Molecular and Integrative Toxicology

Rodney R. Dietert
Robert W. Luebke *Editors*

Immunotoxicity, Immune Dysfunction, and Chronic Disease

Humana Press

Molecular and Integrative Toxicology

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Rodney R. Dietert

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This book is dedicated to the immunotoxicologists and clinicians who have contributed to our understanding of immune system toxicity and the realization that the consequences of immunotoxicant exposure may extend beyond transient effects often addressed in immunotoxicity studies conducted for chemical safety assessment. Their research efforts and synthesis of data into compelling hypotheses of multisystem chronic inflammatory diseases as a function of immune system modulation and host susceptibility factors will enhance safety assessment and disease prevention efforts.

Rodney R. Dietert
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Preface

Chronic diseases are the leading cause of deaths worldwide and, according to the World Economics Council and the Harvard School of Public Health, the cost of chronic diseases is expected to reach a staggering 48% of global gross domestic product by the year 2030. The urgency of the issue was demonstrated in 2011 when for only the second time in its existence, the UN General Assembly brought a health issue to the floor for consideration: chronic diseases.

To date, most considerations of the issue have approached the topic from the vantage point that chronic diseases are a myriad of largely unconnected diseases and conditions arising in diverse tissues, organs, and physiological systems. This volume, *Immunotoxicity, Immune Dysfunction, and Chronic Disease*, deviates from that prior model. It considers the interconnectivity of chronic diseases with both environmental insult of the immune system and subsequent immune dysfunction and inflammatory dysregulation as the underlying basis for many, if not most, chronic diseases.

This change in the perception of environment–immune linkages to chronic disease is significant and has immediate implications both for the prevention of disease as well as for the development of more effective therapeutic approaches. Rather than considering environmental factors and types of reported immune alterations (e.g., depressed humoral immunity) as is common in books involving immunotoxicity, this book approaches the environment–immune–disease triad from the standpoint of the disease. Each chapter emphasizes one or more specific immune dysfunction-based chronic disease(s) or condition(s) (e.g., asthma, atherosclerosis, multiple sclerosis, lupus) and describes: (1) the suggested environmental risk factors, (2) the underlying immune dysfunction(s) associated with the disease, and (3) the overall health consequences of the disease.

This volume is an early entry for a new Toxicology book series for Springer titled: *Molecular and Integrative Toxicology (MaIT)*. The series will feature detailed research information, but in the context of a more integrative or holistic framework. As part of this framework, the chapters will contain a section on "Key Points" as well as "Recommendations" where appropriate. The goal is to cover the most timely,

state-of-the-art issues in toxicology as well as to ensure that the information is maximally accessible for research scientists, teachers, physicians, and students.

We are particularly grateful to the numerous chapter authors for providing comprehensive and expert disease-oriented contributions. We are also appreciative of their willingness to consider their material not as disparate pieces of what has become a major health crisis, but rather as key pieces in a network of apparently interconnected health challenges.

Ithaca, NY, USA — Rodney R. Dietert
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Part I
Introduction

Chapter 1
Introduction to Immunotoxicity

Keith D. Salazar and Rosana Schafer

Abstract Recognition that the immune system is vulnerable to adverse effects after exposure to xenobiotics led to the discipline of immunotoxicology and the subsequent addition of immunotoxicology testing to regulatory guidelines for toxicity. Immunotoxic effects can result in immunosuppression, immunostimulation, hypersensitivity, and autoimmunity. The complex nature of the immune system is such that all of these outcomes can potentially affect any number of components of the immune system. In turn, these immunotoxic effects can lead to immune dysfunction that may ultimately contribute to chronic diseases. This chapter will provide a very basic overview of the components of the immune system including innate immunity and adaptive immunity. A number of model systems and assays are described that are commonly used to assess the immunotoxicity of xenobiotics on the immune system. Although these are primarily in vivo based immunoassays, in vitro assays are having a greater role in determining immunotoxic effects and mechanisms of toxicity. Several regulatory agencies have begun to incorporate immunotoxicology testing in their guidelines. The current status of six regulatory guidelines is presented.

