation bias and placebo effect. Thereafter, mainstream of migraine opinion moved away from the causative role of allergy. Nonetheless, in 1952 Unger and Unger published a paper entitled "Migraine Is an Allergic Disease" (15). Of interest, the preceding article in that issue was captioned "Is Migraine an Allergic Disease?" (16). Schwartz detailed his extensive epidemiological work in Denmark involving 241 asthmatics, 200 non-allergic controls, and their 3815 relatives spanning four generations. He found no difference in the frequency of migraine in relatives of asthmatics and normal controls, commenting that because migraine was so common, it was not unexpected to find it occurring in allergic kindreds.

Unger and Unger (15) investigated 55 patients with skin tests, elimination diets, food diaries, and the "feeding test" to identify migraine-provoking foods. All foods ingested for 24 hours before the onset of migraine were recorded. The patients were challenged with the suspected food after 2 weeks on an elimination diet. If no reaction occurred within 1 hour, a second portion was given, the patients recording symptoms for 24 hours. With this protocol, 35 of 55 patients achieved complete relief of migraine symptoms, nine had $\geq 75\%$ relief, and another two had 50%–65% improvement. No benefit was experienced by nine patients. Food skin testing in this study was not helpful, identifying a provoking food only five times. This study was reminiscent of earlier work, in that it was an open study, but certain findings repeatedly appeared. A substantial number of migraineurs improved markedly on elimination diets. Recurrence of headache coincided with reintroduction of certain foods, and the onset of the headache could be delayed 3–6 hours after ingestion of the provoking agent. Food skin tests were of varying help in defining diets.

A smattering of open studies over the next 25 years supported the value of elimination diets in migraine but offered little insight into mechanisms. Grant in 1979 (17) reported remarkable results in 60 patients placed on a strict lamb-and-pear elimination diet. Of an initial group of 126 migraineurs, 35 discontinued the diet, and data were reported on only 60. After 5 days of the diet, foods were reintroduced singly, with symptoms and pulse rate monitored up to 1.5 hours. This technique led to improvement in all the patients, and complete resolution in 51 (85%). Foods found to provoke symptoms for each patient ranged from 1 to 30, with a mean of 10. No blinded challenges were performed, and these results no doubt reflect substantial placebo effect. Likewise, the use of the pulse test has no documented validity and could lead to unnecessary elimination of numerous foods. Finally, the 31 patients who continued the diet but were not included in the data analysis presumably had less striking results.

Monro and coworkers (18) reported 47 migraineurs managed with elimination and rotation diets. Twenty-three of 36 patients completing the diet phase were able to identify provoking foods. Subsequently, the radioallergosorbent test (RAST) to a battery of foods found migraine provokers to have higher RAST titers than foods not producing headaches. In a further report, the same workers presented nine migraine patients with reproducible food sensitivity documented by elimination diets with open challenges (19). High dose oral cromolyn blocked headache in five patients and placebo did not. The benefit of a strict milk protein-free diet for classic migraine was reported in 1983 (20). Of 26 patients, 18 improved on the diet, all of which had documented lactase deficiency. One additional deficient patient did not improve on the diet; the remainder was not lactose intolerant. Hughes and colleagues (21) placed 21 migraine patients on a "semi-elemental" diet for a week and 19 had a marked reduction of headache severity during the week of observation. These unblinded studies suggested that a large percentage of migraineurs would benefit from elimination of specific foods, and the more stringent the diet, the more likely the success.

There have been only a small number of studies using DBPC challenges, necessary in an area where cause and effect are being assessed by subjective symptomatology. A preliminary report by Vaughan and colleagues (22) in 1983 linked the value of food skin tests and DBPC food capsule challenges in adult migraine patients. Also that

year, Egger and associates (23) studied 99 children who suffered from at least one migraine per week for a minimum of 6 months. They were maintained for 3–4 weeks on an "oligoantigenic" diet: one meat, lamb or chicken; one carbohydrate, rice or potato; one fruit, apple or banana; one vegetable, brassica; and water and vitamin supplements. If no benefit was derived (more than one headache per week in the last 2 weeks of the diet), the alternate foods were tried. Those improving on the diet then reintroduced foods in normal portions daily for 1 week. Those who could identify a provoking food entered the DBPC challenge phase. Eighty-eight completed the diet, and 78 recovered fully, 4 were greatly improved, and 6 received no benefit with the diet. Of the 82 who improved, 74 had migraines with one or more foods, with median onset of headache 2 days after reintroduction of the responsible food. DBPC food challenges were performed with 40 children. Twenty-six responded to the active agent alone, two to the placebo, four to both, and eight to neither ($P < .001$). Skin prick testing (SPT) was not helpful: testing identified all of the precipitants in only three subjects. Eighty-nine percent of the children completing the diet phase recovered completely, and in 29.5% of those children, at least one provocative food was verified on DBPC challenge.

A negative DBPC study was reported by Atkins and coworkers (24). They studied 36 children by history, physical examination, and a battery of 20 food SPTs. Sixteen suspected a food or additive, two of which had a positive skin test. Suspected foods were studied with a total of 19 DBPC challenges: none provoked a migraine attack. Twenty patients could not identify any precipitants, and of these only five had more than two headaches per week. These five were placed on an elimination diet and two became headache free. However, headaches did not recur on resumption of a normal diet. The differences between the outcomes of these two studies may be explained by differences in protocol and patient selection. Egger placed all his patients on the elimination diet, probably dealt with a more severely affected group, and challenged with larger amounts of foods over several days. The more prolonged challenge might lead to more false positives because of the recurring nature of spontaneous migraine. Because headache may be delayed several hours in onset, patients may not identify such agents, and testing only history-suspected items would falsely lower the response rate.

Mansfield and coworkers (25) reported on 43 consecutive migraine adult patients referred from a neurology clinic. Following an 83-food SPT battery, positive foods were eliminated from the diet for 1 month. Patients with negative skin tests were placed on a wheat, corn, milk, and egg elimination diet. Patients experiencing at least a two thirds reduction in headache frequency underwent single-blind challenges with capsules containing a total of 8 g of desiccated food or a similar number of placebo capsules. Positive challenges returned for DBPC challenges. Thirteen (30%) of 43 had the two thirds reduction in severity while on the diet. Of seven who underwent DBPC challenges, no patient responded to placebo, five had migraine with the active challenge, and two were without headache for either challenge.

Vaughan and associates (26, 27) performed a study of 104 adult migraine subjects in another DBPC protocol. All patients had migraine verified by a neurologist, and documented headache frequency of at least three per month on a regular diet using a symptom-food diary. All foods suggested by history, results of an 83-food skin test battery, and wheat, corn, milk, and egg and were eliminated for 1 month. Patients were studied further if they had a greater than 50% reduction in headache frequency. Foods were reintroduced in an open fashion and eaten three times daily. Those who felt they could identify at least one provoking food entered into the DBPC phase. Capsule foods were given three times daily, with the challenge sequence comprising two placebo (P) and two active days (A). On the basis of Egger's report that some patients reacted only on a second day of challenge with larger amounts of the incriminated food, the order was randomized but with the two active days always together (23). A positive challenge was defined as headache occurring on both days or on the second challenge day, and any response to placebo was ruled a negative challenge.

Forty (38.5%) of the 104 patients had a greater than 50% reduction in migraine frequency, eight becoming headache free. Twenty-seven of 36 undergoing open challenges identified at least one precipitant, with a range of one to four. Of 24 patients with DBPC challenges, 15 had migraine on both active days and two on the second day only. Three patients reported headache on placebo, and four had none. Therefore, more than one third of 104 consecutive adult migraine patients improved on an elimination diet, and 17 (16%) had reproducible DBPC demonstration of food-induced migraine. Food skin testing was not uniformly helpful, because it was positive for less than half of the documented triggers (Table 37–1), and neither

Table 37–1.
Value of Double-Blind Food Challenges and Skin Tests in Migraineurs*

Patient #	*Positive Open Challenges*	*Skin Test Results*	*Positive Double-Blind Challenges*
1	Egg	1+	Egg
	Milk	0	
	Wheat	1+	
2	Coffee	2+	Coffee
	Maple syrup	ND	
3	Wheat	3+	Wheat
4	Black-eyed peas	4+	Black-eyed peas
	Pinto beans	3+	
5	Egg	1+	Egg
	Chocolate	0	
6	Egg	0	Egg
	Milk	0	
7	Wheat	0	Wheat
	Cheese	0	
8	Wheat	0	Wheat
9	Wheat	0	Wheat
	Chocolate	0	
10	Milk	0	Milk
	Wheat	0	
	Chocolate	0	
	Cheese	0	
11	Cheese	0	Cheese
	Chocolate	0	
12	Corn	0	Corn
	Wheat	0	
13	Coffee	0	Coffee
14	Cheese	0	Cheese
	Chocolate	0	
15	Corn	0	Corn
	Soy	0	
16	Wheat	0	Wheat
	Egg	0	

From (26–28).

consistently identifying migraine-provoking foods nor migraineurs more likely to benefit from dietary manipulation.

It has been suggested that some placebo responses may be due to materials within the placebo challenges (28).

Pharmacologic Triggering Agents

In 1925, Curtis-Brown (29) proposed that defective protein metabolism was responsible for migraine headache, leading to "protein poisoning" by certain foods such as chocolate, eggs, fruit, tomatoes, mushrooms, and meats. Migraine could thus occur on the first exposure, and patients improved on restrictive diets. Although this theory fell from favor, it introduced the concept that food intolerance in migraine patients could be due to pharmacologic action of a constituent.

In the 1960s, a syndrome of severe pounding headache was described in patients on monoamine oxidase (MAO) inhibitors when they ingested certain foods containing tyramine. Hanington (30) noted that migraine sufferers frequently incriminated such foods as causing their headaches. A double-blind challenge in 45 migraine patients showed an 80% response of headache to 125 mg of tyramine, and an 8% response to placebo (31). Some studies followed that confirmed tyramine sensitivity in migraineurs, but others could not demonstrate a significant role for tyramine. In a DBPC trial, Moffett and coworkers (32) studied eight migraine patients who believed that tyramine precipitated their symptoms, another 10 migraineurs without this history, and seven patients with migraine and epilepsy. The patients with presumed tyramine headache had symptoms as often with placebo as with tyramine, a patient with epilepsy had a tyramine-induced headache, and none of the other migraineurs had headache. Forsythe and Redmond (33), in a blinded challenge, used 100 mg of tyramine and found that 12 (20%) of 61 children reacted; a second group of 38 children had only five (13%) reactors to tyramine. Ziegler and Stewart (34) used a higher dose, 200 mg of tyramine, in 80 patients. Forty-nine patients had no symptoms, 12 had symptoms with both tyramine and placebo, 11 with placebo, and only 8 with tyramine alone. Tyramine-free diets have also failed to affect headache frequency (35).

Traditional provokers of migraine such as chocolate, cheeses, and red wine may not contain tyramine, rather phenylethylamine (36). This vasoactive amine crosses the blood-brain barrier and can cause large changes in cerebral blood flow. Five of six patients with histories of chocolate-induced migraine developed headaches within 8 hours of an open challenge of 100 g of chocolate (37). Sandler and associates (38) studied 36 patients who believed that chocolate precipitated headache. They received either 3 mg of phenylethylamine or of placebo in a single-blinded fashion. Eighteen (50%) patients reported headache with the amine, whereas six (17%) reported headache with placebo, a statistically significant difference. However, Schweitzer and coworkers (39) analyzed a number of chocolate varieties and found about 150-fold less phenylethylamine in their samples than in the preparations tested by Sandler. These authors postulated that either chocolate-induced migraine was not due to phenylethylamine, or migraine sufferers were sensitive to extremely low levels of this substance. Another DBPC study examined 25 patients

with a history of chocolate- or cocoa-induced migraine (40). Eight patients reported headache with only chocolate, five with only placebo, one with both, and 11 with neither. Fifteen patients underwent repeat challenges with different chocolate and placebo preparations, and five had migraine with chocolate alone, only two of whom had reproducible results. The authors concluded that chocolate on its own was rarely a precipitant of migraine.

Wantke and colleagues (41) reported symptom improvement in 28 patients with chronic headache by the institution of a histamine-free diet. The patients avoided alcoholic beverages, fish, cheeses, sausages, and pickled cabbage for months. After four weeks, four lost their headaches, 15 had > 50% improvement, and nine had no change; after 1 year, eight of these nine continued to be improved. Salfield and coworkers (42) reported a trial of high-fiber diet in 39 children with migraine, with half of the children randomly allocated to a diet also low in dietary vasoactive amines. There was no influence of dietary vasoamines because both groups improved equally, with significant decreases in headache, reinforcing the need for double-blind studies. These reports demonstrate that although there probably are patients sensitive to substances such as tyramine or phenylethylamine, it is difficult to demonstrate appreciable numbers of reactors in controlled settings.

Lai and associates (43) performed clinical assessments and EEGs on 38 patients with diet-induced migraine. After a control day, the patients were challenged with a combination of red wine, chocolate, and sharp cheddar cheese; 16 developed headache, four with scotomata. Abnormalities in the EEG were demonstrated but generally did not separate headache responders from nonresponders. All of the patients with headache showed photic driving of the EEG, whereas only 64% of the nonresponders did so ($P < .01$); the significance of this finding is unclear.

A number of people experience headache after the ingestion of hot dogs or cured meats. The incriminated vehicles are nitrites, which are added to meats as coloring agents. High concentrations of nitrites are found in hot dogs, bacon, ham luncheon meats, smoked fish, and some imported cheeses; it is not uncommon to find levels much higher than the FDA recommended levels of 200 ppm. The headache usually begins within minutes or hours of ingestion, is bitemporal or bifrontal, and is pulsatile about half of the time (44). The mechanism is unclear.

Alcohol is commonly identified by migraineurs as a precipitant. Headache usually appears within 30–45 minutes of consumption, similar to the onset of cutaneous vasodilatation. Alcohol has little to no effect on cerebral blood flow, however; therefore, intracerebral vasodilatation is not the mechanism by which alcohol causes headache. Depression of brain serotonin turnover by high levels of alcohol may play a role (4, 5, 36). Red wine is incriminated more often than other forms of alcohol. Littlewood and associates (45) assembled 19 migraineurs who believed that red wine but not other forms of alcohol provoked headache. Chilled red wine and vodka were consumed in a blinded fashion, and the incidence of headache compared. The alcohol content of the two preparations was similar; the tyramine content of the wine was 2 mg/L, and that ingested less than 1 mg. The wine produced significantly more headaches than the vodka. The authors felt that alcohol and tyramine were not responsible for the migraine headaches, suggesting ingredients such as phenolic flavonoids (found in higher quantities in red than white wine) as possible triggers.

The "Chinese restaurant syndrome" induced by monosodium glutamate (MSG) comprises headache, facial tightness, warmth across the shoulders, and less often, dizziness, nausea, and abdominal cramps (36). Approximately 30% of people ingesting Chinese food have symptoms, usually beginning about 20 minutes after ingestion. Thresholds vary from 1.5 to 12 g, but are commonly below 3 g, the amount found in a portion of wonton soup. Symptoms are presumed to be due to central nervous system (CNS) neuroexcitatory effects.

Since its introduction in 1981, the artificial sweetener aspartame has provoked numerous reports of adverse reactions. A large number included headache or were of a neurologic or behavioral nature (46). In 1987, a DBPC crossover study in 40 subjects reporting aspartame-induced headaches showed no differences in headache induction between the sweetener and placebo (47). The following year, however, another study demonstrated differing results (48). Twenty-five subjects began a 13-week study, but only 11 completed the protocol. A 4-week baseline period was followed by randomized sequential 4-week periods with either aspartame 300 mg four times daily or placebo, with the crossover periods separated by a week washout. Headaches occurred twice as frequently on aspartame as on placebo or during the baseline period ($P < .02$). The differences were accounted for by a

marked increase of headaches in four of the 11 subjects.

Mediators and Immunologic Mechanisms in Migraine

Immunologic studies have been generally unrewarding in migraine. Medina and Diamond (35) reported no differences in total IgE between migraineurs and the normal population. Merrett and colleagues (49) examined IgE levels in 74 adults with dietary migraine, 45 with non-dietary migraine, 29 with cluster headache, and 60 normal controls. They found no differences in specific and total IgE in the groups with the exception of a higher total IgE in the cluster headache patients, which they attributed to a higher percentage of smokers. Specific IgE for cheese, milk, and chocolate showed no difference between dietary and nondietary migraine. Pradalier and coworkers (50) performed duodenal biopsies for immunocyte enumeration in patients with common migraine. Twenty consecutive migraineurs, 11 with food-induced migraine, and nine without, had mid-duodenal biopsies examined for lamina propria plasmocytes containing IgE, IgG, IgA, or IgM. There were no differences between the two groups for histologic appearance, total plasmocytes, or subsets. Ratner and associates (51) have linked dietary migraine with lactase deficiency, and represented data on elevated IgM in 11 such migraine patients. Martelletti and coworkers (52, 53), using a C1q binding assay, showed an increased incidence of circulating immune complexes in 21 patients with food-induced migraine (29% vs 10% in the control group). Activated T cells increased 4 hours after challenge then decreases at 72 hours. The authors speculated on the role of IL-2 receptors in food-induced migraine.

Three studies have examined mediator release in dietary migraine. Three patients in the Mansfield adult migraine study (25) returned for repeat challenges and histamine plasma levels. Headache was provoked only with the active challenge and was associated with increases in the histamine levels coinciding with or preceding the onset of the headache. Placebo challenge on two revealed no or little change in histamine. Steinberg and colleagues (54) reported an extensively evaluated case of beef-induced migraine in a young woman. A threefold increase in histamine was noted as was an increase of a PGF2α metabolite coinciding with the onset of the migraine after the ingestion of beef. Increased intracerebral blood flow was demonstrated with xenon computerized tomography (CT) and Doppler ultrasonography. SPT and RAST to beef were negative.

Olson and colleagues (55) reported serial histamine and prostaglandin (PG) D levels during DBPC challenges in five patients with food-induced migraine. Placebo challenges produced no changes; with active challenge, all five had a threefold to 38-fold increase in plasma histamine as well as increases in PGD_2 before or coinciding with the onset of symptoms. A second increase in the PGD_2 was noted 4–6 hours after ingestion, whereas histamine did not show this late increase. This discordance suggests the late recruitment of nonbasophil inflammatory cells. Skin tests in this group were negative.

Summary

A wealth of clinical data supports the contention that dietary migraine is a bona fide entity, with both pharmacologic and immunologic mechanisms involved in subsets of migraineurs (Table 37–2). Certainly, these are not mutually exclusive conditions, and both may exist in the same patient. The exact pathophysiology of these reactions remains unclear, although release of immediate hypersensitivity mediators has been convincingly demonstrated. The variable results of immediate skin testing suggest that although some reactions may be IgE mediated, many are probably anaphylactoid, akin to radiocontrast media reactions. Why release of these mediators causes migraine in susceptible persons and not more traditional allergic manifestations is unclear.

The frequency of dietary migraine in migraineurs as a whole is not settled. Studies suggest that 15% may have reproducible triggers under con-

Table 37–2.
Incriminated Agents in Dietary Migraine

Presumed Pharmacologic Action
Tyramine
Phenylethylamine
Phenolic flavonoids
Ethanol
Nitrites
Caffeine
MSG
Aspartame
Immunologic or Uncertain Action
Food proteins

trolled situations, but that twice that number may benefit from dietary restriction. Although the majority of headache patients believe that there are connections between food intake and their headaches, fewer than half have this relationship addressed by their physicians, and fewer modify their dietary practices (56). Evaluation of such patients seems indicated, and should begin with the appropriate history and physical examination and the exclusion of migraine-mimicking conditions. Once bona fide migraine has been established, and pharmacologic control achieved, it is reasonable to pursue possible dietary triggers. Global dietary restrictions as have been suggested by some authors are likely not indicated. Although history may identify a number of triggers, some patients with reproducible headaches on DBPC challenges could not separate the causative agents during a normal diet.

Food skin testing is likely to present both false positives and false negatives, and should not be relied on alone; RAST is of little value. This leaves the prospect of food diaries and elimination diets. For patients with infrequent migraines, a diary listing foods ingested in the 48 hours previous to a headache may be useful. A diet eliminating wheat, corn, milk, and egg for a period of 2–4 weeks may be helpful. Patients benefiting from such a diet should reintroduce foods singly and for 3 consecutive days. Foods not provoking symptoms should be returned freely to the diet. Suspect foods should be eliminated and rechallenged. In patients with numerous suspected positives, it is wise to perform challenges under blinded conditions to remove expectation or anxiety as confounding factors, and to avoid unnecessary restriction of the diet. Consulting with a nutritionist is warranted for the rare patient who has multiple documented dietary triggers.

Epilepsy

Earlier in the last century, epilepsy was compared to the similarly episodic syndromes of anaphylaxis and the atopic disorders. Schwartz, in his monumental epidemiologic study of asthma and atopy in 4256 subjects and relatives in Denmark, also collected data on migraine (as mentioned above) and epilepsy (57). He found very few cases of epilepsy in the kindreds he studied, and no evidence for genetic correlation between epilepsy and the atopic disorders. Nonetheless, a number of reports have linked allergy (frequently food-induced) and epilepsy. In 1927, Ward and Patterson (58) food skin-tested 1000 epileptics and 100 controls, finding patient reactivity between 37% and 67%, and only 8% reactivity in the controls.