Disclaimer This chapter has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Key Points

- Immunotoxicology is defined by four adverse events: immunosuppression, immunostimulation, hypersensitivity, and autoimmunity.
- Assays for immunotoxicity testing are predominantly conducted in vivo.
- The importance of immunotoxicity testing has increased in regulatory guidelines over the past decade.
- Developmental immunotoxicity and in vitro assays are likely to have an increased role in future guidelines.

1.1 Introduction

Recognition that the immune system is vulnerable to adverse modulation by xenobiotics led to the discipline of immunotoxicology and the subsequent addition of immunotoxicology testing to regulatory guidelines for toxicity. In general, immunotoxic effects are categorized into four possible outcomes: immunosuppression, immunostimulation, hypersensitivity, and autoimmunity. The complex nature of the immune system is such that all of these outcomes can potentially affect any number of components of the immune system. In turn these immunotoxic effects can lead to immune dysfunction that may ultimately contribute to chronic diseases as discussed in later chapters.

This chapter will provide a very basic overview of innate and adaptive immunity. The primary characteristics of the innate immune system that distinguish it from the adaptive immune system are rapid (minutes to hours) responses that are antigen nonspecific and do not result in "memory" of the stimulus. This is in contrast to the adaptive immune system, which is antigen specific, slow to develop (days), and results in long-lasting memory for the specific antigen that provides a rapid response if the antigen is reencountered. Cells and tissues of the immune system and their organization and function are briefly discussed. These form the basis for humoral and cell-mediated immune (CMI) responses that depend on a complex system of cooperation, communication, and regulation for a successful immune response. A number of model systems and assays are described that are commonly used to assess the immunotoxicity of xenobiotics on the immune system. Although these are primarily in vivo immunoassays, in vitro assays are having an increasing role in determining immunotoxic effects and mechanisms of toxicity.

In addition, research over the last several years has clearly demonstrated a need to adapt immunotoxicity testing to determine effects on the developing immune system. Several regulatory agencies have begun to incorporate immunotoxicology testing in their guidelines. Tiered testing protocols are frequently utilized for risk assessment in regulatory guidelines. The current status of guidelines for six regulatory agencies is presented.

1.2 The Innate Immune Response

Both anatomic and physiologic barriers, such as keratinized epithelium and complement, provide immediate protection against pathogens. In the event that pathogens breach these barriers, the innate immune response provides our first line of defense against invading pathogens and is critical for survival (Janeway and Medzhitov 2002). The innate immune system is comprised of a number of components that provide for rapid recognition and elimination of invading microbes.

1.2.1 Cells in Innate Immunity

Macrophages are the premier phagocytic cell in the body and can also function as antigen presenting cells (APC) in the adaptive immune response (Janeway and Medzhitov 2002). Macrophages can be found in the blood or as resident cells in tissues such as the liver, spleen, lung, lymph nodes, and bone marrow. Macrophages are generally found in a lower metabolic resting state. In response to infection, bacteria and host proteins provide signals that prime and activate macrophages to enhance the functional activity of the cells. In general, as macrophages become more activated, their capacity to kill invading pathogens increases. In addition, macrophages produce a number of proinflammatory cytokines including interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), and IL-8.

Neutrophils, or polymorphonuclear leukocytes (PMN), comprise up to 60–70% of the leukocytes in human and are a short-lived cell with a life span of 3–5 days (Janeway and Medzhitov 2002). They are highly phagocytic and are crucial in the initial stages of infection to eliminate pathogens. Neutrophils typically circulate in the blood stream until they receive signals generated during an inflammatory response that recruit them to sites of infection and inflammation. Macrophages and neutrophils utilize oxygen-dependent mechanisms (reactive oxygen and reactive nitrogen species) and oxygen-independent mechanisms (lysozyme, defensins, and proteolytic enzymes) to eliminate pathogens.

Natural killer (NK) cells are specialized lymphocytes that play a role in the early response to viral and bacterial infections, but unlike other lymphocytes do not respond to antigens or generate clones of memory cells (Janeway and Medzhitov 2002). Instead, NK cells bind to the target cell, recognize that normally expressed self-antigens are missing from infected and transformed cells, and deliver a “lethal hit” that causes lysis. NK cells also produce interferon-gamma (IFN-γ), which in turn activates macrophages for enhanced killing of pathogens.

1.2.2 Receptors and Recognition by Cells of the Innate Immune System

The mechanism of innate immune recognition has evolved to rely on a limited number of molecules known as pathogen-associated molecular patterns (PAMPs) (Janeway and Medzhitov 2002). These molecules share several key features: (1) highly conserved among pathogens, (2) crucial to the survival of the organism, and (3) exclusively produced by microbes. Pattern recognition receptors (PRR) are the receptors for PAMPs present on cells of the innate immune system. There are several families of PRRs including Toll-like receptors (TLR), RIG-I-like receptors (RLRs), and Nod-like receptors (NLRs) (Kawai and Akira 2010; Medzhitov 2007). PRRs are located in different cellular compartments and recognize distinct pathogens. Interaction of PRRs with their specific ligand triggers activation through signaling pathways, which results in the production and secretion of a number of soluble factors including cytokines and chemokines. These factors can then direct the cellular response and the differentiation of lymphocytes in the subsequent adaptive immune response (Medzhitov 2007).

1.2.3 The Complement System

Complement is an important mediator of immunity and participates in the inflammatory response. The complement cascade is a family of enzymes that can be activated through three distinct pathways: (1) the classical pathway activated by antigen–antibody complexes, (2) the alternative pathway activated by components of microbial membranes such as lipopolysaccharide (LPS), and (3) the lectin pathway activated by the binding of mannose-binding lectin to mannose on the surface of a pathogen. The three major functional activities of complement are (1) the formation of a membrane attack complex (MAC) that leads to killing of bacteria, (2) complement component C3b acts as an opsonin to enhance phagocytosis and complement components C5a, and (3) C3a act as chemotactic factors to enhance neutrophil chemotaxis to sites of infection or inflammation (Walport 2001; Rus et al. 2005).

1.3 Cells and Tissues of the Adaptive Immune System

1.3.1 Cells of the Adaptive Immune System

1.3.1.1 Antigen Presenting Cells

The three classes of APC are dendritic cells (DC), macrophages, and B cells. DC are the most efficient APC and are comprised of multiple subsets characterized by their location and surface expression of CD11c, CD11b, CD8, B220, CD103, and

langerin (Manicassamy and Pulendran 2011). APC endocytose or phagocytose antigens, process them into antigenic peptides and re-express peptides in association with major histocompatibility complex (MHC) molecules on the cell surface for recognition by the T cell receptor (TCR).

1.3.1.2 T Lymphocytes

T lymphocytes are a diverse group of cells that initially develop in the bone marrow and migrate to the thymus to undergo selection and differentiation. Mature T cells further differentiate into T helper (Th) cells, regulatory T (Treg), or T cytotoxic (CTL) cells. The development of these subsets and their function is determined by the cytokines delivered by the APC and the environment of the differentiating T cell.

Th cells are characterized by the expression of CD4 on their surface and are divided into several subsets based on cytokine production and expression of specific transcription factors. These subsets include Th1 cells, Th2 cells, Th17 cells, and Tfh (follicular helper) cells (O'Shea and Paul 2010; Zhou et al. 2009; McHeyzer-Williams et al. 2009). Th1 cells produce inflammatory cytokines and promote cell-mediated immunity. Th2 cells promote humoral and mucosal immunity. Recently identified Th17 cells drive autoimmune diseases and allergies through the production of IL-17. Tfh cells assist in the development of mature B cells in the germinal centers of the lymphoid tissue.

Treg cells express CD4 and CD25 on their surface and the transcription factor FoxP3. Treg cells can be classified as natural Treg or adaptive Treg (Bluestone and Abbas 2003). Treg cells play a critical role in the regulation and homeostasis of the immune response as well as tolerance. CTL express CD8 on the surface, secrete inflammatory cytokines, and are able to lyse infected host target cells and tumor cells through release of perforin and granzyme.