In 1951, Dees and Lowenbach (59) reported on 37 children with epilepsy who were treated with antiallergic therapy, environmental avoidance measures, and elimination diets as well as anticonvulsant therapy. Of these, 22 met criteria for "allergic epilepsy": personal and family history of allergy, blood eosinophilia, positive skin tests, and no organic disease of the CNS. The remainder had possible allergic disease, but did not meet all criteria; half had eosinophilia. Twenty of the "allergic" group and 13 of the "nonallergic" group had positive food skin tests. The predominant EEG finding was occipital dysrhythmia (73% of both groups), a rhythm that the authors had found to be present in some allergic children without an overt seizure disorder. Thirteen of the allergic group was treated with allergen immunotherapy as well as the dietary and medical manipulations. Convulsions were controlled in 18 (82%) of 22 allergic children and 6 (40%) of 15 "nonallergic" children; anticonvulsant therapy could be stopped in 13 of the former and 1 of the latter group. The authors felt that in certain cases epilepsy could have an allergic basis, and therefore could conceivably be controlled with appropriate antiallergic therapy. They did not, however, provide any indication of how many epileptic children were surveyed to arrive at their study group, so although this is an interesting observation, it is difficult to place it in proper perspective.

Egger and colleagues (23), in their assessment of food factors in migraine, had several patients who had epilepsy and/or behavioral problems that also appeared to respond to the oligoantigenic diet. In a further communication, they investigated children who either had epilepsy alone or in association with migraine, and all had symptoms that were difficult to control (60). None of 18 with epilepsy alone improved on the oligoantigenic diet, whereas 40 (89%) of 45 with both epilepsy and migraine reported improvement of one or more symptoms. In follow-up ranging from 7 months to 3 years, 25 patients had complete control of their epilepsy. Thirty-two patients had seizure during reintroduction of incriminated foods. In double-blind challenges of 16 children, seven reacted to the suspected food only, none to placebo only, and one to both.

One variant of reflex epilepsy is caused by the act of eating rather than the food ingested. This entity is called "eating epilepsy," and although quite rare, appears to be more common in kindreds in Sri Lanka and the Indian subcontinent (61–63). The seizure type is usually complex partial, does not occur with all meals, and usually happens at home. Many episodes are linked to the ingestion of rice, but since this is a staple of the diet, it is likely that this is not truly specific (61). It has been postulated that stimulation of areas of the brain that receive sensory input during eating may lower the seizure threshold (64).

Diet Manipulation in Epilepsy

Seventy-five years ago it was observed that many epilepsy patients were free of seizures while fasting, and the benefit persisted after return to a normal diet; it was suggested that this effect was due to ketonemia, and a "ketogenic" high fat, low carbohydrate diet was proposed for treatment. The diet was rigid, requiring strict nutritional supervision, and was perceived as unpalatable and difficult to maintain (65, 66). However, it appeared useful, especially in younger children and in those with seizures not responsive to anti-epileptic medications. A report by Kinsman and associates (66) showed benefit from the diet in 58 epileptic children requiring multiple medications. Seizure control improved in 39 (67%), with reduced medication in 25 (64%), greater alertness in 14 (36%), and improved behavior in 9 (23%). Seventy-five percent of these improved patients were able to maintain the diet for at least 18 months. A medium-chain triglyceride diet was found to be more ketogenic than the fat in the traditional diet, and seemed more palatable; Sills and colleagues (65) reported on their success with such a diet in 50 epileptic children. Eight achieved complete control of seizures (four without medication), four children had seizures reduced by 90%, and 10 children by 50%–90%. Extra dosing of the medium-chain triglycerides at bedtime was useful for control of nocturnal seizures. The mechanisms remain unclear. Possibilities include alterations in acid-base balance, water and electrolyte distribution, or lipid concentrations, and direct action of ketone bodies (66).

Epilepsy and Migraine

The link between migraine and epilepsy is apparent, but the nature of the relationship unclear. An editorial by Wilson, addressed several overlapping issues (67). If attacks and auras are brief, especially if the attacks are stereotyped, a diagnosis of epilepsy is preferred; if attacks with prodrome are longer, and if the impact on consciousness is primarily confusion, migraine may be more likely. Therapeutic trials of migraine prophylaxis and anti-epileptic drugs may help clarify the diagnosis. Several migraine-epilepsy syndromes have been identified: 1) seizures with typical migraine prodrome, 2) migraine with later development of epilepsy, and 3) alternating hemiplegic migraine. In the first case, impairment of cerebral blood flow associated with migraine may precipitate the seizure. In the second situation, repeated ischemic insult may lead to an epileptogenic focus. Despite such cases, the relationship between epilepsy and migraine remains obscure. Can one condition trigger the other, in a dually susceptible individual, or is epilepsy an epiphenomenon in a vascular disease (67)? Both mechanisms may occur in different patients.

Summary

Although the role of food is important in provoking attacks of migraine, less is known about dietary factors in epilepsy. The efficacy of ketogenic diets is well established, but the manner in which they operate remains uncertain. That bona fide allergic reactions, or anaphylactoid reactions, could trigger convulsions in susceptible patients appears likely, but DBPC studies are absent that would be helpful in validating the clinical observations to date. And certainly, studies investigating mediator release are needed.

Vertigo

In 1976, Dunn and Snyder (68) reported their experience with 33 pediatric cases of benign paroxysmal vertigo, a syndrome of sporadic brief episodes of disequilibrium, nystagmus, and/or vomiting. During infancy, this often manifested by paroxysmal torticollis. While food allergy was considered in all cases, in only four cases was it deemed likely. Three children had histories suggestive of milk allergy, and attacks were eliminated by removing milk from the diet, with vertigo reappearing with milk challenges. In another child chocolate was suspected, but could not be confirmed on challenge. The authors do not state whether these were open or blinded challenges.

Therefore, at best, a tenth of the cases had evidence for a food etiology.

A food cause for adult vertigo or Meniere's syndrome has been postulated. In 1923, Duke (69) reported that five cases of Meniere's improved on elimination diets. No well-performed double-blind studies exist to confirm this result. Older reports are limited to the nonreproducible technique of provocation-neutralization. A survey of Meniere's patients who returned a questionnaire revealed that many underwent allergen immunotherapy and/or elimination diets (70). An analysis of pre- and post-treatment symptoms revealed improvement in both frequency and severity of vertigo, tinnitus, and unsteadiness ($P < .005-.001$). Unfortunately, the mode of diagnosis of food allergy was by both skin testing and provocation-neutralization, and those that received diet manipulations were not segregated from those that received immunotherapy. Also, a quarter of the patients acknowledged not following the diet, 30% following it "sometimes," and about 45% following the diet "almost always." So this survey, at best, suggests an association between diet and vertigo. Whether a food role can be substantiated in this area will require appropriately controlled studies.

Hemiplegia

Several case reports exist of transient neurological deficits following presumed allergic reactions to foods. Cooke (70) reported transient third cranial nerve palsy associated with hemiparesis, followed by an episode of contralateral blindness and paresthesia in a food-allergic patient. Symptoms resolved with avoidance of beef and pork, challenges were not performed. In 1951, Staffieri and colleagues (71) reported a case of right-sided hemiplegia immediately after a meal, and associated with angioedema, urticaria, purpura, and peripheral eosinophilia ranging from 34%–40%. A wheat elimination diet was attended by resolution of the symptoms within a few days. To rule out coincidence, a total of four wheat challenges (apparently single-blinded) were performed over the ensuing four months, resulting initially in headache, with purpura and angioedema, and ultimately in the skin manifestations alone. Passive transfer of skin sensitizing antibodies was not successful. Such reports are fascinating, but probably reflect that anaphylactic reactions may be attended by edema almost anywhere, to include the central and peripheral nervous systems.

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38

Behavior and Adverse Food Reactions

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Introduction

In the last four decades, the immunopathology of allergic diseases has been unraveled, improving both diagnosis and treatment. Furthermore, the application of a rigorous scientific approach has facilitated the identification of foods as a cause of acute allergic disorders ranging from catastrophic anaphylaxis, angioedema, and urticaria, to more chronic disorders such as atopic dermatitis (AD) and enteropathies. The scientific evidence supporting the role for foods in many allergic disorders is compelling enough to convince even the most skeptical clinician. However, difficulties remain where no underlying mechanism can be found to explain the association between exposure to food and the reaction. As a result, no objective diagnostic test exists beyond dietary exclusion and double-blind placebo-controlled food challenge (DBPCFC). The latter procedure is now well established and can reliably identify individuals with food intolerance as a cause of a range of physical disorders (1). The concept, however, becomes strained when the reaction to food cannot be measured as a change in function or in physical symptoms, but as a change in behavior.

Unsubstantiated claims, made primarily in the lay media rather than in scientific channels, about "debilitating and chronic symptoms of ill health coming from an intolerance to certain foods" (2) have polarized medical opinion against the concept that foods might play a role in affecting behavior. The danger is that the profession's rejection of such claims will provide no help for individual patients and ignores the fact that there are some proven associations between ingestion of food and aberrations in behavior. "You cannot separate the body from the mind" is an appropriate aphorism to apply in considering such relationships.

Food and Behavior

Interest in the possibility that dietary variation might modify behavior is not new. Indeed, it is fundamental to the practice of Ayurvedic medicine. A survey of allergists in North America conducted in 1950 indicated that over half had noticed changes in personality when patients known to be allergic had been exposed to a provoking food (3). Food-induced tension-fatigue syndrome was first suggested as a clinical entity in 1922 (4). At that time, the scientific basis of allergy had not been unraveled and, therefore, it was not surprising that associations between exposure to food and changes in behavior were lumped into the all-embracing description of food allergy. Nevertheless, some of the early workers did recognize that other factors might be relevant. It was clear that behavior was sometimes affected by the discomfort of the primary allergic response such as urticaria, AD, or gastrointestinal (GI) reactions to food. Consequently, elimination of the food was often associated with a dramatic improvement in affect. It is not surprising that the tired, withdrawn

child who is kept awake most of the night with pruritus due to eczema, will become happy and friendly after major allergens have been eliminated. It has been found that parental reports of sleep disturbance do relate to behavior problems as rated by teachers in schools and in other settings (5). In our practice, we have been amazed by the wide range of behavioral responses in children during double-blind challenge procedures. Sometimes the underlying physical reaction has not been detected by the parents. Thus, children with urticaria only occurring on the torso in response to a food challenge are sometimes perceived by their parents to become extremely irritable and naughty (6). It is only by meticulous challenge procedure combined with careful, thorough examination of the patients that such associations can be validated. When no physical abnormality is evident, it becomes more difficult to measure behavior change objectively using reproducible methods over short periods, and thereby document responses to food challenge. Until such techniques are developed, controversy will continue about associations between isolated behavior disorder and reactions to food.

There are three potential mechanisms by which food ingestion and behavior disturbance might be linked (Fig. 38–1). The first is that the discomfort of symptoms associated with allergic disease causes secondary emotional reactions. This is clearly a mechanism common to many chronic illnesses and is not specific to allergic disease. Second, psychological problems may either directly cause or exacerbate allergic symptoms. This could be a common phenomenon among food allergy sufferers, whereby the onset of symptoms following exposure to a food allergen leads to intense anxiety and significant worsening of the symptoms. Finally, there could be a common causal mechanism—genetic, neuroendocrine, immunologic, or environmental—behind both psychological problems and allergic disease.

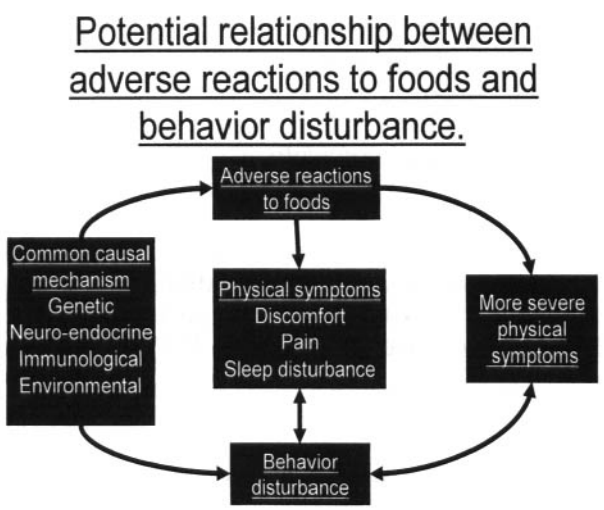

Figure 38–1. A flow diagram demonstrating the potential relationship between adverse reactions to foods and behavior. The direction of the arrows indicates the probable direction of effect.

Evidence for a Link

Much of the work linking the psychological state to allergic diseases has not specifically focused on adverse reactions to foods but to atopy in general. One study that examined adults with psychiatric problems reported that patients with depression had a higher prevalence of detection of specific IgE antibodies than those patients with a diagnosis of alcoholism or schizophrenia (7). Similarly, in a population of women, a positive correlation was demonstrated between the detection of IgE antibodies and either shyness or depression (8). A more recent study in children, however, has failed to find an association between attention deficit hyperactivity disorder (ADHD) and atopy in children (9).

Several studies have examined psychological profiles in groups of asthma sufferers. Asthmatic adults have been suggested to be less dominant, more anxious, and more depressed than equivalent controls (10). Among college students, current depression has a higher prevalence among those suffering from asthma compared with those without this problem (11), and in the same group of students, shyness was reported more frequently in hay fever sufferers (12).

Studies in children are rather more confusing. Some suggest there is an increase in internalizing problems in children with asthma (13–15); others do not (16–19). The question that arises from all these studies is whether an individual's psychological state alters both their perception of illness and their coping style or response to the symptoms and to the recommended medical management (20). Alternatively, the symptoms of the disease may affect the individual's ability to cope with other aspects of life, thereby affecting behavior. Indeed, some studies have suggested that the severity of allergic symptoms explains most of the variations in behavior (17, 21). However, other

studies have failed to establish an association between severity and psychological disorder (22).

Similar conflicting data have been generated in relating AD to psychopathology. Studies have variously reported no associations (12), and significant problems often related to evidence of sleep disturbance due to pruritis (23). The prevailing view arising from the above studies is that there is a bidirectional relationship between psychopathology and allergic disorders. A meta-analysis of the psychological effects of chronic childhood illness identified maternal maladjustment, poor child self-concept, decreased child IQ, and decreased family cohesion as the strongest predictors of psychological adjustment problems with illness severity being a less strong predictor (24).

However, this view may require further reevaluation as a result of a number of recent studies. In a large birth cohort study in Boston, parental stress when the infants were 2–3 months of age was associated with an increased risk of subsequent repeated wheeze in the children over the subsequent year. This effect was independent of parental smoking, breast-feeding, allergen exposure, birth weight, and lower respiratory infections. This suggested a more direct relationship between stress and induction of wheeze (25). The explanation for this observation may be found in another very recent study investigating airway allergen challenges in college students with mild asthma during a period of low stress in mid-semester and during stress in the final examination week. There were significant differences in the levels of sputum eosinophils and sputum eosinophil-derived neurotoxin at 6 and 24 hours post-allergen challenge during the stress period compared with the low stress period. This suggests that anxiety and, indeed, depression, which was highlighted in the students during stressful periods, directly enhanced eosinophilic airway inflammation in response to allergen (26). One might conclude, therefore, that emotional stress enhances the expression of disease. This might also explain the observations recently made in the Early Treatment of Atopic Child (ETAC) study (27). This prospective study of children with AD from the second year of life through to age 4.5 years showed that the onset of asthma was predicted by a prior elevated rate of behavior problems. These problems may indicate stress in the child. Importantly, the possible role of stress in disease expression was indicated in this same sample by the absence of an increase in behavior problems subsequent to asthma onset.

Genetic Factors

Because both certain psychological traits and atopy have a genetic basis, the two may be associated. Hyperactivity in children has a heritable component (28). A school-age twin pair study assessed a range of behaviors in relation to allergy symptoms. A strong within-subject correlation was found between an allergy score and a range of externalizing and internalizing behaviors. Because the correlations were far greater for monozygotic than for dizygotic twins, the investigators estimated that 77% of the covariance was explained by genetic factors (29). However, this observation does not exclude the possibility that the behavioral abnormalities actually caused or aggravated the allergic symptoms (30). The only way in which the relationships can be disentangled is by doing whole-family studies. One study has investigated allergy in first and second-degree relatives of young children with inhibited (shy) behavior. Although prevalence of asthma, food, or drug allergy was not increased in any of the relatives, there was a greater prevalence of hay fever and eczema. The investigators postulated that complex genetic factors mediating extreme degrees of shyness may be responsible for influencing immunological vulnerability to eczema and hay fever (31).

Pharmacological Effects of Food

Caffeine

It is self-evident that certain foods contain pharmacologically active substances that can affect behavior in all individuals to some extent. Caffeine in coffee is the most obvious example; it exhibits potent pharmacological properties by directly activating the cerebral cortex to maintain wakefulness and improve concentration. Individual variation exists in sensitivity to the stimulant effects of caffeine, with some individuals becoming intensely anxious after exposure to high doses. Furthermore, regular moderate dosing with caffeine can lead to physical dependence, and withdrawal produces a range of symptoms including depression, anxiety, fatigue, listlessness, sleepiness, decreased alertness, and headaches (32). Whether these concepts of variations in sensitivity and dependence can be extended to other pharmacologically active ingredients in foods is more difficult to establish. Some investigators have suggested, without any objective evidence, that this

adverse reaction to caffeine is one of the greatest health problems of the age (33).

Chocolate

Chocolate has been regarded as another food containing pharmacologically active substances, including a range of vasoactive amines such as histamine, tryptophan, and serotonin, as well as methylxanthine and theobromine. Individuals claim "addiction" to chocolate, in which abstinence produces symptoms of withdrawal, but these reports are very inconsistent. Milk chocolate apparently contains less of the pharmacologically active substances such as methylxanthines, but appears to be the variety that is most consumed by so-called chocoholics (34).

Other Foods

Clearly, certain foods contain high levels of vasoactive substances such as tyramine in cheese and histamine in various fermented foods and poorly stored scombroid fish (35). Individuals predisposed to irritable or difficult behavior may tend to show a greater degree of response to similar quantities of such products in foods than relatively placid individuals. Furthermore, peptides in milk and wheat contain exorthine-like activity, which might be predicted to affect behavior (36).

Vasoactive Mediators

Amino acids such as tryptophan, a precursor for serotonin, may be predicted to affect behavior, mood and appetite if administered to individuals in high concentrations (37). It has even been suggested that the amount of carbohydrate-rich foods consumed affects the production of serotonin which, in turn, influences the degree of hunger for carbohydrates (38). This hypothesis has led to a very tenuous line of argument about relationships between ingestion of food, changes in behavior, and the development of dependence and addiction to the food (33).

Some foods have been hypothesized to induce changes in brain perfusion that can mimic the abnormalities reportedly found in individuals with developmental learning difficulties (36). Varying the intake of precursors of vasoactive mediators might accentuate abnormalities in individuals with pre-existing brain disorders that affect behavior.

Histamine-Releasing Foods

High doses of the food coloring agent tartrazine can directly affect basophil histamine release (39). It is, therefore, not surprising that a large dose of tartrazine administered in a double blind food challenge produces an increase in circulating histamine. Such challenges might accentuate hyperactive behavior by a direct pharmacological mechanism (40). Similar effects may occur with strawberries, tomatoes, pineapple, and alcohol (41).

Where a pharmacological effect of food is implicated, it is likely that only high doses of the putative food will cause a significant problem. Thus, the concept of total dietary exclusion is inappropriate. Furthermore, diagnosis is unlikely to be achieved by any standard tests for allergy.

Other Mechanisms

Reactive Hypoglycemia

It has been suggested that individuals with a high sugar intake develop reactive hypoglycemia several hours after ingestion, which in turn produces an aberration in behavior and cognitive performance (42). Diabetologists are only too familiar with the wide range of behavior disturbances that occur in individuals who become hypoglycemic. This observation has led to the claim that reactive hypoglycemia commonly causes neuropathologies ranging from schizophrenia to criminal behavior (43). The most widely publicized use of this diagnosis was in a court of law. An argument was put forward by the defense that the defendant, who was accused of murder, had diminished mental capacity as a result of over-consumption of sugar-containing "junk" foods. The conviction was eventually for manslaughter rather than first degree murder (32). No compelling evidence has subsequently been generated that would support this concept. Indeed, some very well conducted challenge studies have failed to find any association between dietary sucrose or aspartame and effects on childhood behavior or cognitive function (44–46).