A separate lineage that develops in the thymus is NKT cells (Balato et al. 2009). They are a distinct population that expresses NK cell lineage markers and a TCR, which recognizes glycolipid molecules presented by the MHC-like molecule CD1d. The function of NKT cells is diverse as they have been demonstrated to play a role in both host defense and in autoimmune diseases.

1.3.1.3 B Lymphocytes

B lymphocytes develop and differentiate in the bone marrow to become mature B cells each able to produce antibodies of a unique specificity (Batista and Harwood 2009). B cells express surface immunoglobulin that serves as the B cell receptor (BCR) for its specific antigen. During an adaptive immune response, B cells interact with Th cells to become antibody-producing plasma cells or memory B cells. Additional subsets of B cells include B-1 cells that predominate in pleural and peritoneal cavities, and marginal zone B cells in the spleen.

1.3.2 Tissues of the Immune System

The site of mammalian lymphocyte precursor differentiation into immunocompetent lymphocytes occurs in the primary lymphoid organs, which include the bone marrow, thymus, and fetal liver (Akirav et al. 2008). Secondary lymphoid organs include the spleen, lymph nodes, and the mucosal associated lymphoid tissue (MALT). The major function of the secondary lymphoid organs is to trap antigen, facilitate the induction of antigen-specific T cells, and generate an antibody response.

1.3.2.1 Bone Marrow

The bone marrow is the site of hematopoiesis (Akirav et al. 2008). All blood cells are derived from common progenitor cells referred to as pluripotent hematopoietic stem cells. These stem cells give rise to other progenitor cells such as the common lymphoid progenitor (CLP), which are responsible for producing lymphocytes. In addition to hematopoiesis, the bone marrow serves as the site for B cell development and tolerance in mammals.

1.3.2.2 Thymus

Precursor T cells exit the bone marrow and migrate to the thymus cortex (Akirav et al. 2008). Immature cells that recognize an individual's major histocompatibility antigens (MHC; see below) are positively selected by delivery of a survival signal and migrate to the thymic medulla where a second round of MHC-dependent selection takes place. Cells that bind MHC too avidly are negatively selected by delivery of an apoptotic signal, a necessary step in the prevention of autoimmune disease. Approximately 95% of precursor cells are lost to selection, primarily in the positive selection process; the remaining 5% complete the maturation process to become naïve T cells and are exported to the circulation. Production of T cells in the thymus is most active before puberty and diminishes in adulthood.

1.3.2.3 Spleen

The spleen is a major secondary lymphoid organ and the major site of antibody production (Akirav et al. 2008). Microorganisms and effete cells in the circulation are filtered by the spleen and come in contact with lymphocytes and APC that initiate a specific adaptive immune response. The spleen largely consists of red pulp interspersed with white pulp regions. Red blood cell elimination occurs in the red pulp and antibody production occurs in the white pulp. The white pulp contains distinct regions of T and B cells, which facilitate antigen presentation and B cell activation. Activated lymphocytes in the spleen give rise to germinal centers, which are composed primarily of rapidly dividing B cells and supported by T cells. Emerging from the germinal centers are plasma cells and memory B cells.

1.3.2.4 Lymph Nodes

Lymph nodes are widely distributed throughout the body, connected by vessels of the lymphatic system and act as filters for microbes and debris in lymph (Akirav et al. 2008). Lymphocytes and APC in lymph nodes are found in three anatomically distinct regions: the cortex, the paracortex, and the medulla. B cells are found mainly in the cortex and T cells are found mainly in the paracortex with few lymphocytes in the medulla.

1.3.2.5 Mucosal-Associated Lymphoid Tissue

Most immunotoxic chemicals enter the body through mucosal surfaces and thus may affect mucosal immune responses. In particular, the respiratory tract and gastrointestinal tract are the initial sites of exposure where immunotoxicants are absorbed from the environment. MALT includes secondary lymphoid tissues in the intestine (referred to as gut-associated lymphoid tissue, GALT), the respiratory tract (bronchus-associated lymphoid tissue, BALT and nasal-associated lymphoid tissue, NALT), the genitourinary tract from the urethra to the gonads and bladder, the mammary gland, and the eye (Cesta 2006). The lymphoid tissues of the intestinal tract are the best characterized of the various MALT sites and constitute the largest mass of lymphoid tissue in the body.

In general, MALT is described as inductive or effector tissue. Inductive sites include local lymph nodes or lymph node-like structures where antigen activation of specific lymphocytes is initiated. These activated lymphocytes differentiate and migrate to effector sites, which include the regions just below the epithelial lining (referred to as the lamina propria) and within the epithelial lining. Both B cells and T cells generally populate the lamina propria, while the epithelium is usually highly enriched in T cells, most commonly $CD8^+$ T cells. Lymphocytes in the gut are found in the Peyer's patches, the lamina propria, and the epithelium.

1.4 Recognition and Activation by the Adaptive Immune System

1.4.1 Antigens and Antibodies

1.4.1.1 Antigens

Antigens are molecules that are recognized by, and react with, the immune system. The important properties that define antigens are molecular size, chemical composition, heterogeneity, degradability, and foreignness. Epitopes, or antigenic determinants, are specific sites recognized by the immune system after processing of the antigen by APC.

1.4.1.2 Antibodies

Immunoglobulin monomers consist of 4 polypeptide chains, 2 identical light chains and 2 identical heavy chains (Luning Prak et al. 2011). The antigen-binding site is formed by the association of heavy chains and light chains. The heavy chain confers the biological function. The light chains are one of two types designated with the Greek letters *kappa* (κ) or *lambda* (λ). There are five different types of heavy chains, which are designated *gamma* (γ), *mu* (μ), *alpha* (α), *epsilon* (ε), and *delta* (δ). The Ig molecules that bear these heavy chains, or isotypes, are IgG, IgM, IgA, IgE, and IgD, respectively. In addition, there are four subclasses of IgG (IgG1, IgG2, IgG3, IgG4 in humans; IgG1, IgG2a, IgG2b, IgG3 in mice) and two subclasses of IgA (IgA1, IgA2 in humans).

There are three main functions of antibodies in humoral immunity: (1) neutralizing the binding of microbes or toxins to host cells (IgM, IgG, IgA), (2) enhancing phagocytosis (IgG, IgA), and (3) activating the classical complement pathway by forming antigen–antibody complexes (IgM, IgG). In addition, IgA is the predominant isotype in external secretions and MALT. IgE is induced in response to allergens and drives immediate Type I hypersensitivity reactions.

1.4.2 Antigen Presentation

The MHC is a set of highly polymorphic and codominantly expressed genes separated into two major categories: MHC class I and MHC class II (Medzhitov 2007). In humans, the products of these genes are referred to as human leukocyte antigens or HLA molecules. The MHC molecules that are expressed on the cell surface are integral to T cell activation. MHC class I gene products are expressed on all nucleated cells in the body, including APCs. The primary function of Class I antigens is to present bound, processed antigen to cytotoxic T cells, thus activating the cells. MHC class II antigen expression is limited to APCs, including B lymphocytes, dendritic cells, thymic epithelial cells, and macrophages. MHC Class II molecules bind peptides for presentation to $CD4^+$ T helper cells. The peptides that bind to the MHC molecules are generated in a complex process referred to as antigen processing and presentation. For MHCI, intracellular proteins in the cytosol are broken down by the proteasome enzyme, delivered to incomplete MHC I molecules in the endoplasmic reticulum and presented on the cell surface. For MHC II, extracellular antigens are endocytosed, degraded in endocytic vesicles, and presented by class II molecules. Cytotoxic and helper T cells recognize and respond to processed antigen only when presented in the context of MHC molecules. This process is referred to as MHC restriction and plays a key role in tissue transplantation and rejection.