Squash Drinking Syndrome

One possible alternative explanation is more credible and certainly requires further investigation. A survey of the drinking habits of 2–7 year old children found that very few now drink water. Three quarters of preschool children never drink water. Squash, a soft drink, was by far the most frequently consumed drink, and in some preschool children this constituted as much as 50% of recommended daily energy intake (47). A group of eight children were described who were referred for a range of problems including poor appetite, behavioral problems, poor weight gain, and loose stools. The subjects received a high percentage of their daily energy requirement in the form of high-energy drinks. The authors hypothesized that the children's appetites had been poor during meals as a consequence of the high-energy drinks they consumed during the day. A reduction in the energy ingested in drinks as a fraction of their diet resulted in an increased dietary intake of a range of other foods, which in turn decreased stool frequency and improved all other symptoms, including behavior. They suggested that the features of the condition were sufficiently well characterized to be accepted as a clinical entity, called "Squash drinking syndrome" (48).

Amino Acids and Immune Responses

An intriguing study has investigated the health and immune status of normal control subjects consuming diets free of tyrosine and phenylalanine. This regimen decreased the plasma tyrosine levels significantly and was associated with a decrease in platelet aggregation in response to adenosine diphosphate and in platelet activating factor. Natural killer, T helper, and T cytotoxic suppressor lymphocyte numbers proportionately increased relative to neutrophils (48). This investigation sought to delineate why diets limiting tyrosine and phenylalanine intake were sometimes associated with decrease in tumor size and metastases. They proposed that the increase in natural killer cell activity and decrease in platelet aggregation might be the explanation. These alterations in immune competence might also have an impact on allergic phenomena.

Hyperkinetic Syndromes

Hyperactivity is a behavioral style characterized by short attention span, impulsive behavior, and overactivity, and is often associated with a range of other problems including aggressiveness, disinhibition, and sudden mood changes. Children who show this behavior in an extreme form are labeled as having hyperkinesis (or the hyperkinetic syndrome) or ADHD. The behavior may become apparent at home, in school, or in other social situations, or it may be pervasive in all environments. In school, these syndromes typically lead to underachievement and disruptive behavior, and often result in their exclusion from conventional schooling. The definitions, terminology, and diagnostic criteria vary in different countries (50). Even using standardized criteria for diagnosis, there is considerable variability depending on who is the assessor (e.g., doctors, school teachers, or parents). The disorder is more common in boys than girls. The prognosis for the condition itself is good but there is evidence of persistence into adult life for some individuals and a high risk of antisocial behavior and alcoholism (51).

In the US, the more inclusive definition of ADHD is used. In the UK, the ICD-10 diagnosis of the more severe and less prevalent hyperkinetic syndrome is adopted that is often associated with other neurological deficits. The label of "conduct disorder" should be used to describe children with antisocial or disruptive behavior, and this may or may not be comorbid with extreme hyperactivity. This distinction is supported by a study that showed antisocial and disruptive behavior in these children was independent of the classic features of hyperkinetic syndrome (52). Recent studies have suggested that a major determinant of hyperkinetic syndrome is a delay aversion. Such children have a self-imposed limitation on presentation time that makes them more likely to reduce overall delay levels during tasks, thereby achieving frequent small rewards rather than opting for larger rewards after more prolonged delay (53). This tendency has facilitated the development of a computerized system for recording delay aversion and might provide one of the first truly objective criteria by which to monitor responses in food challenges. Trials of this technique will be awaited with great interest.

It has been shown that genetic factors play an important role in childhood hyperactivity (54). The underlying mechanisms of the behavior include those related to deficiencies in behavioral inhibition and to delay aversion (55). These two mechanisms appear to act independently in contributing to the hyperactivity (56).

The Feingold Hypothesis

Feingold devised a diet excluding artificial food colors and flavors and naturally occurring salicylates, which he claimed led to improvement in behavior disturbances in as many as 50% of both normal and neurologically damaged children. He proposed a pharmacological, rather than allergic, mechanism (57). The diet was enthusiastically embraced by organizations representing the interests of families with hyperactive children. The rationale for the natural salicylate exclusion has been shown to lack foundation, with many excluded foods containing no salicylate and some foods remaining in the diet containing significant quantities (58). Attempts to confirm or refute the concept have encountered major difficulties. Most of the studies were not truly double blind and questions have been raised about diagnosis, case mix, type of elimination diet, timing and dose of challenge, lack of acknowledgment of carry-over effect, and the objectivity of the ratings of behavior change (51, 59).

The most commonly used rating system was devised by Conners, who has done a number of meticulously conducted double-blind crossover challenges (59). The results obtained from these studies have been conflicting showing either no difference between placebo and active challenge or significant worsening of behavior during challenge periods (60–63). Two excellent studies by Harley and colleagues could not confirm any dramatic changes with diet, although small differences were suggested in relation to elimination of additives (64, 65). The NIH Consensus Development Panel concluded in 1983 that a limited positive association existed between the use of diet and decreased hyperactivity, but only a small proportion of children showed a response (66). The panel also accepted that hyperactivity increased in a few children when challenged with artificial food colors but not placebo. On the other hand, it was very critical of many of the diet/behavior studies.

Further attempts have been made to elaborate on this problem. Egger and colleagues recruited 76 children from a special clinic for hyperactive children who had high Conners' scores (67). An unusually high proportion had other associated allergic problems and neurological disabilities. Sixty-two of the children appeared to improve on a so-called oligoantigenic diet, and 28 subsequently participated in a DBPC single crossover challenge. The challenge included various foods and very high doses of tartrazine and benzoic acid. The symptoms appeared to be worse in the active challenge period than the placebo, but a considerable order effect was observed and the significance of difference between the single active and placebo challenge was not great. Reactions mostly involved the artificial colors and preservatives, which provides some consistency with previous studies. The same group has repeated this study with a very similar outcome (68). However, the authors admit that, just as in their previous study, there were a high proportion of children with physical symptoms and of parents with a particular interest in following a dietary approach. This cannot, therefore, be extrapolated to all children with behavior disorders.

The author's group conducted a study on 39 children referred to an allergy clinic with behavior problems supposedly associated with food colorings (40). The patients exhibited poor concentration, excessive fidgeting, and poor school performance. The parents asserted that even small doses of food colorings immediately exacerbated problems. Of the 39 children, only 19 completed a DBPC challenge with a mixture of 125 mg of various food colors or placebo in a 7-week challenge protocol with 2 of the weeks randomized to daily active challenge. The mean daily behavioral scores, based on a 10-item Conners' checklist, were significantly higher in the active weeks compared with placebo, whereas somatic symptoms did not differ significantly between the two periods. Furthermore, the small changes in behavior scores had no relationship to changes in somatic symptoms or to atopic status in the children. Only two parents were able to identify all the challenge weeks correctly. The majority could not detect any change in behavior during the course of the challenge. The high dropout rate leaves the study open to criticism but reflects the nature of the problem. Several of the dropouts occurred within the first few days of challenge with parents asserting severe reactions, which were equally distributed between active and placebo periods. The other criticism of this study was that the Conners' scores were relatively low and, therefore, would not have normally been included as a diagnosis of true hyperkinetic syndrome (51). Nevertheless, these children do reflect the experience that might be expected in a general pediatric or allergy clinic rather than a psychiatric setting.

Recently we completed a food challenge study to determine whether artificial food colorings and a preservative in the diet of 3-year-old children in the general population influenced hy-

peractive behavior (69). Subjects were taken from the general population, which was therefore unaffected by biases in the referral process. In addition, the study attempted to establish whether any effects of additives on behavior were limited either to children with atopy, and therefore mediated by an allergic process, or to a small group of vulnerable children already demonstrating elevated levels of hyperactivity. A total of 1873 children were screened at their third birthdays for the presence of hyperactivity (HA) and 1246 were screened in addition for atopy (AT). Children were matched in quartets to form the following groups: HA/AT, not-HA/AT, HA/not-AT, and not-HA/not-AT (N = 277). Using a within-subject double-blind crossover design and after baseline assessment, children were subjected to the withdrawal from their diet of artificial colorings and benzoate preservatives. This was followed by, in random order, periods of dietary challenge with a drink containing artificial colorings (20 mg daily) and sodium benzoate (45 mg daily) (active period), or a placebo mixture supplementary to their diet. Behavior was assessed by a tester blind to dietary status and by parents' ratings. Hyperactive behavior was significantly reduced during the withdrawal phase. There were significantly greater elevations in hyperactive behavior during the active than the placebo period based on parental reports. These effects were not influenced by initial hyperactive status or by the presence of atopy. No significant differences were detected by testing in the clinic. The study shows a general adverse effect of artificial food coloring and benzoate preservatives on the behavior of 3-year-old children that is detectable by parents outside the clinic. Subgroups are not made more vulnerable to this effect by their prior levels of hyperactivity or by atopy. The effects of the dietary challenge on the mean levels of hyperactivity would be sufficient to reduce the prevalence of hyperactivity in the general population from 15% (based on the definition used in this study) to 6%. The study clearly needs replication but the findings indicate that potentially considerable public health benefits could be gained by the withdrawal of these artificial food colorings and preservatives from young children's diets.

One published study has investigated the value of so-called hyposensitization for children with apparent, albeit not objectively substantiated, food-induced hyperkinetic syndrome. The enzyme-potentiated vaccine contained 45 foods and 10 food colors, which was prepared by one of the authors and would therefore be impossible to replicate. The significant improvement on active treatment remains difficult to explain and clearly requires further controlled study by independent groups. It is, however, salutary to note the authors' closing sentence in the paper, which states "restricted diets are socially disruptive, expensive and because of nutritional inadequacy may be dangerous. . . ." (70).

A recent publication described a well-constructed blinded, placebo-controlled challenge with various doses of tartrazine or placebo in 34 children with behavior problems and 20 controls. Twenty-four of the index cases did show a response to tartrazine, which appeared to be dose related (71).

Our own conclusion from a study of the literature on this topic is that the conclusions of the NIH Consensus Development Panel may need to be revised in the light of more recent evidence. There certainly is evidence that high doses of artificial food colors can produce an adverse effect on some children with behavior disorders, whether they have true hyperkinetic syndrome or conduct disorder. In most cases, the effects are small. However, for some age groups, such as preschool children, the effects may be more marked and may be shown by substantial numbers within the general population. Little, if any, evidence supports the involvement of a range of other foods. It seems reasonable to recommend a reduction in the intake of foods containing a high level of artificial colorings irrespective of the mechanisms by which additives might produce a problem. This approach should not detract from the children being given other therapy, which may well be more effective. Nevertheless, only one study has attempted to compare standard pharmacotherapy with dietary modification. The effect of stimulant medication was statistically significant but the dietary effects were variable (72).

Food Aversion

Food aversion is very common. Indeed, food phobias are probably universal. Psychologically based food intolerance occurs where a conditioned response is elicited by the recognition, appearance, smell, or taste of a particular food. It can arise following an unpleasant genuine reaction, for instance, an episode of gastroenteritis or even a previous genuine food intolerance. Such a reaction cannot be reproduced when the food is disguised in a double-blind challenge. At its most severe level, food aversion is associated with

anorexia nervosa and the bulimic syndrome (73). In pediatric practice, it may be exhibited as part of the Munchausen syndrome by proxy. Parents, usually mothers, present children with multiple problems, leading to inappropriate extensive investigation and treatment. "Allergy" figures very prominently among the supposed problems and children are often on very abnormal diets. The symptoms are fabricated to apparently fulfill a psychological need in the mother. Not only are the diets nutritionally inadequate but the children become socially isolated and learn a disease model from an early age that may persist throughout life (74). Some of these children are eventually submitted to worse forms of abuse (75).

It is imperative that food aversion is distinguished from genuine intolerance. Unfortunately, many clinicians will assert that it is impractical to carry out double-blind food challenges. Few clinicians feel they have enough experience or time to devote to such meticulous diagnostic procedures; this creates opportunities for practitioners of fringe medicine to capitalize on the failure of conventional medicine to address a common and significant problem. Therefore, double-blind challenges should be performed not only for research, but also as an objective evaluation of the need for a continuing diet. Formal challenge may highlight a genuine underlying problem. If the challenge is negative, it will hopefully facilitate the introduction of more appropriate treatment.

Failure to address the problem has significant consequences. A retrospective study of 11 children who were failing to thrive as a consequence of parental beliefs about multiple food allergies were identified from a sample of 700 children referred for evaluation (76). Skin tests were negative in seven and DBFCs in nine of the 11. Two children reacted to food, one to milk and one to both milk and eggs. However, both children had more than 12 other foods excluded from their diets. Thus, the parental beliefs about food allergy in these children had resulted in significant failure to thrive, which could have major long-term effects. It is, therefore, imperative that professionals not collude with such beliefs, but carry out formal evaluation.

When inappropriate diagnoses of food intolerance are established by dubious techniques, which lead to major complications such as nutritional deficiencies, it is perfectly understandable that conventional medicine should aggressively highlight the problem (77). However, patients and their caretakers might become dissatisfied with medical care if their beliefs are not sensitively addressed. One report of patients attending an allergy clinic noted that those in whom food hypersensitivity could not be confirmed by appropriate investigation had high levels of neurotic symptoms and low levels of classical atopic problems (78). Individuals with what might be described as pseudo-allergy suffer from a range of underlying psychiatric problems but present with an initially confusing array of symptoms involving many organ symptoms and ostensibly associated with exposure to foods (79). Successful treatment depends on recognition, sensitive handling, and demonstration by appropriate double-blind challenge that food is not the primary cause of the problem (80).

This misperception is particularly frequent in infants. Normal changes in an infant's stool character and frequency, or activity and sleep cycles, may be wrongly considered abnormal and due to changes in diet. Parental conviction is reinforced not only by media publicity but also the medical profession's inability to handle such concerns appropriately. It is often easier for medical staff to collude with the parents' belief, setting the scene for an escalation of dietary avoidance for any subsequent problem that the child may suffer (81). Indeed, one clinician commented, "Not since mesmerism and phrenology were in vogue in the 19th Century has the public appeared so gullible and so vulnerable to fashionable nostrums." (82).

Conclusions

An excellent review on food sensitivity and the nervous system noted that " . . . there is, indeed, scientifically sound evidence to support an association between foods and abnormal behavior in children. However, the frequency of this is less than that claimed by some psychologists, psychiatrists and allergists." (51). Notably the conclusions of this detailed study of the literature did not support the concept of foods affecting adult behavior. The most compelling evidence links a transient effect of high doses of artificial food colorings on hyperactivity. More work is required to clarify whether associations with other foods or additives are genuine and appropriately treated by dietary modification. Where patients have genuine food associated behavior problems, additional underlying atopic diseases such as eczema, urticaria, and asthma are typically identified. More commonly, behavior disturbances have a psychosocial cause and require psychosocial solutions. Continued preoccupation with diet only detracts from the principal cause of the problem and its resolution.

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Food Allergy: Psychological Considerations

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Introduction

Food has been central to our physical and social development from our earliest memory as individuals and as a society. Since childhood, the sight, smell, and taste of food is inextricably linked to experiences that shape our personalities and how we relate to the world. It is therefore small wonder that food is involved in numerous psychological and somatic disorders with psychological overtones such as anorexia, bulimia, obesity, and many others (1). Food-related behavior has not only been the means of expression of psychological disorder, but food itself has been implicated in the causation and exacerbation of emotional and psychological problems, such as attention deficit disorder, autism, and the controversial area of idiopathic environmental intolerance (IEI) or multiple chemical sensitivity syndrome (MCS) with its associated multiple food sensitivities. The concept of food allergy or food "sensitivity" as a cause of psychological problems and of ill-defined polysymptomatic syndromes such as IEI/MCS has been propagated in the mass media, and most recently through the Internet, to an uncritical public.

This chapter aims to clarify these issues and provide the practicing allergist with an approach in managing and counseling patients with psychogenic food reactions. It is worth noting Pearson's observation (2) that one of the important contributing factors in the majority of the patients he studied with these reactions was a history of failure of diagnosis and treatment of a recognizable medical problem or failure of communication with the patient by a medical practitioner.

Definition of Terms

In 1984, the Royal College of Physicians and the British Nutrition Program formed a joint committee to address the public's concern about food processing and food allergies. In their report (3), they defined two main disorders: food intolerance or adverse physical reaction to a specific food or food ingredient that is reproducible under blinded challenge conditions; and food aversion or "pseudo food allergy," as Pearson called it (4), which includes psychological avoidance of food and psychogenic physical reactions to food due to emotions associated with the food rather than a physical response to the food itself, that is not reproducible in a blinded challenge. Food allergy is classified under food intolerance or adverse reaction with characteristic clinical and immunologic abnormalities that may be immediate IgE-mediated or non-IgE mediated. Adverse reactions to food may also be due to toxic reactions, enzyme defects, or pharmacologic intolerance, which are not mediated by immune mechanisms.

The key features differentiating the person with food aversion or food sensitivity, as they are currently called, from the person with a true food allergy or adverse food reaction are 1) the absence or inconsistent finding of recognized signs and symptoms, physical findings, and laboratory evaluation supportive of an allergic, toxic, enzymatic, or pharmacologic reaction to a specific food, and 2) the inability to reproduce symptoms or physical changes under adequately controlled double-blind food challenge conditions. Double-blind placebo-controlled food challenge (DBPCFC) in an

appropriate clinical setting is the gold standard in the diagnosis of food allergy (5) and is the best method to avoid patient and observer bias (6).

Psychologic Reactions to Allergic Disease

The most common abnormal psychological responses to physical illness include denial, anxiety, anger, depression, and dependency. These psychological states are a reaction to loss of health. The extent of psychopathology and impaired somatic functioning depends on the degree to which emotional issues related to the illness are resolved (7). With this in mind, allergic patients have not been found to have a significantly increased prevalence of psychological problems compared to nonallergic controls (8). If psychiatric illness does exist in asthmatic and other allergic individuals, it is usually related to the severity and chronicity of the associated physical disability (9, 10). It is also recognized that the experience and expression of illness reflects the interaction between the physical and psychological states of an individual, such that an individual's mental state can influence physiological changes, including the reactivity of the immune system (11, 12). Moreover, the response to physical stimuli, such as the size of the wheal and flare reaction to intradermal testing (13) and hyperreactivity of the bronchial airways (14), are reported to be influenced by mental events. Psychologically mediated allergic changes can be classified into a nonspecific autonomic nervous system response to emotional arousal, such as an asthma attack due to fright or violent emotion, and changes due to suggestion or conditioning to specific stimuli (4). It has been reported that nasal, eye, and airway symptoms as well as changes in eosinophil levels, nasal secretion, bronchoconstriction, and gastrointestinal (GI) and skin blood flow can be experimentally induced by suggestion alone (15, 16). These findings emphasize the importance of performing diagnostic tests, particularly challenge/provocation procedures, under blinded, placebo-controlled conditions.

Food Allergy and Psychological Disorders

The experimental induction of hypersensitivity by Portier and Richet (17) in 1902 stimulated scientific interest in the different manifestations of food allergy. In the 1920s, experimental and clinical observations described the characteristic manifestations of food allergy as consisting of anaphylaxis, asthma, urticaria, angioedema, and GI disturbances (18). However, many physicians at that time distrusted the accuracy of skin tests and preferred to base their diagnosis of food hypersensitivity on their own clinical assessment of the association between symptoms and food reactions (19). Thus arose several papers claiming central nervous system (CNS) involvement in food allergy and sensitivity reactions with symptoms including "nervous" complaints, migraine, irritability, sleep disturbance, hyperactivity, moodiness, poor concentration, apathy, fatigue, and various other general nonspecific complaints (20–22). Treatment for these complaints consisted of elimination diets.

In 1950, Randolph (23) proposed that these subjective nonspecific complaints were due to specific maladaptation to foods in susceptible individuals who pass through various phases of reactivity. This concept, so far, appears to have no recognized or demonstrable immunologic basis.

Autism

Childhood autism is characterized by significant abnormal or impaired development in social interaction and communication, and restricted repertoire of activity and interests (1). Immunologic abnormalities, gluten sensitivity, and food allergy have been proposed to play a role in the pathogenesis and management of autism (24–26).

Increased basophil degranulation to food allergens was reported in 10 autistic children by Bidet and colleagues (27). In 1995, Lucarelli et al (28) studied 36 autistic patients on a cow's milk elimination diet. They reported that 13 (36%) of the autistic patients were skin test-positive to various food antigens such as casein, lactalbumin, β-lactoglobulin, egg white, rice, and soy, compared to only 5% (total of 20) of the control group. They also found that the autistic patients had elevated total IgE levels, IgA-specific antibodies to casein, lactalbumin, β-lactoglobulin, and ovalbumin; IgM and IgG to casein; and IgM to lactalbumin. All the autistic patients were then placed on a cow's milk protein elimination diet together with elimination of other foods found to be positive on skin test, for 8 weeks. At the end of the period, they were evaluated for symptomatic or behavioral modification using the Behavior Summarized Evaluation (BSE)

scale, which was then compared to the BSE performed at the beginning of the study. Patients observed to have improvement underwent a DBPC challenge with the suspected food. Patients were observed for 4–6 hours after the administration of the oral challenge and were also re-examined after 2 weeks if they did not have any immediate reactions. The authors reported significant improvement in behavioral disturbances in five of the seven categories of the BSE with the elimination diet but worsening in only three of seven categories after oral challenge, which they attributed to the brevity of the post-challenge observation period. It is interesting to note that, although only 36% of the autistic patients had positive skin tests to the food antigens and all the patients underwent the 8 week cow's milk elimination period, no correlation was reported between skin test positivity and improvement on the BSE after the elimination diet or subsequent deterioration after oral challenge. Sponheim (29) studied seven autistic children on a gluten-free diet. Three patients underwent DBPC challenge with gluten while four patients were on an open gluten-free diet for 6 months. The Visual Analogue Scale and Real Life Rating Scale were used to evaluate behavior at baseline, during and after the study period. No improvement in behavior was reported with the gluten-free diet. Renzoni and colleagues (30) also studied the immunologic and allergic characteristics of 43 autistic patients compared to a sex- and age-matched population of developmentally delayed non-autistic controls. They did not find any significant increase in prevalence of elevated total IgE (> 200 kU/L) and number of patients with specific IgE to common foods such as milk, fish, eggs, wheat, peanuts, and tomatoes in the autistic patients. They did find an increased prevalence of eosinophilia (blood eosinophils > 5%) compared to controls, which was not associated with an increased prevalence of atopy in the autistic patients. The authors concluded that they were unable to demonstrate a higher prevalence of hypersensitivity to common food allergens in autistic children.

The association of celiac disease (CD) and autism was studied by Pavone and colleagues (31) in a 1997 study. They evaluated 120 patients with CD and 20 controls for features of autism based on DSM III-R criteria. The authors also evaluated 11 autistic patients and 11 age- and sex-matched controls for CD with IgA and IgG antigliadin antibody and endomysium-specific antibody assays with jejunal biopsies in the case of positive antibody tests. The authors did not find any evidence of CD in the autistic patients, although two patients had slightly increased gliadin- and endomysium-specific antibody levels with normal jejunal biopsies. They also did not find any CD patients to be autistic or to exhibit autistic-like behavior. Other studies (32, 33) have also failed to demonstrate an increased prevalence of CD in autistic patients using antigliadin antibody assays and jejunal biopsies.

In summary, the scientific evidence supporting an association between autism and food allergy/hypersensitivity is sparse. A key concern in studies of this kind, particularly those involving long periods of diet elimination, is dietary compliance, which has not always been addressed and monitored in previous studies. The implementation of unproven treatment modalities such as elimination diets may divert the autistic patient's family from more useful treatments and may contribute to poor nutrition and further social isolation in these patients and families already facing great difficulties.

Schizophrenia

In 1966, it was proposed by Dohan (34) that gluten played a significant role in aggravating the symptoms of schizophrenia and that a gluten-free diet was of therapeutic value in these patients. Studies of the rate of mental hospital admissions for schizophrenic women and change in wheat consumption during World War II in the US, Canada, Norway, Sweden, and Finland reported a high correlation further supporting this hypothesis (35, 36). Dohan and other researchers (37–39) also reported improvement in schizophrenic patients when placed on a gluten-free diet with improvement seen as early as 1 month and others needing 6–12 months, and deterioration when given a gluten challenge. However, only some schizophrenics, those who were chronically ill, had a poor prognosis, and had nuclear schizophrenia, seemed to respond best. Reports that the incidence of antigliadin antibodies is elevated in schizophrenic patients, and that wheat gluten has endorphin-like and opioid antagonist polypeptides properties that can cross into the brain in experimental animals seemed initially to support this hypothesis (40–42).

Subsequent studies of gliadin antibody levels in schizophrenics and follow-up intestinal biopsies in antibody-positive patients did not find an increased incidence of CD in schizophrenic patients (43, 44). Other studies have failed to find any improvement in schizophrenic patients with

a gluten-free diet. Potkin (45) studied 8 schizophrenic patients who were placed on a closely supervised gluten-, cereal grains-, and milk-free diet for at least 13 weeks. They then underwent DBPC gluten challenge for a period of 5–8 weeks. No deterioration in clinical status was observed using the Brief Psychiatric Rating Scale (BPRS). Other researchers (46, 47) were also unable to demonstrate any improvement with a gluten-free diet for a period as long as 9 months.

Milk was reported in one case to be associated with psychotic symptoms in a 14-year-old female with a history of GI intolerance to milk who developed symptoms on double-blind challenge (48). Elevated IgA antibodies to gliadin, β-lactoglobulin, and casein were reported in 25 schizophrenic patients compared to controls, but the clinical relevance of this finding is unclear (49). Other researchers (50) have not found elevated food antibodies in schizophrenic patients.

Celiac Disease and Psychiatric Disorders

CD, or gluten-sensitive enteropathy, is a chronic disease of the small intestinal mucosa with intermittent diarrhea, abdominal pain, distension, and irritability induced by gliadin, the prolamin protein of wheat (51). Aside from the resulting weight loss and malabsorption, neurological and psychiatric illnesses have also been reported in patients with CD (52, 53).

A high prevalence of anxiety and depression has been reported in adult patients with CD (54–56). The prevalence of these disorders has been attributed to the reduction in the quality of life due to chronic disease in these patients (54, 57) and secondary to reduction in brain monoamine metabolism due to either malabsorption or impaired transport (58, 59). Hallert and Sedvall (60) reported significant increases in monoamine metabolites and tryptophan in the cerebrospinal fluid in patients with CD after being on a gluten-free diet for 1 year. De Santis and colleagues (61) reported a case of a patient with undiagnosed and untreated CD with psychiatric disorder. The patient's psychiatric symptoms disappeared and frontal cortex abnormalities normalized as documented by single photon emission computed tomography (SPECT) after beginning a gluten-free diet. Addolorato (54) studied 35 patients with CD, anxiety, and depression for 1 year on a gluten-free diet. They reported a significant decrease in anxiety state to values similar to controls after 1 year on the gluten-free diet without significant reduction in depression. They attributed these findings to the fact that anxiety in CD patients is predominantly reactive, and related to poor quality of life due to chronic illness, whereas depression is a characteristic of CD. They recommended that patients with CD obtain psychological support to improve compliance to treatment and limit related disease complications. Hallert (62) studied 12 patients with CD and depression and also reported no improvement in depressive symptoms after 1 year on a gluten-free diet despite improvement in small intestinal biopsies. However, he reported significant reduction in depression as evaluated by the Minnesota Multiphasic Personality Inventory (MMPI) after 6 months on oral pyridoxine (vitamin B6) therapy (80 mg/day). Their findings suggest that the metabolic effects of pyridoxine deficiency may influence central nervous mechanisms regulating mood in CD.

Somatoform Disorders

In 1984, Rix and colleagues (62) studied the psychiatric characteristics of 19 patients who believed they had allergies to multiple foods but were subsequently found not to be allergic on skin testing and double-blind provocation. These patients attributed to food allergy a variety of symptoms such as lethargy, head pain or tightness, abdominal discomfort, nausea, depression, and irritability, among others. The authors found this group to be almost identical, in terms of psychiatric symptoms, with a group of new psychiatric patients who attended an outpatient clinic. The majority of these patient had depressive neurotic complaints, which under current classification criteria could be categorized under the somatoform disorders.

The characteristic feature of the somatoform disorders is the presence of multiple physical symptoms that cannot be explained by a medical condition or by another mental disorder, and that cause significant social or occupational dysfunction (1). Somatization disorder, conversion disorder, pain disorder, hypochondriasis, and body dysmorphic disorder are included in this category.

Somatization disorder is of special interest because food intolerance is a common complaint in these patients. Patients with this disorder complain of numerous physical problems over several years with onset before age 30. These complaints cannot be fully explained by any known medical condition, or if they occur in the presence of a medical condition, the resulting functional impairment is in excess of what would be expected.

Criteria for diagnosis require that the patient report at least four pain symptoms, two GI symptoms (which may include multiple food intolerance), one sexual symptom, and one pseudoneurological symptom. Patients with this disorder have increased suggestibility and are more likely to complain of multiple problems (63). Other studies (64–66) have also found increased frequency of somatoform disorders, depression, and anxiety in community samples of professionals and students reporting intolerance to foods that are not confirmed by allergy skin testing or oral challenge. In their study, Rix and colleagues (62) observed that these patients were initially hostile to the suggestion that they had psychological problems, belonged to a higher social class, and were highly suggestible, prone to self-diagnosis on the basis of reports in the media, and sought numerous other medical opinions. Their prognosis was related to the strength to which they held the belief in a food allergy etiology as the cause of their symptoms in spite of scientific evidence to the contrary. The patients who accepted the evidence that food allergy was not the cause of their symptoms improved with supportive therapy and were able to go off the restrictive diets to which many were limiting themselves.

Patients with somatoform disorders are the most frequently encountered type of patient who present with an unconfirmed food allergy and nonspecific symptoms. They present a special challenge to the physician and require extra effort and support in terms of time, education, and attempts to build rapport, since most patients will reject a psychiatric referral if they do not have a good relationship with their physician and if they feel that their emotional and physical problems are not being taken seriously.

Depression

Seggev and Eckert (67) reported that three patients who believed they had food allergies related to multiple vague physical complaints, with negative skin tests to implicated foods. Two patients were found to be depressed and the third had psychotic ideation. All three patients improved with antidepressant and/or antipsychotic medication and psychiatric therapy. The authors point out that although the antidepressants have potent antihistamine activity, this was not responsible for the improvement in these patients. They also observe that depression leads to increased focus on physical symptoms that may be considered "allergic" by the patient who then limits their diet, which makes their lives even more isolated and preoccupied.

There is no observed increased incidence of food allergy in depressed patients (67, 68). Ossofsky (69) and Nasr (70) reported an increased incidence of atopic disease such as allergic rhinitis and asthma in depressed patients, an observation that warrants further study.

Panic Disorder and Environmental Intolerance

Self-reported multiple food intolerances/sensitivities have been reported to be frequently associated with IEI, formerly called MCS (66, 71, 72). In 1987, Cullen (73) introduced the term "multiple chemical sensitivities," which he defined as "An acquired disorder characterized by recurrent symptoms, referable to multiple organ systems, occurring in response to demonstrable exposure to many chemically unrelated compounds at doses far below those established in the general population to cause harmful effects. No single widely accepted test of physiologic function can be shown to correlate with symptoms." Other terms for IEI are cerebral allergy, chemically induced immune dysregulation, total allergy syndrome, and ecologic illness (74).

The most common complaints are fatigue, headache, nausea, malaise, pain, mucosal irritation, disorientation, and dizziness, which are mostly non-specific. No gross or microscopic evidence of inflammation or other objective signs of pathology have been associated with IEI. As in somatoform disorders, these patients have multiple chronic symptoms and have previously consulted with numerous physicians and other health care professionals without satisfaction nor any finding of underlying immunologic, autoimmune, or any physical disease to explain their symptoms (75). Patients attribute their illness to exposure to a combination of environmental chemicals, multiple foods, and drugs. A unique feature of IEI is the general absence of a dose-response curve in the provocation of symptoms. The length of exposure to environmental chemicals and foods required to stimulate symptoms has varied from a few seconds to more than 20 years with no association of presumed dose and length of exposure to severity of reported symptoms (76). The diagnosis of IEI has often been popularized by practitioners who do not normally deal with occupational health is-

sues. Some practitioners have championed this diagnosis and have advocated extreme lifestyle changes leading to reinforcement of the underlying psychiatric pathology, vocational impairment, and social isolation (77, 78).

Various theories, such as failure of the body to adapt to synthetic chemicals (79), immunologic and autoimmune mechanisms (80), and time-dependent sensitization (81), have been put forth by proponents of IEI. However, acceptable objective and scientific documentation supporting any of these various hypotheses has yet to be presented (82).

Evidence is growing in support of a causal role of underlying psychiatric illness, specifically somatoform (83), depression, and panic disorder in IEI (84, 85). IEI and panic disorder share common symptoms such as chest tightness, breathlessness, and palpitations; apprehension; and avoidance of situations that have been associated with onset of symptoms. Panic attacks may temporally occur with non-noxious stimuli that are then associated with symptoms by the patient and are subsequently considered the cause of the symptoms (86). Reports of placebo-controlled studies using saline infusions (86), carbon dioxide inhalation (87), and provocative challenges (88) note that these approaches provoke symptoms suggestive of panic disorder and anxiety syndrome with hyperventilation in IEI patients. Evidence for a common neurogenetic basis linking IEI and panic disorder was reported by Binkley and colleagues (89) in a study of 11 IEI patients who were found to have a significantly increased prevalence of cholecystokinin B (CCK-B) receptor alleles, which are known to be associated with panic disorder, compared to age-, sex-, and ethnic background-matched controls.

Approach to the Patient with Psychologic Symptoms Attributed to Food Allergy

Epidemiologic research has found a large discrepancy between the high prevalence of self-reported food allergy symptoms in the general population and the low prevalence of actual food allergy as documented by skin testing and oral challenges. Up to 20% of the population report some form of food intolerance or food allergy, whereas the prevalence of documented immunologic food reactions is around 2% (90, 91). As previously discussed, many patients who attribute their symptoms to food allergy without scientific basis after appropriate allergy evaluation, may have an underlying psychiatric disorder. The most commonly reported disorders are somatoform disorder, depression, or panic disorder. The stigma placed on psychiatric disorders in our society makes it more acceptable to attribute symptoms to an organic cause such as allergy rather than a psychiatric etiology. Physicians may contribute to this perception by paying selective attention to physical symptoms. Patients may also be hesitant to reveal psychological issues if they sense the doctor has negative attitudes toward psychiatric problems or is uncomfortable dealing with emotional distress (92). Every effort should therefore be made to maintain good rapport and communication with these patients. It is important that they feel that their physician takes them and their symptoms seriously.

When taking the history, psychosocial cues from the patient such as description of symptoms worsening around stressful situations should be noted and explored if the patient is willing. Physicians should be alert to the presence of paroxysmal episodes of symptoms which involve a combination of physical and psychological symptoms (palpitations, nausea, sweating, tension, fear), since they may be suggestive of a panic or anxiety disorder. Multiplicity of symptoms is also suggestive of a psychiatric disorder. A linear association has been found between the number and severity of somatic complaints such as myalgia, tiredness, and pain, changes in sleep and energy levels, and psychological distress (93).

The importance of performing blinded placebo-controlled challenges, as opposed to open challenges, to evaluate suspected psychogenic food reactions cannot be stressed enough due to the multiple, non-specific character of these complaints, the increased suggestibility in the majority of these patients, and to avoid patient and observer bias. It is also important to perform only investigations that the physician feels is warranted based on the history and physical examination, as further investigations may serve only to reinforce the patient's belief in an organic pathology and to delay appropriate treatment (94).

Although DBPC challenges are the gold standard in the diagnosis of food allergy and should be performed whenever feasible, single-blind placebo-controlled (SBPC) challenges may also be performed to confirm neuropsychological complaints associated with food ingestion, as long as guidelines are followed. The Scripps Clinic has performed SBPC food challenges in patients with

neuropsychological reactions who met the following conditions: 1) identification of at least one specific food or substance with a consistent association with symptoms; 2) identification of the specific dose of the suspected food or substance that produces symptoms; 3) that the specific symptom or symptom constellation is consistently associated with exposure; and 4) that a consistent time frame exists during which symptoms occur after exposure to the suspected food or substance. A series of at least five challenges are performed, with placebo challenges occurring at least on the first and last challenge and randomly interspersed with challenges with the active substance. Placebo is given on the first and last challenges because psychophysiologic reactions are most likely to occur at these times. The patient should be informed as to the duration of the entire challenge procedure (half day, full day, or several days) and that at least one of the challenges will be with the suspected food/substance. He or she should not be informed of the exact number of challenges and the exact combination of active and placebo challenges as this may increase the likelihood of a psychophysiologic reaction to the last challenge. If the patient has at least two reactions to the suspected food or substance, and no reaction to at least three placebos, then a cause-and-effect association between substance exposure and symptoms is established. This procedure has also been useful in chemical- or aroma-associated reactions using nose clips to prevent olfactory stimulation, but allowing oral inhalation, of the suspected chemical.

The experience at the Scripps Clinic with this challenge protocol has been very positive. According to R Simon, the protocol has been effective in screening patients with psychogenic food reactions and in overcoming the patient's belief system that there is a cause-and-effect relationship between exposure to the substance and onset of symptoms.

Part of the discussion of negative challenge results should include an explanation that the patient's symptoms may be due to a "conditioned reflex" association. This type of association may have been established when the patient experienced symptoms that coincidentally occurred in the presence of the suspected substance. The patient may then have mistaken a temporal association between substance exposure and onset of symptoms with a cause-and-effect association. Repeated episodes of substance exposure paired with symptom onset reinforces this association. After a sufficient period, whenever the patient believes he or she has been exposed to the food or substance, symptoms are triggered. As previously mentioned, the patient should not be told that they were "imagining" or "making up" their symptoms. They should be informed that they were in fact experiencing symptoms, but these symptoms were not caused by exposure to the suspected substance. Most patients will accept and be reassured by explanations that an allergic etiology is not involved in their symptoms and that there is no serious organic pathology found on evaluation.

When the physician feels he or she does not have the expertise to manage more serious psychiatric disorders or to address psychosocial issues, a referral to a psychiatrist with an interest in patients who present with somatic complaints would be appropriate. The manner in which the referral is made is crucial to the success of future treatment, because patients may be reluctant or even hostile to the idea of seeing a psychiatrist. Insensitively handled psychiatric referrals will add to the patient's distress and loss of confidence in orthodox medicine and may lead them to seek help from unorthodox practitioners instead.

Patients may be more receptive to accept psychologically based treatment if they are reminded of the complex interactions between psychological, social, and physical influences, and if there is a discussion of how psychological issues can contribute to symptoms (92).

Close liaison and communication between the referring physician and the treating psychiatrist is important to enhance communication between the physicians and the patient. These patients present a special challenge to the allergy specialist, and it is our task to counsel them with compassion and guide them toward more appropriate and effective therapy for their problem.

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40

Foods and Connective Tissue (Rheumatic) Diseases

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Some immunologic or hypersensitivity reactions to foods develop over a period of several hours or days (or longer). They are referred to as "delayed reactions" and are mediated by immunologic mechanisms other than IgE-mediated, immediate-type hypersensitivity (1–9). Because such reactions are difficult to investigate and confirm, most alleged delayed food allergies, including those related to connective tissue, are controversial. Information presented in this chapter was derived from selected published reports that are reasonably well documented, and will focus on the relationship of food to two specific rheumatic processes, namely arthritis and vasculitis.

Historical Perspective

Considerable literature about food and rheumatic diseases began to appear early in the last century. Most observers were unimpressed that diet or food had a consistent relationship to rheumatic diseases (10–12) (summarized in Table 40–1). For example, Weatherbee in 1932 analyzed 350 cases of arthritis and concluded that "Dietary treatments of all types had been tried in many cases [but] . . . little definite improvement from dietary management alone was reported" (13). Minot in 1933 wrote: "There exist many peculiar facts concerning diets for arthritis" (14). In 1935, Walter Bauer stated, "there exists no unanimity of opinion concerning the correct diet for an arthritic patient," although he felt that allergy to foods provoked arthritis in certain patients (15). An authoritative, comprehensive review of the English language rheumatology literature in its time—1940—concluded, "The incidence of food allergy among rheumatic patients is not significant" (16). This same group also wrote, "We cannot approve the emphasis laid on the factor of food allergy in cases of atrophic arthritis; it is neither common nor do we consider it important. Variations in articular systems are so common from day to day that it is easy to blame erroneously some food for the day's ill feeling. Cases of atrophic arthritis with undoubted and repeated articular exacerbations from foods are few and far between" (17). On the basis of this literature, one of the last rheumatology textbooks that extensively considered the relationship between food and arthritis, in 1954, included the statement "it is almost universally acknowledged that rheumatoid arthritis cannot be overcome by any dietary manipulations which have thus far been proposed" (18). The Arthritis Foundation, too, in a 1981 informational pamphlet for patients, summarized: "The possible relationship between diet and arthritis has been thoroughly and scientifically studied. The simple proven fact is: no food has anything to do with causing arthritis and no food is effective in treating or 'curing' it" (19).

However, there have been provocative observations supporting the notion that specific dietary manipulation ameliorated arthritis. These were predicated on the hypothesis that foods or food additives products were injurious and caused or perpetuated arthritis. Most of these data may be inadequate because controlled, prospective, blinded experimental studies were not carefully conducted as would be expected today. Also, different types of arthritis were considered together, making conclusions about specific disease difficult.