1.4.3 T Cell Activation

Activation of T cells and B cells during an adaptive immune response primarily occurs in lymph nodes and the spleen when they encounter an APC expressing their

specific antigen. In contrast to innate immunity, adaptive immunity normally takes several days (7–10 days, typically) for an optimal response to develop (Smith-Garvin et al. 2009). T cell activation is dependent on the interaction of an antigen-specific TCR with antigen presented in the context of the appropriate MHC molecule on an APC. The majority of T cells express a TCR that is composed of two disulfide-linked, transmembrane glycoproteins: the α and β chains. The two-chain structure includes constant regions and variable regions with the hypervariable region forming the antigen-binding site. The TCR repertoire (estimated to be up to 10^{15} specificities) is generated by random gene rearrangement in the thymus during T cell development. The TCR is normally expressed on the T cell surface in association with either the CD4 or CD8 molecules that bind MHC class II or MHC class I, respectively. In addition to the TCR, there are a number of costimulatory ligands (CD28 and CTLA-4) and adhesion molecules (integrins) that contribute to T cell activation and function.

The first signal for T cell activation is the interaction between the TCR and an APC expressing the appropriate peptide self-MHC molecule. The second signal is the interaction between CD28 on the T cell with its ligand, B7 (CD80, CD86), on the surface of the APC. The two steps initiate a cascade of signaling pathways that result in the expression of cytokines and other proteins that mediate T cell functions. Both interactions must occur for the T cell to be activated. In the absence of the second signal, the T cell will not differentiate and proliferate; a state referred to as anergy or tolerance. The primary response initiated by the second signal through B7-CD28 interaction is the expression of the high affinity receptor for IL-2 and increased IL-2 production, a critical T cell growth factor. A second important consequence of T cell activation is the expression of an additional molecule, CTLA-4 (CD152), which binds tightly to B7. This interaction sends a negative signal to the activated T cell to turn off production of IL-2 and dampens the immune response.

1.4.4 B Cell Activation

B cells express surface bound immunoglobulin that functions as the BCR for antigen (Harwood and Batista 2010). The majority of antigens requires T cell help for the production of antibodies and are referred to as thymus (T)-dependent antigens. The generation of a diverse pool of B cells takes place in the bone marrow during development and differentiation. Mature B cells exit the bone marrow expressing surface-bound IgM and IgD. B cells functioning as APC for T cells also express MHC class II molecules, CD40, and the costimulatory molecule, B7 (Batista and Harwood 2009). Antigen bound by the BCR is endocytosed, processed, and expressed on the surface of the B cell in association with MHC Class II molecules. Upon interaction of the B cell with the antigen-specific T cell, a T–B cell conjugate is formed. In addition, the costimulatory molecule B7 binds to CD28 on the T cell. A third important interaction, which is crucial for isotype switching, is the interaction of CD40 with CD40 ligand (CD40L) on the T cell. Ligation of CD40 on the B

cell promotes B cell survival thereby assisting continued B cell differentiation. After activation, T cells secrete a number of cytokines, including IL-4, IL-5, and IL-6, which are necessary for proliferation and differentiation of the B cell into antibody-secreting plasma cells. Activated B cells then undergo somatic hypermutation and affinity maturation in germinal centers, which results in the production of high-affinity antibodies.

1.5 Immune Effector Systems and Immune Dysfunction

1.5.1 Immune Regulation and Tolerance

Tolerance is broadly defined as a state of unresponsiveness to an antigen, be it self or foreign (Singh and Schwartz 2006). Central tolerance occurs during development in the thymus and bone marrow. The primary mechanisms of central tolerance include clonal anergy, receptor editing, and clonal deletion. In the cells of the thymus, the transcription factor, autoimmune regulator (AIRE), controls the expression of several hundred tissue-specific genes at low levels. This serves as an additional mechanism for the elimination of self-reactive tissue specific cells by clonal deletion in the thymus. Peripheral tolerance is induced in mature lymphocytes in the periphery and can also occur through clonal anergy or clonal deletion. Specialized T cells, referred to as T regulatory (Treg) cells, participate in maintaining self-tolerance by suppressing activity of autoreactive cells (Wing and Sakaguchi 2010). Treg cells act through direct cell contact and the production of suppressive cytokines such as IL-10 and transforming growth factor β (TGF-β). The importance of Treg cells in tolerance was demonstrated in mice depleted of Tregs, which lead to the development of autoimmune diseases; reconstitution of Tregs ameliorated the disease (Lahl et al. 2007).