Table 40–1.
Food, Diet, Nutrition, and Rheumatic Diseases

Reference	*Observations*	*Comments*
Pottenger 1928 (63)	Allergies frequent among arthritis patients	Uncontrolled arthritis undifferentiated
Weatherbee 1932 (13)	Dietary management ineffective for arthritis	Uncontrolled arthritis undifferentiated
Minot 1933 (14)	"There is, of course, no standard diet for arthritis"	Review
Lewin and Taub 1936 (66)	Allergic synovitis due to walnuts	Uncontrolled, unblinded
Berger 1939 (67)	Intermittent hydrarthrosis improved on elimination diet	Unproven
Hench et al 1941 (16, 17)	Food allergy suspected but unproven in palindromic rheumatism	Review
Hench and Rosenberg 1941 (20)	Food allergy suspected in 16 patients with palindromic rheumatism	Unproven
Vaughn 1943 (68)	Palindromic rheumatism in 2% of allergic patients	Unblinded, uncontrolled
Miller 1949 (cited in [94])	Allergic (palindromic) arthritis	Three anecdotes
Zeller 1949 (21)	Possible food allergy "as a factor" in RA	Four anecdotes
Kaufman 1953 (64)	"Food induced allergic musculoskeletal syndromes"	Anecdotal
Zussman 1966 (22)	Foods suspected of causing inflammatory arthritis	Four patients
Epstein 1969 (70)	Sodium nitrate associated with palindromic rheumatism	Blinded, controlled challenges
Millman 1972 (65)	"An allergic concept of the etiology of RA"	Review
Rowe 1972 (23)	Food allergy caused arthralgias/arthritis	10 patients
Marquardt et al 1973 (71)	Behçet's syndrome associated with black walnuts	Incompletely blinded, controlled
Randolph 1976 (69)	RA and myalgias associated with foods	Anecdotes
Skoldstam et al 1979 (cited in [94])	Fasting ameliorated RA symptoms	Controlled
Mandell and Conte 1980 (79)	"Rheumatic joint" reactions in 88% of patients	Abstracted and presented Questionable controls, blinding
Parke and Hughes 1981 (77)	RA exacerbated by milk	Incompletely blinded, controlled
Williams 1981 (cited in [94])	Dairy products associated with arthritis	Anecdote
Sundqvist et al 1982 (cited in [94])	Fasting improved RA	Controlled, unblinded
Wraith 1982 (80)	RA-like arthritis associated with food (tartrazine)	Double-blind challenge
Hanson et al 1983 (cited in [94])	No therapeutic benefit from evening primrose oil	Prospective, open trial
Panush et al 1983 (28)	Restriction diet comparable to placebo diet for RA and OA	Prospective, controlled, blinded
Reidenberg et al 1983 (75)	SLE associated with hydrazine	Unblinded challenge
Stroud 1983 (51)	Fasting antirheumatic; foods exacerbated arthritis	Incompletely blinded, controlled
Roberts and Hayashi (73)	SLE exacerbated by alfalfa (l-canavanine)	Anecdotal
Uden et al 1983 (50)	Fasting improved RA	Controlled
Bourne et al 1985 (cited in [94])	Six patients with arthritis and celiac disease	Food-related?
Jantti et al 1985 (cited in [94])	No appreciable therapeutic benefit from sunflower oil (linoleic acid) for inflammatory arthritis	Prospective, single-blind, placebo-controlled; 10 patients
Kremer et al 1985 (cited in [94])	EPA modestly improved some subjective symptoms of RA	Double-blind, controlled prospective
O'Driscoll et al 1985 (cited in [94])	Normal incidence of atopy in RA	
Ratner et al 1985 (83)	Milk associated with arthritis in lactase deficiency	Incompletely blinded, controlled
Darlington et al 1986 (84)	Food elimination improved and challenge worsened some RA patients	Unblinded, controlled
Panush et al 1986 (29)	RA-like arthritis and immunologic hypersensitivity to milk	Prospective, double-blind, controlled, repeated challenges on clinical research unit
Kremer et al 1987 (cited in [94])	EPA modestly improved subjective symptoms of RA	Double-blind, controlled, prospective
Moore et al 1987 (cited in [94])	EPA did not benefit SLE	Randomized, controlled
Sperling et al 1987 (cited in [94])	EPA modestly improved some symptoms of RA	Double-blind, controlled
Tanner et al 1987 (cited in [94])	28% of RA patients noted association of food with clinical status: 11% unfavorable, 6% favorable, 10% both	Prospective survey
Belch et al 1988 (cited in [94])	Fish oil (EPA) reduced NSAID requirement in RA	Controlled
Beri et al 1988 (cited in [94])	Diet restrictions ameliorated RA	Incompletely described
Cleland et al 1988 (cited in [94])	Modest subjective, symptomatic benefit from fish oil for RA	Double-blind, non-crossover study
Hafstrom et al 1988 (cited in [94])	Antirheumatic effects of fasting, possibly mediated by neutrophils	Prospective crossover study
Malone and Metcalfe 1988 (86)	IgE-dependent, mast cell-mediated "arthritis" in rats	Experimental model
Kremer et al 1990 (cited in [94])	Fish oil (EPA) improved RA tenderness and swelling in dose-dependent fashion	Prospective, randomized, controlled
Panush 1990 (34)	Food definitely but infrequently induced palindromic, RF(-) inflammatory arthritis	Prospective, double-blind, controlled, repeated challenges on clinical research unit
van der Tempel 1990 (cited in [94])	Fish oil was modestly beneficial for RA	Randomized, double-blind, placebo-controlled crossover
Westberg and Tarkowski 1990 (cited in [94])	Max EPA was not beneficial for SLE	Randomized, double-blind, placebo-controlled, 9-month crossover trial
Clark et al 1990 (cited in [94])	Max EPA therapy led to biochemical but not clinical change in lupus nephritis	Open, unblinded, uncontrolled
Lassus et al 1990 (cited in [94])	EPA/DHA provided subjective benefit for psoriatic arthritis	Uncontrolled, unblinded

(continued)

Table 40–1.
Food, Diet, Nutrition, and Rheumatic Diseases (*Continued*)

Reference	*Observations*	*Comments*
Perez-Mareda et al 1991 (cited in [94])	Antibodies to milk proteins in RA cross react with epitopes on type I collagen, C1q, and vitamin D	Intriguing
Kjeldsen-Kragh et al 1991 (60)	Notable benefit for RA patients from individually adjusted diet modifications	Important methodologic limitations
Walton et al 1991 (cited in [94])	Max EPA was beneficial in SLE	Prospective, double-blind, crossover
Fahrer et al 1991 (cited in [94])	Fish (4–6 times weekly) produced lipid changes similar to fish oil	Normal volunteers
Panush 1991 (12)	A comprehensive review of nutrition and rheumatic disease	21 contributions
Kjeldsen-Kragh et al 1992 (cited in [94])	Mild benefit of EPA/DHA for RA	Randomized, controlled comparison vs naproxen
Lunardi et al 1992 (cited in [94])	4 of 5 patients with hypersensitivity vasculitis and personal or family history of allergy remitted and 1 of 5 benefited from elimination diets	3-week elimination diets followed by open and double-blind food challenges
Karjalainer et al 1992 (cited in [94])	Patients with insulin-dependent diabetes mellitus have antibodies cross-reacting with bovine albumin and a beta cell surface protein	Also intriguing
Stammers et al 1992 (cited in [94])	Cod liver oil was ineffective for OA	Double-blind, placebo controlled
Epstein et al 1992 (cited in [94])	Urticarial vasculitis resolved in association with elimination diet	Single patient, uncontrolled
Shigemasa et al 1992 (cited in [94])	SLE improved in association with vegetarian diet	Single patient, uncontrolled
Skoldstam et al 1992 (cited in [94])	Modest benefit of fish oil for RA	Randomized, controlled blinded
Panush 1993 (25)	Questionable arthritis remedies	Review
Leventhal et al 1993 (cited in [94])	Modest benefit from plant seed derived γ-linolenic acid for RA	Randomized, double-blinded, placebo controlled, 24-week trial
Rossi and Costa 1993 (cited in [94])	Fish oil prevented miscarriages in women with spontaneous abortions and antiphospholipid antibodies	Uncontrolled, unblinded
Lau et al 1993 (cited in [94])	Fish oil reduced NSAID requirement in RA	Randomized, single blind, placebo controlled
Geusens et al 1994 (cited in [94])	Significant clinical benefit of fish oil for RA	12-month, randomized, double-blind, controlled trial
Appelboom and Durez 1994 (76)	Many spondyloarthropathy patients responded to milk elimination	Incompletely controlled
Haugen et al 1994 (cited in [94])	Some RA patients may respond to elimination of foods	Small study (17 patients), small differences in groups
Leventhal et al 1994 (cited in [94])	Black walnut seed oil suppressed disease activity in RA	Randomized, double-blind, placebo controlled, 24-week trial.
Cleland et al 1994 (cited in [94])	"Diet and arthritis"	Review
Kavanagh et al 1995 (cited in [94])	Elemental diet improved RA: This was not sustained on individualized diets	Unblinded, incompletely controlled
Bengtsson et al 1996 (cited in [94])	Instances of alleged food-associated arthralgias and joint swelling, Rheumatic symptoms on questionnaires of consecutive patients in allergy clinic	
Kjeldsen-Kragh et al 1995; 1996 (cited in [94])	Follow-up observations of RA patients on vegetarian diet (60) suggested no link between food antibodies and clinical response and perhaps a role for *Proteus mirabilis* in RA. See prior comments.	Needs independent confirmation
Shapiro et al 1996 (cited in [94])	RA may be less common in women consuming fish rich in omega-3 fatty acids, case-control study based importantly on recall of dietary habits years earlier	
Hansen et al 1996 (cited in [94])	Possible mild benefit of "specialized" diet (adjusted to body weight, with fish meal and antioxidants) for RA	Prospective , single-blind, 6-month study with many drop-outs
Peltonen et al 1997 (cited in [94])	Vegan diet changed fecal flora. Might this have been associated with clinical improvement in RA?	Randomized, prospective trial
Trollmo et al 1997 (cited in[94])	Short-term starvation increased mucosal B cell responses	Controlled
James and Cleland 1997 (cited in [94])	Omega-3 fatty acids and RA	Review
Slotkoff et al 1997	Might fibromyalgia reflect "multiple chemical sensitivities" syndrome?	Serious methodological shortcomings; uncontrolled, nonrandomized, unblinded, unvalidated, self-reported questionnaire
Nenonen et al 1998 (cited in [94])	Uncooked vegan diet, rich in lactobacilli, subjective symptoms of RA	Controlled
Dykman et al 1998 (cited in [94])	Might nutritional supplements help symptoms of fibromyalgia?	Methodological shortcomings

DHA, docosahexaemoic acid; EPA, eicosapentaenoic acid; OA, osteoarthritis; NSAID, nonsteroidal anti-inflammatory drug; RA, rheumatoid arthritis; RF, rheumatoid faactor; SLE, systemic lupus erythematosus.
Modified from (94) Panush RS. Diets, other complementary and alternative therapies, and the rheumatic diseases. In: Koopman WJ, ed. Arthritis and Allied Conditions—A Textbook of Rheumatology. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:965–986, with permission.

In 1941, Hench and associates concluded that food allergy occasionally caused "atrophic arthritis" (rheumatoid arthritis [RA]) (16, 17). In describing palindromic rheumatism, it was suggested that allergy may be an etiologic factor in some of these cases (20). Several allergists wrote about the relationship of allergy and "rheumatism." Zeller related four case reports to support his thesis that ingested foods exacerbated RA, and that dietary exclusions improved its course (21). However, some patients failed to improve on exclusion diets. Zussman (22) also presented patients whose histories suggested an exacerbation of their musculoskeletal problems with ingestion of certain foods. Two hundred (20%) of 1000 consecutive adults with allergic complaints (e.g., asthma, hay fever, or urticaria) had rheumatic complaints. Most of these had allergic symptoms attributed to food, although 27 of the original 1,000 had rheumatic symptoms exacerbated by ingestion of specific foods. Rowe reviewed literature from 1917 to 1972 and concluded that "Food allergy as a cause of arthritis pain or arthralgia and swelling occurs not infrequently." He cited substantiating literature and personal cases (23).

Thus both physicians and patients have long been intrigued by the possibility that some foods might provoke arthritis and others ameliorate it. If true, then arthritis would be expected to respond to appropriate nutritional therapy (12, 24–32). Diet therapy for rheumatic disease, however has been generally considered "quackery," and now often falls within "complementary" and "alternative" medical practices. Indeed more than 90% of arthritis patients spend billions of dollars annually on this and related questionable therapies (12, 25, 26). Surprisingly, despite the skepticism of rheumatologists and the fervor of its advocates, until recently little objective information existed about nutritional therapy for rheumatic diseases. This was considered an important issue and in 1985 was identified among major future clinical advances anticipated in rheumatology (33). Most conclusions have been based on improper study design or inadequate data (10–12, 24–32).

Possible Relationships Between Nutrition and Rheumatic Diseases

A relationship between nutrition and inflammatory/immunologically mediated rheumatic diseases could occur through two possible mechanisms that are not mutually exclusive (10–12, 24–32). First, nutritional factors might alter immune and inflammatory responses and thus modify manifestations of rheumatic diseases. Second, food antigens might provoke hypersensitivity responses—food allergies—leading to rheumatologic symptoms.

This brief and necessarily selective review considers the evidence for the latter possibility: that certain patients with rheumatic diseases may indeed have food-related symptoms. The published data are largely but not exclusively anecdotal. And some of the anecdotes (which may be a valid basis for generating scientific hypotheses) are persuasive. There are several reasons for considering the hypothesis that rheumatic diseases might relate to food allergy: 1) foods may evoke immune responses; 2) foods may cause immunologically mediated symptoms; 3) immunologic mechanisms of tissue injury are important in the pathogenesis of rheumatic diseases; 4) those antigens that might trigger abnormal immune events in rheumatic diseases are largely unknown; and 5) rheumatic diseases have been associated with foods in anecdotal reports (34).

The etiology of RA and most forms of inflammatory rheumatic diseases remains unknown. There has been much speculation about the putative role of microbial and other environmental agents in the pathogenesis of these disorders. It seems no less reasonable to consider food or related antigens as candidates for initiation of immunologically mediated inflammation for certain patients. Studies in this area offer the possibility of identifying antigens capable of inducing or perpetuating inflammatory arthritis and elucidating pathogenic pathways for such patients. This information may affect the study of patients with rheumatic diseases.

Food sensitivity as a cause of rheumatic disease has been controversial, in part due to unsubstantiated claims in the lay press based on inadequate data. Nutritional therapy for disease, in general, also has been controversial. Inappropriate advocacy of this notion prejudiced its consideration as a potentially useful approach for selected situations. There is now, however, firm evidence of nutritional modulation of experimental autoimmunity. Food sensitivity in rheumatic disease is now an issue in clinical immunology.

Food Allergy, Rheumatic Disease, and the Gastrointestinal Tract

If arthritis can be caused by hypersensitivity to foods, then food antigens would have to cross

the gastrointestinal (GI) barrier and circulate in an immunogenic form until they were recognized by effector or intermediary cells in the immune system. Experimental data indicate that food antigens indeed cross the GI barrier and circulate both as food antigens and as immune complexes (35–38).

In immunologically compromised persons, intrinsic abnormalities of the GI mucosa may permit the transport of larger quantities or different types of antigenic material (e.g., peptides). Selective IgA deficiency has been sometimes associated with both GI disorders and immunologic disease. Increased titers of antibodies to cow's milk were found in IgA-deficient subjects and correlated with the presence of circulating immune complexes (39) and with serologically defined autoimmune disease (35). Patients with hypogammaglobulinemia had both increased absorption of bovine milk antigens and prolonged persistence of these antigens in the circulation (40). Furthermore, some studies have suggested that patients with rheumatic disease have abnormal digestive and absorptive function (41, 42). Increased fecal fat and vitamin A, and decreased D-xylose absorption, supported the idea that the GI tract in patients with RA may be more penetrable to food antigens than that in normal individuals, but this finding has been inconsistent (43, 44). Abnormal intestinal permeability may be due to effects of nonsteroidal anti-inflammatory drugs (NSAIDs) (45).

Antigens of the intraluminal contents, other than food antigens, stimulated lymphocyte transformation and production of leukocyte inhibitory factor in patients with spondyloarthropathies (46). Some patients with jejunoileal bypass for obesity developed an arthritis indistinguishable from rheumatoid and displayed immunologic abnormalities including increased circulating immune complexes (47). These observations were consistent with a possible role for the GI tract in possible food-induced rheumatic diseases.

Fasting, Elemental Nutrition, and Diets

These and related studies are summarized in Table 40–1 (13–94) and Table 40–2. Fasting seemed to ameliorate disease in some patients with rheumatic disease (48–50). Five of 15 patients with classic RA who fasted for 7–10 days benefited, whereas only 1 of 10 control subjects improved. Fasting patients showed lessened pain, stiffness, and medication requirements, and decreased Ritchie index score and finger size. Continuation of a lactovegetarian diet was without consistent benefit (48). Another controlled study on the effects of fasting on patients with RA involved 14 patients who fasted for 7 days and then were crossed over to a control regimen. The majority experienced clinical improvement during fasting, and their condition remained unchanged or worsened during the control period. Improvement while fasting was accompanied by decreased erythrocyte sedimentation rate, Ritchie and Lansbury indices, morning stiffness, and joint counts. Serum cortisol levels did not change (50). A prospective study investigated the effects of complete fast on patients with RA. This was performed in a specialized, "environmentally controlled" unit. Forty-three patients underwent a water fast lasting 7 days. Tenderness, swelling, grip strength, dolorimeter scores, joint circumference, functional activity, and erythrocyte sedimentation rate improved significantly during the fast. It was suggested that short-term fasting may induce rapid clinical improvement in RA (51). Our own studies also found that short-term fasts were antirheumatic for some patients with RA (28, 29). Improvement might have been caused by reduced GI permeability, decreased neutrophil function, depressed lymphocyte response to mitogens, or increased cortisol concentrations during fasting (52–55).

In other studies we examined a specific prescription "arthritis" diet (no red meat, fruits, dairy products, herbs, spices, preservatives, additives, or alcohol). We reported that outpatients who had

Table 40–2.
Diets, Fasting, Elemental Nutrition, Vitamins, Minerals, and Foods for Rheumatic Diseases

- Fasting had short-term antirheumatic effects (23, 34, 44, 48, 50–55, 93, 94).
- Elemental nutrition has been inconsistently antirheumatic (23, 28, 34, 54, 55, 62, 93, 94).
- Specific diets have not been consistently beneficial for patients with rheumatic diseases (28, 56–62, 89, 90, 92–94).
- Vitamins, minerals, or nutritional supplements have not been consistently antirheumatic (12, 24–26, 32, 93, 94).
- Rare patients with rheumatic disease have had clinical symptoms convincingly documented to be associated with food or food-product sensitivity (16–23, 28–30, 34, 51, 57, 61, 64–88, 91, 93, 94).

Modified from (94) Panush RS. Diets, other complementary and alternative therapies, and the rheumatic diseases. In: Koopman WJ, ed. Arthritis and Allied Conditions—A Textbook of Rheumatology. 14th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:965–986, with permission.

long-standing, progressive, active RA fared no better than comparable patients receiving a placebo diet. Some patients did improve on the experimental diet, however, and experienced recurrence of symptoms when they deviated from it (28).

Limited information is available about other specific diets for arthritis. Various types of elimination, vegetarian, or other diets were utilized in some other studies, usually not entirely controlled, with possible but unpredictable benefit (58, 60, 89, 90, 92–94). Elemental diets were also beneficial in some cases (12, 29, 34, 57, 58, 60, 62, 92–94) (Tables 40–1 and 40–2).

Vegetarian and vegan diets have been shown in uncontrolled studies to benefit some arthritic conditions (56, 68, 92–94). In a Scandinavian study, 27 adults with RA followed a vegan diet that also reduced coffee, tea, spices, and sugar intake. After 4 months, 60% of patients reported an improvement in pain and stiffness (56). It was suggested that the combined reduction in animal fats, removal of possible food allergens, or the inclusion of more antioxidant vitamins often achieved when subscribing to a vegan diet, may have accounted for the improvement noted. Based on the above rationale, some patients with rheumatic conditions choose to follow a macrobiotic diet. The Zen macrobiotic diet has ten stages. As a person advances through the ten stages, progressively more foods are eliminated and replaced with grain products. Lower-level macrobiotic diets, if carefully planned, may meet nutritional needs, but strict adherence to higher levels of the diet can result in serious malnutrition and growth retardation. These diets are especially hazardous to growing children since it is difficult to obtain adequate calories, vitamins, and minerals while adhering to such a strict dietary regimen (71).

Limited information is available concerning other specific diets for arthritis. Currently there is no proven role for the use of special "fad diets" or many individual dietary supplements in managing patients with rheumatic diseases (15). There are a variety of fad diets for arthritis, some of which include the use of foods or food substances such as Brewer's yeast, apple cider, honey, wheat germ, garlic, alfalfa, cod liver oil, molasses, ginger, and bromelaine. The emphasis on a single component should alert the consumer and physician that these diets are typically unproven. Most diet studies for rheumatic disease patients should be considered as suggestive, or hypotheses-generating, at best, unless they are rigorous controlled, detailed, and blinded (which unfortunately most are not).

Food Allergy and Palindromic Rheumatism, Systemic Lupus Erythematosus, and Other Systemic Rheumatic Diseases

Several clinical observations suggested relationships between food intake and systemic rheumatic disease (16, 17, 20–22, 63–69) (Table 40–1). Reports included that of a dermatologist who documented his own palindromic rheumatism to be due to sodium nitrate hypersensitivity (70). Black walnut ingestion was linked to clinical exacerbations of Behçet's syndrome and to abnormal cellular hypersensitivity responses (71). Ingestion of alfalfa seed and sprout has been associated with systemic lupus erythematosus (SLE) in both humans and monkeys (72, 73). The disease induced in primates was characterized by an autoimmune hemolytic anemia with low complement levels, positive antinuclear antibodies, anti-DNA, positive lupus erythematosus cell preparations, and the deposition of immunoglobulin and complement in the skin. Induction of the disease was attributed to L-canavanine, a nonprotein amino acid component of alfalfa. Antibody to the alfalfa seed was found to cross-react with DNA and may have activated B lymphocytes (74). SLE in a young woman was linked to environmental exposure to hydrazine (75). Milk elimination might have been beneficial to patients with spondyloarthropathy (76) (Tables 40–1 and 40–2).

Food Allergy and Inflammatory Arthritis

Parke and Hughes reported a well-described challenge test in a woman with progressive RA for 11 years (77) (Table 40–1). She showed a poor response to NSAIDs and daily prednisolone, and could not tolerate penicillamine, gold, or azathioprine. She gave a history of eating cheese daily for the past 18 years. A milk-free diet resulted in improvement within 3 weeks. Challenge in the hospital with dairy products produced marked exacerbation of her arthritis as measured by increased pain, Ritchie index score, morning stiffness, swelling, and decreased grip strength. Stroud, in a largely uncontrolled study, reported an antirheumatic effect of fasting and also found that chemical or food challenges, particularly wheat, corn, and beef, caused deterioration of grip strength and dolorimeter and arthrocircameter scores in patients with RA (51).

A preliminary report of a prospective study of patients with definite or classic RA indicated that

withdrawal of allergenic substances identified by an elimination diet resulted in a response varying from "complete success with total abolition of all rheumatic symptoms to deterioration" (78). A double-blind provocation study, also reported in a preliminary fashion, found that challenges "with food extracts and other incitants including alternaria, house dust, tobacco smoke and petrochemicals induced 'rheumatic' joint and muscle reactions, indistinguishable from presenting complaints . . . in . . . (87.5% of 40) . . . subjects" (79).

Other presentations related food sensitivity in at least five patients with RA, including one in whom double-blind challenge studies implicated tartrazine. Many of these patients had circulating immune complexes containing IgE and IgG anti-IgE; it was speculated that these were related to the pathogenesis of symptoms (80). Hill (81) and Brostoff and coworkers (82) have reported, respectively, on patients with juvenile and adult rheumatoid-like arthritis with food sensitivities. Ratner and coworkers have suggested that lactose-intolerant individuals may be susceptible to milk-induced arthritis (83). Darlington and colleagues extended their earlier observations (78) and reported that some patients with RA benefited from food elimination and underwent symptomatic deterioration with reintroduction of offending foods (84). Golding (87) reported three patients who had recurrent joint pain, sometimes associated with swelling, precipitated by milk in two and by cheese and egg in one. Milk challenge in one subject caused knee swelling within a few hours; 30 mL fluid was aspirated and indicated synovitis. Additional observations suggested two patients had inflammatory spondyloarthritis associated with milk and wheat, respectively (91). These studies were not rigorously controlled, and they did not definitely prove that foods can exacerbate inflammatory joint disease (12, 24–26, 32).

Our own initial studies of effects of food on arthritis naively used a prescription diet (no red meat, fruit, dairy products, herbs, spices, preservatives, additives, or alcohol) and found that outpatients with long-standing, progressive, active RA fared no better than when receiving placebo diet (28). But some patients improved on the experimental diet and experienced recurrence of symptoms when deviating from it. A prospective, blinded, controlled trial in a clinical research center was then initiated to determine whether joint symptoms could be associated with food sensitivities in selected patients. The first patient, with RA, had noted exacerbation of symptoms associated with dairy products and other foods. She exhibited marked, consistent, subjective, and objective improvement during fasting, which was sustained with elemental nutrition. Four different, blinded challenges with milk reproducibly exacerbated symptoms whereas placebo and other foods were without effect. Symptoms peaked 24–48 hours after challenge and resolved over 1–3 days. Immunologic studies suggested the involvement of both delayed and immediate-type reactions, as indicated by cutaneous reactivity to milk, marked increase of anti-milk IgG and IgG4 levels, marginally increased IgG-milk circulating immune complexes, and in vitro cellular sensitivity to milk, but there was no elevation in anti-milk IgE. Symptomatic exacerbation of arthritis and immunologic hypersensitivity to milk coexisted in this patient (29).

In further studies, we noted that 30% of our patients with RA alleged food-related ("allergic") arthritis. Sixteen patients completed 19 double-blind, placebo-controlled (DBPC) food challenge studies, and three demonstrated subjective and objective rheumatologic symptoms following double-blind food challenges; they were virtually asymptomatic on elemental nutrition (30, 34). They were seronegative patients with palindromic symptoms and nonerosive disease. Fasting or elemental nutrition also benefited several of these patients (30, 34). Thus most patients alleging food-induced rheumatic symptoms did not show these on blinded challenge, but some did. We tentatively estimated that immunologic sensitivity to food was an uncommon cause of symptoms among rheumatic disease patients. Such patients have only been identified by controlled challenge studies. These observations suggest a possible role for food allergy in some patients with rheumatic disease. In related work, we and others have noted that substituting cow's milk for water led to inflammatory synovitis in certain rabbit strains (10, 31, 85). One study demonstrated that synovial membrane mast cells (MCs) can be activated by IgE- and non-IgE mediated stimuli (86).

Food Allergy and Vasculitis

Allergic vasculitis constitutes a relatively small group of the vasculitic syndromes. It affects the small blood vessels, mostly of the skin, to a lesser extent the mucous membranes, and occasionally the joints or other organs, and is often associated with fever. The skin lesions are usually in

the form of palpable purpura that can be large. In some patients, it may mimic urticaria or erythema multiforme. Unlike the common IgE-mediated urticaria, the lesions of urticarial vasculitis are usually non-blanching, non-pruritic, and persist for more than 24 hours. However, the differentiation is not always clear, which made some authors consider the two conditions as one disease continuum (95).

Food hypersensitivity has been implicated over the years in causing vasculitis in certain patients, mostly adults. The relationship did not receive wide acceptance, probably because of its rarity and because of the anecdotal nature of most of the reports.

Probably Osler in 1914 was the first to suggest a role of protein sensitivity in certain patients with purpura (96). In 1929, six cases of Henoch-Schönlein purpura were reported as being improved on avoidance of certain foods and recurred on reintroduction of those foods (97). In 1951, a patient was reported with recurrent purpura following eating crab meat; his platelet count was normal and his serum transferred passive hypersensitivity (Prausnitz-Küstner [PK] reaction) to crab (98). In 1953, Ackroyd reported 23 patients with allergic purpura related to specific foods, most common were egg, milk, chocolate, wheat, and beans (99). A few other patients had purpura related to fish (100, 101). Other authors recognized as well that food hypersensitivity might cause vasculitis (102, 103). In a few patients, azo dyes, particularly tartrazine, were implicated (104–107). Lunardi et al (108) described five patients who had allergy and vasculitis for 1–13 years in whom the offending agent was confirmed by double-blind challenge to foods in two, to food additives in two, and to both in one. While they were on an elimination diet, none had a recurrence of vasculitis during the 3-year follow-up.

We reported two children with well-documented severe food-induced vasculitis (109). The first was an 8-year-old girl with a 9-month history of recurrent palpable erythematous eruption and arthritis. A skin biopsy showed leukocytoclastic vasculitis. Her rheumatologic evaluation was negative. However, her serum IgE level was moderately elevated and she had positive skin prick test (SPT) and radioallergosorbent test (RAST) to cow's milk and egg. Her symptoms remitted following strict avoidance of these two foods and recurred following oral challenge tests. The second patient was a 23-month-old girl who at 15 months of age was admitted to the hospital because of a swollen knee and elbow, fever, skin rash, conjunctivitis, upper respiratory infection, and otitis media. She had a normal platelet count, coagulation studies, and urinalysis. She was diagnosed as having erythema multiforme secondary to infection. Improvement was substantial within 1 week of treatment with ampicillin and prednisone. Sudden relapse occurred four times within a few months' duration, with marked vasculitis of the skin, subconjunctional hemorrhage, large petechiae in the oral mucosa, gross rectal bleeding, and swollen joints. Her response to corticosteroid treatment was remarkable. Results of her hematologic and immunologic studies were normal and no circulating immune complexes were detected. Skin biopsy showed leukocytoclastic vasculitis. Possible food allergy was considered, and chocolate was the most suspected by the medical history. Skin tests with chocolate and other common foods were negative, however. A trial of oral cromolyn (50–100 mg three times a day), without strict dietary elimination, was associated with no recurrence for a few months. After discontinuation of cromolyn, a few recurrences occurred and were related to exposure to chocolate, which reinforced strict avoidance of that food.

From the limited information available in the literature and from our own experience, it seems that vasculitis, if caused by food allergy, is rare, even though it is probably underdiagnosed. The underlying immunologic mechanism appears to be predominantly a type III (immune complex or Arthus-type) reaction. In some cases, a type I (IgE-mediated) reaction may be involved as well, causing histamine release, increased vascular permeability, and enhanced deposition of immune complexes (110). Histologic examination of a biopsy specimen taken from the edge of a lesion will verify the presence of vessel wall necrosis, extravasation of red cells, a perivascular polymorphonuclear cellular infiltrate, fibrin deposits, and often nuclear debris, hence the name leukocytoclastic (111, 112). Immunofluorescence studies may demonstrate immune complex deposits in vessel walls along the basement membrane. The nature of the antigen and antibody involved sometimes can be determined. McCrory et al (113) reported a case of glomerulonephritis in which the renal biopsy showed immune complexes that incorporated bovine serum albumin (BSA). Dietary elimination of BSA markedly enhanced the response to pharmacotherapy.

Studies are certainly needed to discover such rare cases of food-induced vasculitis, to delineate its pathogenesis, and to develop simple reliable tests for its diagnosis.

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Unproven Diagnostic and Therapeutic Techniques

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Introduction

The process of diagnosing and treating allergic disease is complex and at times elusive. It requires a thorough history and physical examination and, in certain situations, complementary laboratory tests. Most of the tests performed today have undergone rigorous scientific evaluation for proof of effectiveness and safety. They must also have established physiological significance when used to diagnose a particular disease. Nevertheless, there are a growing number of unconventional, unproven, and inappropriate procedures used by some to diagnose allergic disease. Some of these "tests" are legitimate but are misused in their diagnosis of allergy. Others have no basis in the pathophysiology of allergic disease. In this chapter we will address several of these controversial procedures, including provocation, neutralization, cytotoxic testing, applied kinesiology, IgG levels, lymphocyte subsets, body chemical analysis, electrodiagnosis, urine autoinjection, and rotation diets among others. It is important that those practicing allergy and immunology become familiar with these "tests." They are generally unsuitable for allergy diagnosis for several reasons. First, many are based on unproven theories. Others are legitimate tests used inappropriately. Some procedures cannot diagnose any disease at all. It is apparent that standardization and controlled evaluation of procedures before their use is imperative for proper patient care. The following information is useful because an increasing number of patients seek "alternative" forms of therapy for a variety of diseases. These patients may present at the beginning of their search with a multitude of questions regarding these procedures, or they may present already having been subject to a number of unproven and often expensive procedures for questionable diagnoses.

Definitions

Standard practice is that which is performed by the majority of physicians today. It encompasses procedures and treatments that have been scientifically proven to be effective and safe. Before describing and critiquing the following procedures and therapies, it is important that we first categorize them. First there are those that are "unproven." These types of tests or treatments are those that are not based on known allergy pathophysiology, and in our judgment their effectiveness is not supported by adequate scientific evidence. Although they appear well constructed, they do not appear capable of diagnosing nor treating allergic disease. Some of the procedures listed have been adapted from proven methods that are currently available in the diagnosis and treatment of allergic disease. One reason that these tests may not have been examined scientifically is that their methodology is vague and often difficult to reproduce. Other procedures are categorized as being "inappropriate," meaning that the test itself is a validated test used to diagnose certain conditions, but in the diagnosis of allergic disease they are not helpful.

"Controversial" Tests

Skin Endpoint Titration

During the 1940s, Rinkel (1) developed the method of endpoint skin testing. He found that this method was a useful guide in determining a patient's sensitivity, and the information found could be used to determine a safe and effective dose for immunotherapy. Variations of this method have been used for both diagnosis and treatment of inhalant and food allergies.

Method: The procedure usually involves intradermal testing with fivefold serial dilutions of extract. A 7-mm whealing response is considered reactive. The endpoint is defined as the weakest dilution that produces a positive skin reaction and initiates a progressive increase in the diameter of the wheals with each stronger dilution tested (2). The optimal starting dose is thought to be 0.01–0.02 mL of extract. The optimal therapeutic dose, defined as the dose at which symptoms are controlled on immunotherapy, is reached after the endpoint dilution is given weekly in increasing increments. Rinkel anticipated relief of patient symptoms at a dose of 0.5 mL of the endpoint dilution.

Conclusion: There have been several trials over the years that looked at the efficacy of the Rinkel method. Van Metre et al published several studies that supported the Rinkel method in quantifying skin sensitivity to ragweed pollen, and found the method comparable with in vitro leukocyte histamine release and radioallergosorbent test (RAST) (3). Although variations of this skin test method are practiced today without any risks, using the results to determine optimal dosing of immunotherapy is controversial. Most of the time this "dose" may be an underestimate that results in ineffective treatment.

Unproven Tests

Applied Kinesiology

Kinesiology refers to the science of motion techniques. Some believe that certain diseases, including allergic reactions, may cause a weakening of skeletal musculature, and that by using applied kinesiology one may diagnose allergic disease—most commonly food allergy.

Method: Allergens to be tested are placed in stoppered glass bottles. In some cases a glass vial containing a specific allergen is placed on or near the body of the patient, or in other cases, the patient is asked to hold the vial. During allergen "exposure," muscle strength is tested. A positive test is indicated by observed weakening in muscle strength. Variations to the standard test include "surrogate" testing in which a relative of the patient undergoes testing for the patient. This form of testing is commonly seen.

Conclusion: In 1988, Garrow (4) published a blinded and open challenge study of allergen using applied kinesiology to look at the reproducibility and efficacy of the test. The study showed no significant difference between frequency of positive reactions to placebo vs allergen. Therefore, at this time, we are aware of no proof of the efficacy or reproducibility of the method of kinesiology in diagnosing food allergy.

Provocation Testing and Neutralization

Provocation of an allergic reaction by delivering allergen via the transdermal, subcutaneous, intradermal, or bronchial route are a daily part of an allergy practice. These "challenges" provide a wealth of information in the diagnosis of several allergic diseases. These tests include: prick and intradermal skin tests, intranasal, subconjunctival, oral tests, and methacholine challenge. These tests differ from the controversial provocative testing and neutralization, in that they have undergone validation in those studies with both patients and normal controls.

The provocation-neutralization method was introduced by Lee in 1961 for the diagnosis of food allergy (5). Provocation is performed by intradermal, subcutaneous, or sublingual routes. It is currently used to diagnose and treat allergic disease and sensitivities to a wide variety of substances. The items tested are not necessarily those suspected by the history. They can include such chemicals as formaldehyde, phenol, ethanol, and hormones such as progesterone (6).

Method: The patient is given an intradermal or subcutaneous dose of allergen extract using fivefold serial dilutions (Rinkel method). The patient is observed for 10 minutes and any symptoms are recorded. If the patient remains symptom free

then increasing doses of extract are given until symptoms do occur. Once these symptoms occur, the patient is immediately given injections of weaker dilutions of the same extract until symptoms are resolved. This amount of extract is considered the "neutralizing dose" and is then used for future treatment (7).

The technique appears vague and imprecise. There also appears no established protocol for performing the provocative testing and neutralization. In addition, no general consensus exists that defines a positive test. Symptoms are quite extensive and nonspecific and include headache, nasal symptoms, chest symptoms, ear reactions, gastrointestinal (GI) reactions, skin eruptions or itching, or general reactions such as fatigue, chills, muscle pain, or drowsiness (8). There has been no general agreement on the role of wheal diameter in reporting a positive test. Some interpret an increase in wheal size as a further indication of a positive test.

Sublingual provocation testing and neutralization has been advocated by some in the diagnosis and treatment of food allergy. It was first described by Hansel in 1953 (9) as a diagnostic and therapeutic technique. The method consists of placing allergenic extract underneath the tongue and waiting 10 minutes for the appearance of symptoms. If symptoms occur, the patient is given a more dilute solution of the same extract. The neutralizing dose is used as treatment before or after eating meals containing the offending food if the food cannot be avoided.

Given the fact that a single item needs to be tested one at a time and requires waiting 10 minutes between each dilution, it comes as no surprise that a complete provocative-neutralization for a single item may take some time. Testing multiple items may take days. Therefore, this test may be time consuming and can be costly.

Conclusion: About 15 studies have looked primarily at the efficacy of provocative testing and neutralization. Eight of these studies were double blinded. Only one study contained a control group. The majority of the studies were not able to demonstrate any benefit from neutralizing solution compared with placebo. Crawford et al (10) performed a double-blind study in 61 subjects with a history of reactions to five common foods. The authors were unable to reproduce results from sublingual food testing. Kailin and Collier (11), in a double-blind study, compared neutralizing effects of sublingual or subcutaneous food extracts against saline placebo. The authors found that in 70% of patients, treatment with saline placebo was "relieving." In a study of 121 patients with inhalant allergy, Draper (12) found that only 38% of positive provocation tests correlated with a positive food challenge test. One of the best-structured double-blind studies was that by Jewett et al (13). This study of 18 patients with symptoms previously provoked by intracutaneous testing were tested with food extracts or placebo. The rate of positive responses to food extracts was similar to placebo.

In conclusion, these studies do not validate the efficacy of provocation testing and neutralization in the diagnosis and treatment of allergic disease.

Neutralization Therapy

Neutralization of allergic symptoms is an extension of provocation-neutralization testing. This type of treatment, also called "relieving therapy," consists of self-administered doses of allergen extract at a concentration that "neutralized" symptoms provoked during the prior provocation testing (6). This treatment might be used to relieve present symptoms, to prevent anticipated symptoms, or for continuous maintenance doses twice weekly. The doses can be given either by injection or sublingually. Patients can change and discontinue or restart treatment as they deem necessary.

Theory: A number of theories have been brought forth to explain neutralization of symptoms. A common belief among some practitioners is that this type of therapy induces immunologic tolerance. Controlled, double-blind, multicenter studies report that sublingual, provocative food testing did not discriminate between placebo controls and food extracts used in neutralization therapy (8). In addition, no long- or short-term studies have evaluated the efficacy of this therapy.

Conclusion: Because there is no known mechanism for neutralization of symptoms and yet no scientific evidence demonstrating its effectiveness, we do not recommend this form of therapy in the treatment of allergic conditions such as food allergy.

Cytotoxic Leukocyte Testing

Also known as "Bryan's" test, this form of allergy testing was adapted by Bryan in the 1960s. Initially designed to aid physicians in diagnosing allergy, the theory behind the test is that the addi-

tion of specific allergen in vitro to whole blood or to serum leukocyte suspension will reduce the white blood cell count or result in the death of leukocytes. It has been said by some to be useful for the diagnosis of both food and inhalant allergy (14).

Method: Buffy coat is collected from a drop of patient's blood and placed on a microscope slide coated with dried extract of food or other allergen/substance. It is then observed microscopically for alterations in the appearance of the white blood cells (15). Once a fair number of white cells have been located, they are rated for degree of destruction. A single sample of blood can be tested to a panel of foods and other substances.

Conclusion: There appears no theoretical basis for the cytotoxic test. The test itself is generally not standardized and has not been convincingly shown in controlled trials to be effective in the diagnosis of food or inhalant allergy. Franklin and Lowell (16) reported no significant difference in white blood cell counts in blood exposed to ragweed extract vs saline in ragweed-sensitive individuals. Lieberman et al (17) could not demonstrate clinical correlation with test results in study patients and found inconsistent results when patients were tested more than once. Benson and Arkins (18) found the test was associated with a high degree of false positives.

Electrodiagnosis

Some practitioners believe that the presence of specific allergy can lead to a change in the electrical potential of the skin.

Method: In this procedure a sample of food extract is placed in a container in contact with an aluminum plate. This is then placed between the skin of the patient and a galvanometer. Electrical activity of the skin is measured at certain "allergy points." For example, certain points on the lower extremities are thought to correspond to food allergy, and points on the upper extremities that correspond to inhalant allergies (14). These results are entered into a computer that prints a list of allergies for the patient.

Conclusion: This type of procedure appeals to some patients who are reluctant to undergo skin testing. Also, the use of computers, galvanometers and "print outs" appear "state of the art" to some patients. However, there are no recognized studies that demonstrate the efficacy of electrodermal diagnosis.

Body Chemical Analysis

Some practitioners believe that detection of any amount of inorganic or organic chemical in body fluid may indicate a toxic exposure and can explain the presence of disease. They postulate that certain substances may be toxic to the immune system and lead to a state of sensitivity to the environment (1). Some of these substances include vitamins, drugs, chlorinated hydrocarbons, volatile organic chemicals, pesticides, and metals.