1.5.2 Allergic Hypersensitivity Reactions

Hypersensitivity is defined as the pathologic consequence of an inappropriate reaction to an antigen. Hypersensitivity can be expressed as humoral or CMI responses following secondary exposure to certain antigens. The reaction has two phases: a sensitization phase and an elicitation phase. The four major types of hypersensitivity reactions are distinguished by the differences in the nature of the allergen, the primary cell types involved, and the mechanism of tissue damage (Wills-Karp 2008).

Type I hypersensitivity reactions (also known as immediate hypersensitivity or allergy) are mediated by antigen-specific IgE antibodies that bind to high affinity Fc receptors for IgE on mast cells and basophils. Re-exposure to the allergen

cross-links the IgE bound to Fc receptors, thereby inducing degranulation of the sensitized mast cells and basophils. The release of pharmacologically active substances from degranulated mast cells and basophils occurs within minutes and mediates effects such as smooth muscle contraction, increased vascular permeability, and vasodilation. Type I reactions can induce discomforting localized conditions such as hay fever or eczema, as well as serious life-threatening reactions such as systemic anaphylaxis.

Type II (or cytotoxic) hypersensitivity is mediated by IgM and/or IgG antibodies. Antibodies specific for exogenous antigen may cross-react with structures on the surface of circulating cells or fixed body tissues. Cells or tissues coated with auto-reactive antibodies activate and become the target of complement-mediated destruction.

Type III hypersensitivity is induced by immune complexes that are formed during the course of an immune response but are not subsequently cleared by Fc or complement receptor-mediated uptake and degradation. Activation of the complement cascade by the supervenient immune complexes induces chemoattraction of inflammatory cells at the site of deposition followed by the release of lytic enzymes and tissue damage.

Type IV or delayed-type hypersensitivity (DTH) reactions are CMI responses mediated by DTH T cells (TDTH). $CD4^+$ TDTH cells produce inflammatory cytokines that activate macrophages to release lysosomal enzymes resulting in damage to local tissue. The two phases follow similar kinetics to CMI responses to pathogens and are induced experimentally 1–2 weeks apart. The second phase, which constitutes the inflammatory response, occurs 24–72 h postexposure.

1.5.3 Autoimmunity

Autoimmunity is a breakdown of tolerance that results in an immune response against self-antigens leading to the destruction of host tissue or damage to the function of an organ or tissue (Davidson and Diamond 2001). It is believed to be caused by a combination of genetic predisposition and triggered by environmental factors. Genetic associations with autoimmunity include MHC, immunoregulatory transcription factors and enzymes involved in peptide processing (Gregersen and Olsson 2009). Environmental factors that may potentially act as triggers for autoimmune disease include chemical exposures or infections. The host immune response to certain bacterial epitopes that are similar to host proteins may result in cross-reactivity to healthy host tissue, resulting in autoimmunity. In addition, the incidence of autoimmune disease is higher in females suggesting that gender and hormones may act as noninfectious triggers (Gonzalez et al. 2010). Chemicals may alter normal host proteins so that they are recognized as nonself, or damaged tissues, thereby releasing proteins that are not normally encountered by the immune system and overriding tolerance.

Autoimmune diseases are divided into two groups: antibody mediated and T cell mediated. Antibody-mediated autoimmune diseases occur when antibodies react to

a self-protein or when immune complexes are formed. Antibodies to self-proteins inhibit normal cellular processes and form immune complexes that activate the complement cascade resulting in inflammation and tissue damage. Cell-mediated autoimmune diseases occur when a T cell response is made to a self-antigen. $CD4^+$ T helper cells produce inflammatory cytokines (IFN-γ) and activate macrophages. The activated macrophages, in turn, produce additional inflammatory cytokines (TNF-α, IL-1) that lead to tissue damage. $CD8^+$ T cytotoxic cells that recognize self-antigens can directly destroy host cells.