Method: Specific tests include gas chromatographic mass spectrophotometry analysis of body fluids and tissue, quantitation of chemicals in serum and other body fluids, and breath analysis (2).

Conclusion: These procedures are highly sensitive and are able to identify chemicals in almost every individual, even those who do not report symptoms. This is why a strong clinical correlation is important in conjunction with this type of testing. In certain situations, and in certain individuals, it may be appropriate to evaluate for chemical poisoning to properly diagnose a disorder. It is important to note that some of the labs performing these tests may have quality assurance deficiencies so, for example, contamination of samples remains a major source of error (2).

Inappropriate Tests

IgG Antibodies

Immunoglobulin E antibody in response to allergens cause the release of mast cell (MC) mediators, which are important in the immediate-type symptoms of anaphylaxis or atopic disease. Sensitivities to certain allergens can be diagnosed by detecting IgE in the serum by RAST. Many laboratories can test in a similar fashion for the presence of IgG to certain foods. Some practitioners measure circulating IgG antibody reactive to food antigens in diagnosing food allergy. It is their belief that, although IgG may not be important in the immediate-type reactions to certain foods, it may be more important in delayed-type reactions such

as depression, apathy, fatigue, myalgias, and vague GI complaints (19). Diagnosis of delayed-type reactions is challenging and, although conventional IgE RAST alone cannot diagnose these types of reactions, no double-blind, placebo-controlled (DBPC) studies have been published that demonstrate a relationship of these symptoms to particular foods.

Based on current knowledge, IgG antibodies do not appear to play a role in the pathogenesis of atopic disease and food allergy. Low levels of IgG to food antigens as well as to other environmental antigens can be found normally, and their presence has not been shown to be associated with atopic disease. Therefore, it is has not been recommended as a form of diagnosing food allergy in the clinical setting. In fact, a method for diagnostic challenges in patients whose symptoms are subjective can be found in Chapter 39.

Lymphocyte Subset Counts

Quantitation of leukocytes bearing one or more surface markers known as clusters of differentiation (CD markers) is helpful in the diagnosis of some forms of lymphocyte cellular immunodeficiencies. For example, measuring CD4 lymphocytes is part of the standard procedure for diagnosis and management in human immunodeficiency virus (1). Lymphocyte subset counts are labile and nonspecific. Levels may not be elevated in traditional allergic diseases but may be elevated in individuals with fatigue, depression, or viral illnesses. Use of these tests to diagnose forms of allergy or other presumed immunological disorders has not been proven to be of value and may lead to inappropriate treatment of the patient.

Pulse Test

Coca in 1953 reported that tachycardia occurring 5–90 minutes after exposure to a food or inhaled material is a reliable indicator of food allergy (20).

Method: The test dose can be given by any route including injection. A change of 10 beats per minute is thought to be diagnostic by some, but the procedure has never been standardized. This test seems remote to the pathogenesis of allergic disease.

Unproven Therapy

Neutralization Therapy

This topic is discussed earlier in the section entitled "Provocation Testing and Neutralization."

Rotation Diets

Theory: This type of diet recommends that a certain food not be eaten more than once every 4–5 days (2). If the patient is allergic to most or all foods, by eating them frequently he or she theoretically risks becoming increasingly sensitized to that food and possibly other foods.

Conclusion: If a patient does have clinical sensitivity to a particular food, then he or she will develop symptoms after contact with that food, irrespective of rotation schedule. However, if a patient demonstrates "subclinical sensitivity" to a certain food, i.e., no symptoms but evidence of specific IgE by testing, then each exposure to that food will increase sensitivity and likelihood of a future reaction. There is no scientific data supporting the efficacy of this type of diet.

Orthomolecular Therapy

Theory: This approach is based on the use of supplements and/or vitamins administered in large quantities either parenterally or orally to treat numerous medical and psychiatric conditions (2). Practitioners measure levels of vitamins in the serum or urine to determine the amount needed for correction. This type of therapy has been used in a wide variety of diseases. For example, antioxidant supplements such as vitamin E, vitamin C, and glutathione have been used to treat allergic disease based on the theory that allergic inflammation generates free radicals that can cause oxidative damage to tissues (21).

Conclusion: No controlled studies have evaluated this type of therapy. It is not recommended for treatment of disease at this time. Large doses of certain vitamins can accumulate in the body and lead to toxic effects.

Mercury Amalgam Removal

Theory: Silver-mercury amalgam has been used in dental fillings for over 100 years. There have been many claims from physicians and dentists that certain patients may develop a sensitivity to this material. Subsequently, it has been blamed for a wide array of symptoms (2). These claims have led some to remove these type of fillings.

Conclusion: There is no clear scientific basis for the claims that mercury amalgam is responsible for the development of a multiplicity of somatic complaints.

Urine Auto-injections

In 1930, Oriel and Barber reportedly found protein-like substances in the urine of allergic individuals during acute exacerbations of allergic disease (22). Urine obtained from sensitive individuals applied intradermally to those individuals with the same sensitivities resulted in a positive skin test. This was not the case for the same urine applied intradermally in a nonatopic individual (19). These practitioners felt that these "urine proteins" can be isolated by chemical extraction and be given to the patient as a form of therapy in a series of intradermal or subcutaneous injections.

In 1947, the procedure was reintroduced by Plesch (23). He described a system of collecting fresh urine from a patient, and after sterilizing it, injecting set amounts intramuscularly. Various reactions would occur within hours of injection and include: fever, diarrhea, hypotension, shortness of breath, and vomiting. He reported that by performing these injections in patients with various syndromes such as jaundice, allergic disease, and GI and dermatological symptoms, there was a decrease in symptoms. There are, however, no controlled studies to support the efficacy nor the safety of the procedure. In fact, in rabbits, urine auto-injection may lead to the formation of auto-antibodies to glomerular basement membrane (GBM) and result in nephritis. Although this has not been demonstrated directly in humans, it is possible that receiving these urine auto-injections could induce immune complex disease. In humans, anti-GBM antibodies can lead to the development of Goodpasture's syndrome. Therefore, at this time, the American Academy of Allergy, Asthma and Immunology has reported that this procedure is unproven, without scientific basis, and potentially dangerous (14).

Clinical Ecology

Clinical ecology is generally based on two concepts. One is that a large number of chemicals and foods can be responsible for illness in the absence of abnormal laboratory tests and physical findings; and the other is that the immune system is functionally depressed as a result of exposure to certain chemicals in the environment (24). This is not to be confused with toxic illnesses that produce a number of symptoms and abnormal laboratory tests in response to a particular toxin. Those who practice clinical ecology believe that patients with chemical hypersensitivity syndrome, also known as environmental hypersensitivity disorder, twentieth century disease, or induced immune dysregulation syndrome have symptoms which are a result of low-level, long-term exposure to environmental chemicals. The doses that cause these syndromes are said to be far below those known to cause harmful effects in the general population (25). The agents are sometimes referred to as "incitants" or "offenders," and they include foods, food additives, and synthetic and natural chemicals such as pesticides, detergents, perfumes, vehicle exhaust, and natural gas. Symptoms are often generalized and affect more than one organ system, including cardiac, GI, respiratory, genitourinary, and neurological.

Theory: Clinical ecologists such as Randolph (26) have theorized that environmental illness is a result of sensitivity to novel synthetic chemicals. Others believe that these chemicals act as haptens in inducing IgG and immune complex formation (27). Environmental illness has also been thought to result from a nonspecific autoimmune process. A possible mechanism for this disease process has not been established, but several concepts are used by clinical ecologists to account for patient symptoms. "Total body load" and "chemical overload" draw an analogy between the immune system and a container. The immune system has a limited capacity for handling antigens. Once a patient develops symptoms in response to an environmental antigen, this indicates that the immune system capacity has been exceeded. "Masking" is a concept in which a patient who is sensitive to a certain food may eliminate symptoms by eating

the food on a regular basis. "Spreading phenomenon" refers to sensitivity to one antigen leading to the development of sensitivity to multiple other antigens (2).

Diagnosis: A detailed history and provocation-neutralization testing remains the mainstay of diagnosing environmental illness by clinical ecologists. Occasionally blood tests for immunoglobulin, complement, or specific chemical levels are used to aid in diagnosis.

Treatment: Treatment consists mainly of avoidance, elimination diets, neutralization therapy, and in some cases, as in *Candida* hypersensitivity syndrome, drug therapy.

Anti-Candida Drugs for Candida Hypersensitivity Syndrome

Theory: Candida albicans is a yeast that is part of the body's normal flora. Some people believe that this organism causes a condition termed "yeast hypersensitivity syndrome" or "*Candida* hypersensitivity syndrome." Proponents of this hypothesis believe that the syndrome is caused by an overgrowth of *C. albicans* in the GI tract that causes local inflammation as well as a more generalized toxic response. This response is thought to be secondary to a hypersensitivity reaction to the toxin, which the organism secretes. As a result, symptoms range from recurrent or persistent candidal infections, or chronic GI symptoms such as bloating, diarrhea, constipation, and heartburn. Central nervous system symptoms have also been reported including depression, chronic fatigue, and memory problems (2).

Methods: There is no established method of diagnosing this syndrome. Diagnosis is most commonly made by history alone and not by specific laboratory measures. Some practitioners perform allergy testing to document sensitivity to *Candida.*

Treatment: Patients are first warned to avoid broad-spectrum antibiotics and systemic steroids because these medications may potentiate *Candida.* They are given minute doses of oral nystatin until symptoms have resolved. If symptoms persist, treatment can be changed to another anti-candidal drug such as ketoconazole or amphotericin B. In addition to anti-candidal drugs, patients are also started on yeast-free, sugar-free diets. It is thought by some that eating simple sugars causes an increase in growth of *Candida* in the gut (28). *Candida* allergy shots are also included in the treatment regimen of some patients.

Conclusion: Books and lay press articles have been published, support groups have been formed, all in the hopes of establishing a "yeast connection." However, a scientific basis for this syndrome has never been satisfactorily established. The reports that do circulate are largely anecdotal. In 1990, Dismukes et al (29), published the first randomized, double-blind, crossover study looking specifically at the effect of treatment with oral and vaginal nystatin compared with placebo in 42 premenopausal women presumed to have *Candida* hypersensitivity syndrome. Results from their work showed that although nystatin therapy did reduce vaginal symptoms, the efficacy of treatment for systemic symptoms including depression and chronic fatigue was not established. Systemic symptoms were not reduced significantly compared with placebo. Therefore, the study could not establish a therapeutic benefit of nystatin therapy in a patient with *Candida* hypersensitivity syndrome.

Elimination Diets

Theory: The elimination of multiple foods has been recommended by some practitioners when multiple food allergies have been discovered upon skin testing. This type of diet is also recommended by others who believe that through elimination diets one may "boost" the immune system (2).

Methods: Patients are then diagnosed with sensitivity to multiple foods by unconventional testing or perhaps history. They are placed on highly restrictive diets to prevent further symptoms. Most of the time patients are given supplements of vitamins, minerals, or amino acids (1).

Conclusion: There is no evidence that by eliminating multiple foods one may improve the functioning of the immune system. In fact, placing patients on such restrictive diets may lead to harmful effects from malnutrition.

Multiple Chemical Sensitivity Syndrome

Theory: Multiple Chemical Sensitivity (MCS) syndrome, or Idiopathic Environmental Intolerances (IEI), as suggested by the WHO/IPCS workshop in 1996, has been used to describe a constellation of symptoms that overlap with those of environmental illness but overall is a distinct entity. This disorder is characterized by a wide variety of symptoms including somatic, cognitive, and affective symptoms, caused by low level exposure to environmental chemicals (30). Symptoms commonly involve almost every major organ system and are thought to result from a sensitivity to certain chemicals. Chronic fatigue, depression, headache, and dizziness are commonly reported symptoms. Little is known about the pathophysiology of this condition, but its proponents claim that through certain mechanisms, such as disruption of immunologic or allergy processes, alterations in nervous system function, changes in biochemical pathways, or changes in neurobehavioral function, chemicals cause tissue damage (31). This may be accomplished through processes such as free radical generation, immune complex formation, or hapten formation.

Methods: Patients with this condition manifest certain psychological features such as anxiety, depression, somatization, conversion, and phobia (2). This makes it especially challenging in establishing a diagnosis of MCS. The diagnosis of MCS can be made if symptoms cannot be explained by abnormal tests but are associated with a documented environmental exposure. The lack of objective findings of disease, such as physical exam and laboratory tests, cast doubt on the validity of MCS as a clinical disease.

Critique: The concept of MCSs in the absence of objective data remains its advocates' greatest challenge. At the present time, there is no convincing scientific evidence that MCS should be regarded as a clinical entity, but rather an association of a wide range of symptoms to a particular or varied number of varied environmental chemicals.

Conclusion

Many of subspecialty groups, including the American Academy of Allergy, Asthma and Immunology and the American College of Physicians, have issued position papers on several of the procedures and therapies discussed above. It was the goal of this chapter to provide definitions of controversial, unproven, or inappropriate procedures and treatments, and to provide examples of each so that it might provide insight into remote practices of allergy. In our judgment, there is no convincing scientific basis for any of the treatments, conditions, or procedures discussed. By examining each theory and method, we can become more aware of the importance of scientific evidence and standardization of procedures in our daily practice. The history, physical exam, selective skin tests, and appropriate laboratory tests remain the standard of care in first evaluating the allergy patient. However, as we have seen, this may not always happen. Patients may be asked to undergo rigorous, expensive, invalidated, and even painful testing. They may be given diagnoses and treatments, which may lead to both physical and mental deterioration. We have also seen that many "validated" tests can be misused to diagnose allergic disease. Many supporters of controversial procedures have implied that they have been clinically proven and accepted. Therefore, it becomes the responsibility of physicians to educate patients to educate themselves about such practices and to make informed decisions concerning their use. It also becomes our responsibility to design proper clinical trials to definitively establish the merit or failure of these tests.

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Future Approaches to Therapy

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Introduction

Food allergy affects about 2% of the US population, appears to be increasing, and is now the most frequent cause of anaphylactic reactions treated in emergency departments in westernized countries (1–4). Currently, strict avoidance of food allergens and ready access to self-injectable epinephrine is the "standard of care" for food allergy (5). However, up to one half of patients experience an accidental ingestion and reaction every 3–5 years (6), and food allergy is the single leading cause of anaphylaxis outside of the hospital setting (7). Earlier reports of "desensitizing" patients with subcutaneous injections of food extracts were poorly controlled (8). Likewise, early reports of oral desensitization were not well controlled. More recently, Patriarca et al (9) reported the successful "desensitization" of 12 children to several foods utilizing an oral desensitization protocol, but no post-desensitization food challenges were performed to confirm loss of reactivity. In 1997, Nelson and coworkers (10) published results of a trial of subcutaneous "rush" immunotherapy for the treatment of peanut-allergic patients. In this well-controlled, double-blind placebo-controlled (DBPC) trial of peanut immunotherapy, subjects underwent DBPC food challenges (DBPCFCs) before and after the "desensitization" protocol. Although 4 of 6 treated patients demonstrated increased tolerance to peanut on the second challenge (and there was no change in the placebo group), the maintenance dose had to be decreased in two cases (with a concomitant decrease in tolerance) because of frequent systemic reactions. Given the partial rate of response and high rate of adverse reactions (> 30%) to this more traditional form of immunotherapy, the authors concluded that standard immunotherapy was not likely to be acceptable for the treatment of patients with peanut-induced anaphylaxis.

The generation of allergen-specific IgE antibodies that bind to high affinity receptors (FcεRI) on the surface of mast cells (MCs) and basophils is central to the immunopathology of food allergy and other atopic disorders. In patients with food hypersensitivity, re-exposure to the relevant foods triggers degranulation of MCs and basophils, resulting in the release of histamine and other mediators, which provoke anaphylaxis. As in other allergies, food allergy is mediated by a Th2-type immunologic response. The "hygiene hypothesis" postulates that reduced pathogen exposure during infancy in modern westernized environments influence maturation of the infant immune system from a Th2 to a Th1 type of immune response; the loss of this influence has presumably promoted the recent epidemic of extrinsic asthma and other allergic diseases (11). This knowledge has led to a number of novel immunotherapeutic strategies for the treatment of food allergy aimed at re-establishing the balance between Th1 and Th2 responses or at blocking IgE-MC interaction. In addition, probiotics, which involve colonizing the intestinal tract with certain bacteria to reduce allergen sensitization, and Chinese herbal medicines, which exhibit anti-allergy properties, are beginning to be investigated as alternative treatments of food allergy.

Given the inability to study potential therapies in patients with food-induced anaphylactic reactions, animal models have been developed to test these potential therapies. We developed murine models of IgE-mediated cow's milk hypersensitiv-

ity (12) and peanut anaphylaxis (13) using an oral sensitization protocol and oral challenge. Following oral food challenge, these mice experience symptoms and physiologic changes, and T and B cell responses to food allergens, that resemble those seen in food-allergic patients (14). These animal models are extremely useful for investigating novel therapeutic approaches for food allergy. Several novel immunotherapeutic strategies are being examined as treatment modalities for food allergy: 1) humanized anti-IgE monoclonal antibody therapy; 2) "engineered" (mutated) allergen protein immunotherapy; 3) antigen-immunostimulatory sequence-modulated immunotherapy; 4) peptide immunotherapy; 5) plasmid-DNA immunotherapy; and 6) cytokine-modulated immunotherapy. These approaches will be reviewed in this chapter.

Humanized, Monoclonal Anti-IgE Antibodies

In atopic and food-allergic patients, the generation of allergen-specific IgE antibodies that bind to FcεRI on the surface of MCs and basophils is central to the immunopathology of these disorders. Humanized, recombinant monoclonal anti-IgE antibodies have been developed that bind to the third domain of the Fc region of IgE molecules, which then prevents these molecules from binding to FcεRIs or low-affinity receptors (FcεRIIs) (Fig. 42–1). A number of clinical trials utilizing these antibodies have reported symptomatic improvement in patients with allergic asthma and rhinitis when circulating levels of IgE antibodies are markedly reduced (15–19). In addition, it has been found that basophil histamine releasibility and FcεRI expression are down-regulated during anti-IgE therapy (20). In a recent multicenter trial, patients with peanut anaphylaxis were treated with humanized anti-IgE antibody therapy (21). The quantity of peanut necessary to provoke an allergic reaction was established by DBPCFC, then patients were treated with four subcutaneous injections of recombinant IgG anti-IgE, at doses of 150 mg, 300 mg or 450 mg, or placebo at monthly intervals. Two weeks after the final injection, patients underwent a second peanut challenge. Patients treated with the 450 mg dose demonstrated significantly increased thresholds of sensitivity to peanut by oral food challenge, increasing from an initial challenge threshold dose of 178 mg, or about one half of a peanut, to 2805 mg, or about 9 peanuts. Further studies are under way, but this response to therapy should translate into at least partial protection for most unintentional peanut ingestions. Furthermore, this beneficial effect should be nonspecific and decrease responsiveness to all forms of allergens, i.e., foods, pollens, animal danders, etc.

"Engineered" (Mutated) Allergenic Protein Immunotherapy

Immunologic tolerance is believed to be induced by redirecting the T cell immune response from a Th2 to a Th1 type response (22, 23). In collaboration with Drs. Burks and Bannon, we have developed engineered major allergenic proteins to eliminate IgE binding but retain T cell proliferation. We have isolated and purified the three major peanut proteins Ara h1, Ara h2, and Ara h3; isolated, sequenced, and cloned the DNA that codes for the synthesis of these proteins; and mapped the IgE binding epitopes. With this information, these proteins have been "engineered" to eliminate the IgE binding epitopes. Using site-directed mutagenesis of the allergen cDNA clones, followed by recombinant production of the modified allergen, the engineered DNA codes for proteins that differ by a single amino acid within each of the IgE-binding epitopes (Fig. 42–2A). Ara h1 is a vicilin storage protein that is 626 amino acids in length and has 23 IgE-binding sites (epitopes), Ara h2 is a conglutin storage protein that is 156 amino acids in length and has 10 IgE-binding sites, and Ara h3 is a glycinin storage protein that is 510 amino acids in length and has four IgE-binding sites. The engineered recombinant proteins, modified (m) Ara h1 and mAra h2, bound virtually no IgE antibodies from peanut-allergic patients but promoted comparable T cell proliferation as the native peanut proteins, native (n) Ara h1 and nAra h2 (24). We hypothesized that these engineered proteins would not provoke any allergic reaction when injected in peanut-sensitized mice, but would be able to down-regulate the allergic response, i.e., "desensitize" the mice. As suggested by in vitro studies with Bet v1 and Phl p b5 (25, 26), studies in our peanut-anaphylaxis mouse model suggested that this is true. Following our standard 3-week sensitization protocol with Ara h2 and cholera toxin, mice received three doses per week of either native or engineered Ara h2 for 4 weeks. Mice were sensitized with whole peanut and then desensitized by intranasal administra-

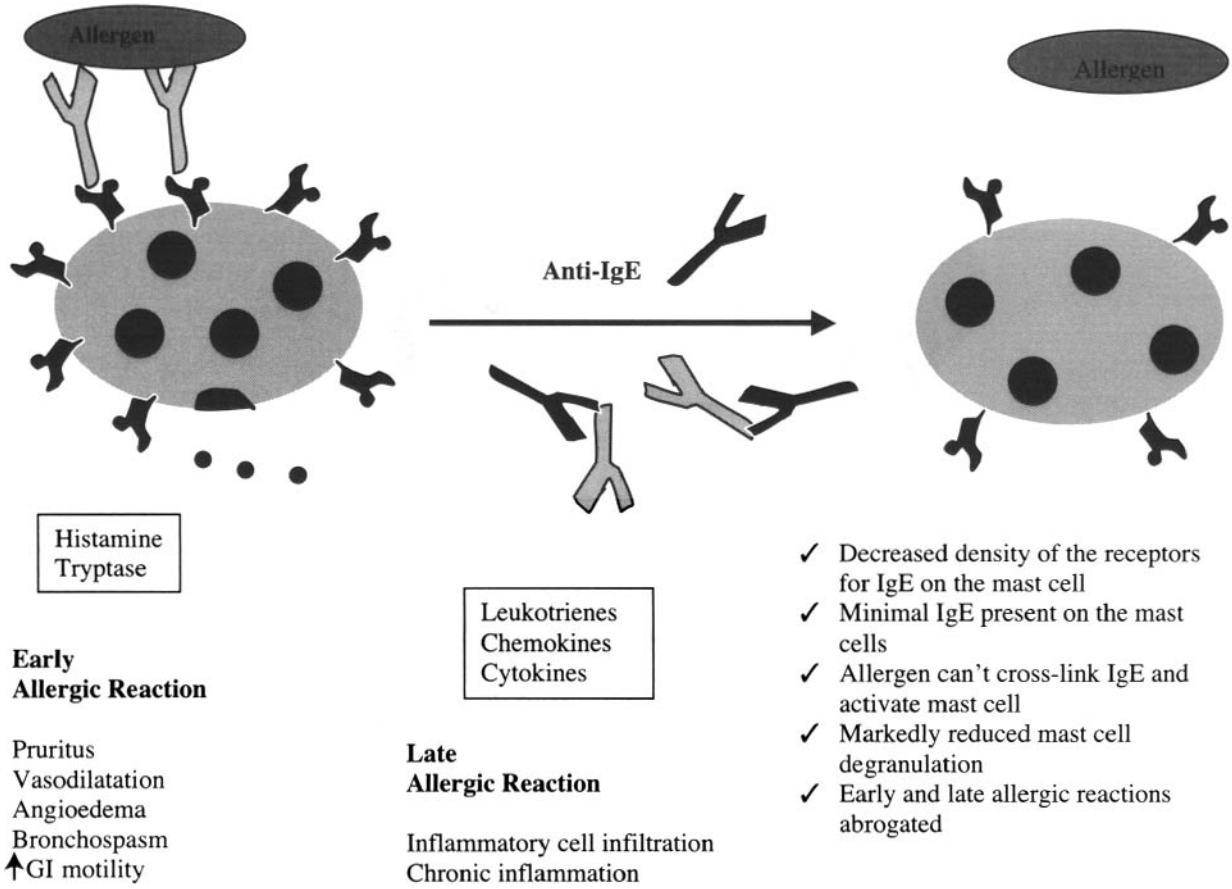
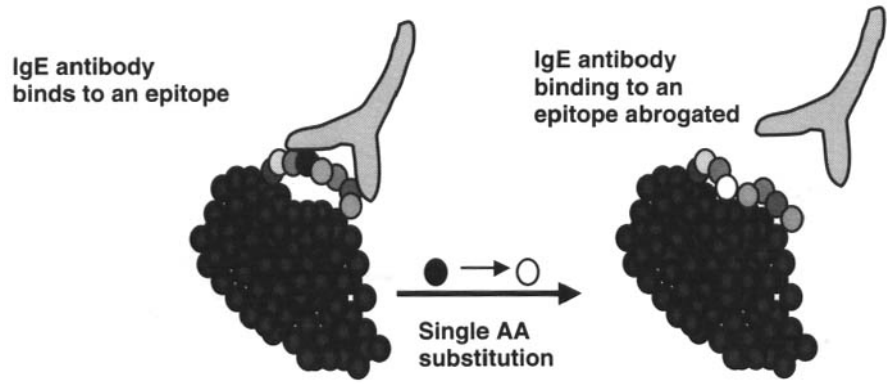


Figure 42–1. Anti-IgE therapy. Humanized anti-IgE antibodies bind to the Fcε portion of "free" IgE preventing it from binding to the Fcε receptor, which eliminates the "trigger" mechanism for activating mast cells and basophils when allergen is encountered.

tion of modified Ara h 2 (three doses per week for 4 weeks). According to unpublished results of one of us (Li), desensitization with the modified Ara h 2 proteins resulted in reduced amounts of serum Ara h2-specific IgE and significantly lowered anaphylaxis scores when compared to controls. In subsequent studies, mice sensitized to whole peanut by the oral administration of peanut extract plus cholera toxin have been successfully "desensitized" with the nasal, subcutaneous, and rectal administration of engineered Ara h1–3. Because recombinant proteins are generated in *Escherichia coli* and bacteria and bacterial DNA that favor Th1 responses, we hypothesized that administering heat-killed *E. coli* containing the purified recombinant Ara h1–3 proteins may be more effective than administering modified Ara h1–3 proteins alone. Several investigators have shown that the use of antigen-linked cytokines (e.g., IL-12, IL-18) (27) or heat-killed *Listeria monocytogenes* (28) could induce deviation of antigen-specific Th2 responses to Th1 responses. Subsequent experiments demonstrated that administering heat-killed *E. coli* containing the modified Ara h1–3 proteins (HKE-Ara h1–3) subcutaneously or per rectum were more effective at "desensitizing" peanut-allergic mice, as determined by post-challenge clinical score, body temperature, airway response, and plasma histamine, than administering modified Ara h1–3 alone in a similar fashion. In addition to administering the heat-killed *E. coli* into an environment replete with *E. coli* and other bacterial organisms, the rectal route of HKE-Ara h1–3 vaccine appeared to require fewer and smaller doses of the engineered protein than the vaccine administered by the subcutaneous route, according to the unpublished results of Li et al. These studies suggest that, in the future, peanut-allergic patients may be able to be successfully "desensitized" utilizing a suppository form of HKE-Ara h1–3.

A. By substituting a single amino acid within the IgE-binding epitope, the IgE molecule no longer recognizes the protein and does not bind.



B. By use of overlapping peptides spanning the length of a protein, all relevant T cell epitopes are represented and can "down regulate" the allergic response.

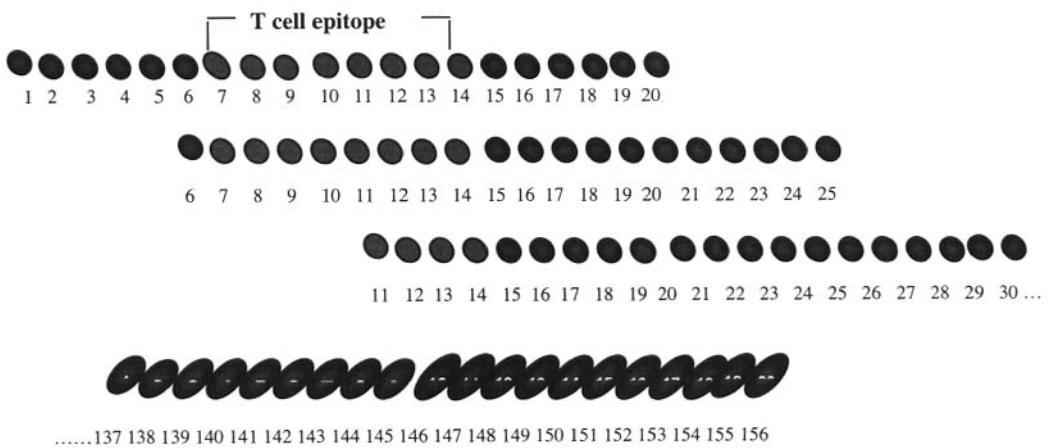


Figure 42–2. Modified recombinant allergens and peptide immunotherapy.

Overlapping Peptides

The use of overlapping peptides has also been investigated as an alternative approach to desensitize peanut-allergic mice. Larche and co-workers have utilized overlapping peptides representing the major cat allergen, Fel d1, to treat cat-allergic patients with asthma (29). They showed that this approach may be a safe and more effective way to "desensitize" allergic patients (30, 31). Vaccines composed of small peptides (10–20mers) that represent the entire length of the major peanut proteins were generated (Fig. 42–2B). For example, for Ara h2, we have generated thirty 20-mers that overlap by 15 amino acids. In this way, antigen presenting cells (APCs) are provided with all the possible T cell binding epitopes, but MCs are not activated because these peptides are unable to crosslink two IgE molecules. Preliminary, unpublished studies in our anaphylactic mouse model show that desensitization with a 20mer Ara h2 peptide mixture (three doses per week for 4 weeks) reduced Ara h2-specific IgE, significantly lowered plasma histamine levels and anaphylaxis scores, and increased interferon-gamma (IFN-γ) production by spleen cells when compared to controls. Although this method has a number of theoretical advantages, the practicality of validating the contents of vaccine preparations containing dozens of peptide fragments representing multiple aller-

genic proteins makes this approach less attractive for the treatment of food allergy in man.

DNA-Based Therapies

Plasmid DNA-Based Immunotherapy

Plasmid DNA-based immunotherapy (DNA vaccine) is a method of generating immune responses by immunizing with bacterial plasmid DNA (pDNA) that encodes specific antigens. DNA vaccination can induce prolonged humoral and cellular immune responses, and is of particular interest in the treatment of allergy because it induces a Th1 response (32) that is attributed to immunostimulatory sequences (ISS) consisting of unmethylated cytosine and guanine motifs (CpG motifs) in the pDNA backbone (33).

Hsu et al (34) reported that intramuscular injection of Brown Norway rats with pDNA encoding house dust mite allergen (Der p 5) prevented the induction of IgE synthesis, histamine release, and airway hyperresponsiveness (AHR) following challenge with aerosolized allergen. A similar protective effect was reported by Broide and colleagues (35, 36). Raz et al (32) showed that intradermal immunization with pDNA encoding β-galactosidase induced a predominant IgG2a response, and reduced β-galactosidase-induced specific IgE antibody levels by 66%–75% in BALB/c mice. These studies suggested that immunization with pDNA encoding allergens had potential for developing a new form of allergen immunization therapy.

In our studies, we found that intramuscular immunization of naïve AKR/J and C3H/HesnJ mice with pDNA encoding Ara h2 before peanut sensitization provided some protective effect in AKR/J mice, but induced anaphylactic reactions in C3H/HeJ mice upon peanut challenge (37, 38). Roy et al (39) demonstrated that oral administration of chitosan embedded plasmid-Ara h2 had some protective effect in AKR mice when administered prior to sensitization. The success in one strain but failure in another suggests potential for problems if this therapy is applied to the genetically diverse human population. Although prophylactic protocols have provided some rationale for the use of immunoregulatory modulators in food allergic disorders, in order to be clinically relevant, therapeutic strategies must ameliorate established allergy. At this time there are no published reports of successful pDNA-based immunotherapy of "established" peanut or other food allergy. We evaluated the possible therapeutic effect of pDNA-based therapy in peanut-sensitized mice using the previously reported prophylactic plasmid-Ara h2 immunization regime and found no reduction in IgE levels and more severe reactions in the treated animals. These results suggest that current forms of pDNA-based immunotherapy are not effective in reversing IgE-mediated hypersensitivity, and that additional methods that improve the efficacy of peanut allergen-DNA immunization need to be developed. Modalities to improve pDNA based-therapy could include the generation of new gene constructs containing additional CpG motifs, addition of modulatory cytokine genes that enhance Th1 response, or utilizing pDNAs that encode synthesis of modified food allergens (that reduce the risk of inducing anaphylactic reactions), and optimizing the gene delivery pathway and DNA doses.

ISS-ODN Based Immunotherapy

Interest is increasing in the use of synthetic immunostimulatory oligodeoxynucleotides containing unmethylated CpG motifs (ISS) for prevention or treatment of allergic disorders such as allergic asthma (40–42). It has been shown that administration of ISS-conjugated antigen was more effective than a mixture of antigen and ISS in suppression of allergic airway responses (43, 44), probably because of enhanced dendritic cell uptake of ISS-allergen (45). In preliminary studies, we utilized ISS-conjugated-Ara h2 (provided by Dynavax, Berkley, CA) in our mouse model of peanut-induced anaphylaxis. C3H/HeJ mice were immunized intradermally with ISS-linked Ara h2 (ISS-Ara h2), or ISS-linked Amb a1 (ISS-Amb a1) as control. Four weeks after initial immunization, mice were intragastrically sensitized with peanut and then challenged with Ara h2 5 weeks later. ISS-Ara h2-treated mice did not develop obvious symptoms following oral challenge with Ara h2 whereas ISS-Amb a1-treated mice did. These findings suggest that ISS-linked Ara h2 immunization had significant preventive effect on peanut-induced allergic response in an antigen-specific manner. Nguyen et al (46) recently found that intradermal immunization with a mixture of ISS ODN and β-galactosidase, but not with either ISS ODN or β-galactosidase alone, provided significant protection against fatal anaphylactic shock induced by β-galactosidase intraperitoneal sensi-

tization and challenge. This effect was comparable to immunization with pACB-LacZ, the pDNA encoding β-galactosidase. This study also found that the protective effect of ISS+β-gal immunization was associated with increase in Th1 (IgG2a/IFN-γ) and reduction in Th2 (IgE/IL-4, IL-5) skewing. Taken together, these data suggest that antigen-ISS ODN immunization may have a prophylactic effect against allergy. However, the ability to desensitize established food allergy remains to be determined.

Cytokine Therapy

Based on their cytokine profiles, at least two functional subsets of CD4+ T cells have been identified (47–50). Th1 cells express IFN-γ, whereas Th2 cells produce the interleukins IL-4, IL-5, and IL-13. Numerous studies have demonstrated that Th2 cytokines play a central role in the pathogenesis of allergic asthma. IL-4 and -13 promote B cell switching to IgE production and MC activation. IL-5 also has been shown to have a potentially autocrine effect on MCs, in addition to its recognized paracrine effects on eosinophils (51–53). A Th2 skewed response in food allergy has also been demonstrated in patients and animal models (12, 54). Schade et al (55) recently demonstrated that T cell clones generated from infants with cow's milk allergy produced high levels of IL-4, IL-5, and IL-13 and low levels of IFN-γ, whereas infants without cow's milk allergy had high levels of IFN-γ and low levels of IL-4, IL-5, and IL-13. Interestingly, Th1 and Th2 cytokines promote the growth and differentiation of their respective subsets and inhibit the growth and differentiation of the opposing subsets (56). Thus, suppression of Th2 responses by directly administering Th1 cytokines (IL-12, IFN-γ), and/or neutralizing antibodies against Th2 cytokines (IL-4, IL-5, or IL-13) or their receptors may be interesting strategies for food allergy treatment. IL-12, a heterodimeric cytokine produced by APCs, promotes differentiation of Th1 cells and IFN-γ production, and inhibits the differentiation of Th0 cells into IL-4-secreting Th2 cells, thereby suppressing IgE production (57–61). Intraperitoneal IL-12 treatment of mice has been shown to inhibit antigen-induced eosinophilic inflammation, airway hyperresponsiveness, and IgE production (27, 62–63), and to switch a Th2 to a Th1-type response in established Leishmania major infections (64). Recently, Lee et al (65) used a mouse model of peanut anaphylaxis to evaluate possible prophylactic and therapeutic effects of orally administrated IL-12 on peanut allergy, and found that oral IL-12 administration initiated 3 weeks after the sensitization protocol as well as at the time of sensitization attenuated anaphylactic reactions triggered by peanut challenge of allergic mice. Symptom reduction was accompanied by reduction in peanut-specific IgE, and reversal or reduction of the IgG1/IgG2a ratio. Furthermore, oral IL-12 treatment increased the IFN-γ/IL-4 and IFN-γ/IL-5 ratios. These results suggest some potential for the use of IL-12, either alone or in combination with other immunomodulatory agents, as a treatment for peanut allergy. Further studies are required to evaluate dose related and long-term effects of IL-12 therapy on peanut hypersensitivity.

In contrast to asthma, there has been no research into the efficacy of anti-Th2 cytokine antibodies, such as anti-IL-4, IL-5 on food allergy. Transforming growth factor-beta (TGFβ), which is classified as Th3 (regulatory) cytokine, is an important immunogulatory cytokine in oral tolerance. It has been found that colostrum TGF-β concentrations were lower in samples from mothers of infants with IgE-mediated cow's milk allergy than in samples from mothers of infants with non-IgE mediated cow's milk allergy (66). In addition, children with atopic dermatitis (AD) were found to have a low-producer TGF-β-1 cytokine genotype more often than controls (67). Consequently it would be interesting to test whether TGF-β administration in high risk children would help prevent food allergy.

Alternative Medicine

Probiotics

Probiotics are live bacteria or components of microbial cells, which reportedly have beneficial effects on the health and well-being of the host (68). The major source of probiotics is in the form of dairy-based foods containing lactobacilli and bifidobacteria. Majamaa et al (68) performed a randomized, double-blind controlled clinical study that found consumption of *Lactobacillus rhamnosus* GG alleviated symptoms of AD associated with milk allergy in children. Symptomatic improvement was associated with the promotion of IgA and suppression of TNF-α synthesis. In a more recent double-blind, randomized placebo-controlled trial, lactobacillus GG was given prenatally to

mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma, as well as postnatally for 6 months to their infants (69). The frequency of atopic eczema in the probiotic treated group was half that of the placebo group, suggesting that lactobacillus GG had some effect in prevention of early atopic disease in children at high risk. The mechanisms underlying the reported effects of lactobacillus GG on AD are not clear. However, it appears that lactobacillus GG has anti-allergic inflammatory properties evidenced by a recent report indicating that *L. rhamnosus* GG increased IL-10 in the sera of children with AD and milk allergy (70), suppressed T cell proliferation (71), and in vitro degraded casein, which suppressed T cell activation accompanied by reduction of both IL-4 and IFN-γ (72).

Traditional Chinese Medicine

Traditional Chinese medicine (TCM) has been used in Asia for centuries and is now attracting a great deal of interest in Western countries as a source of alternative or complementary therapies in a variety of diseases due to its reputed effectiveness, low cost, and relative absence of side effects. Previous studies including ours provided scientific evidence to support the use of TCM for allergic asthma (72–75). Li et al (76) investigated the effect of a TCM herbal formula, FAHF-1 (Food Allergy Herbal Formula-1) for the treatment of peanut allergy in a well-characterized murine model of peanut hypersensitivity, and found that FAHF-1 markedly reduced MC degranulation and histamine release, and completely blocked peanut-induced anaphylactic symptoms. Peanut-specific IgE levels in the serum were significantly reduced following 2 weeks of treatment and at the time of challenge, and remained lower for 4 weeks after discontinuation of treatment. FAHF-1 also significantly reduced peanut-induced lymphocyte proliferation, and IL-4, IL-5, and IL-13 but not IFN-γ synthesis. No toxic effects on liver or kidney functions or immune suppression were observed. Although this animal model is not identical to human disease, this study suggests that FAHF-1, and possibly other herbal formulas, may be useful for the treatment of peanut allergy and other IgE-mediated food allergy. Although the mechanisms are not clear, it is possible that FAHF-1 targets many aspects of the food allergic reaction cascade, such as suppression of antigen-specific B cell, Th2 cell, and MC activation. It is also conceivable that FAHF-1 may reduce intestinal permeability, thereby reducing the amount of peanut allergens available to interact with MCs. These features may prove particularly advantageous, even over other immunotherapies that target only antigen, Th2 cytokines, or IgE antibody. However, before these therapies become useful in man, herbal products will need to be standardized for clinical use. Ideally, some day the active components of these herbal products will be defined and can be given in a more traditional form.

Conclusion

Although avoidance remains the only effective and safe therapy for food allergy, many novel approaches are under investigation. Some of these approaches, e.g. anti-IgE therapy, are undergoing clinical trials and may be available to allergists in the near future.

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Chapter 29

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Chapter 33

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Figure 33-2: Source: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion. http://www.cdc.gov/growthcharts
Table 33-1: Modified from Hamill PVV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth: National Center for Health Statistics percentiles. Am J Clin Nutr 1979;32:607–629. © Am. J. Clin. Nutr. American Society for Clinical Nutrition.
Table 33-2: From Beaton GH, Bengoa JM, eds. Nutrition in Preventive Medicine, Geneva: WHO; 1976:530–555, with permission from World Health Organization.
Table 33-3: Modified with permission from RECOMMENDED DIETARY ALLOWANCES 10th EDITION © 1989 by the National Academy of Sciences. Courtesy of the National Academies Press, Washington, DC.
Table 33-4: Reprinted from Vaughan VC, McKay RJ, Nelson WE, eds. Parenteral Fluid Therapy. In: Nelson Textbook of Pediatrics, Page 251. Copyright 1987, with permission from Elsevier. *Revised from page 442.*