1.6 Immunotoxicology

Immunotoxicology is a relatively recent addition to the standard toxicological testing guidelines despite the immune system having been recognized as a target for xenobiotics for over 40 years. Immunotoxic effects are categorized into four possible outcomes: immunosuppression, unintended immunostimulation, hypersensitivity, and autoimmunity. Immunosuppression was the first adverse effect associated with exposure to environmental chemicals and was initially the primary focus of immunotoxicologists. Suppressed immune systems lead to increased risk of infections and viral-induced malignancies. Unintended immunostimulation results in the nonspecific or skewed upregulation of the immune response, which can induce inflammation, aggravate autoimmune diseases, or lead to hypersensitivity. Compounds can elicit a hypersensitivity reaction by affecting one or both of the two phases: inducing sensitization or triggering an allergic response in a sensitized individual. Thus, chemicals that induce other adverse events may also induce autoimmunity. For example, immunosuppressive effects on cells involved in tolerance early in development could lead to greater numbers of autoreactive lymphocytes. Although the mechanisms underlying autoimmunity are poorly understood, the immune mediators that are involved in autoimmunity are the same as those involved in normal responses to foreign bodies.

1.7 Immunotoxicity Assays

Numerous models and assays have been developed over the past 30 years to assess the competence of the immune response and to detect allergenicity. The assays currently favored by immunotoxicologists for identifying the potential toxicity of chemicals and drugs are performed primarily in animals, with the majority conducted in rats and mice. Ideally, models must produce data that are useful in assessing the risk of a tested compound to cause immunosuppression, immunostimulation, hypersensitivity, and autoimmunity. The assays presented in the following sections are some of the most commonly employed assays in an immunotoxicologist's toolbox.

Histological analysis of the lymphoid organs is one of the initial techniques employed in an evaluation that provides data on the potential immunosuppressive or

immunostimulatory effect of a xenobiotic. Examination is performed on a diverse representation of primary and secondary immune organs, as well as internal and secretory lymphoid organs such as the draining lymph nodes, Peyer's patches, spleen, femoral bone marrow, and thymus (Schuurman et al. 1994). Splenic and thymic organ weights are obtained prior to sectioning since changes in these weights often correlate with immunotoxicity (Haley et al. 2005). In addition, histopathology can be conducted during routine toxicology studies without using additional animals (Germolec et al. 2004). Microscopic examination of hematoxylin and eosin stained 4 or 5 μm sections is frequently conducted on preweighed organs (Schuurman et al. 1994). Microscopic smears are usually performed with bone marrow and blood samples and stained with conventional dyes such as Giemsa stain.

More recently, techniques such as flow cytometry and immunohistochemistry have been applied to develop more quantitative data. However, these techniques are generally not believed to provide additional sensitivity to the qualitative histology already employed (Haley et al. 2005). Despite these more recent innovations, functional assays remain the standard to evaluate immunotoxicity and the only method to identify autoimmunity or hypersensitivity.

1.7.1 Immunosuppression/Immunostimulation Assay

1.7.1.1 Lymphoproliferative Assays

Lymphoproliferative assays assess entry of lymphocytes into the cell cycle, generally measured by the uptake of a labeled nucleoside. Typically, spleen cells are harvested from chemically exposed rats or mice, stimulated in culture for 48–72 h and the responses of exposed and control lymphocytes are compared (Bach and Voynow 1966). In some cases, nonspecific proliferation is induced by T or B lymphocyte-specific mitogens such as concanavalin A (ConA) or LPS to evaluate potential immunostimulative or immunosuppressive effects. Alternatively, proliferation can be assessed in a mixed lymphocyte reaction (MLR) in which T cells are stimulated to divide by foreign antigens on allogeneic lymphocytes that have been chemically treated to prevent proliferation (Elves and Israuels 1965). However, concordance analysis of results from experiments on numerous immunotoxic chemicals determined that lymphoproliferative assays are relatively poor predictors of immunotoxic outcomes compared to other immunotoxicity assays to be discussed (Luster et al. 1992). For example, LPS and ConA had a 50 and 67% concordance with an immunotoxic outcome, respectively.

1.7.1.2 Plaque Forming Cell Assay

The T cell-dependent antibody response (TDAR) is one of the most widely used assays for detecting immunostimulation or immunosuppression (Ladics 2007). The assay is designed to measure primary antibody responses in chemically treated

mice or rats that have been injected with sheep red blood cells or some other T-dependent antigen such as keyhole limpet hemocyanin (KLH). Splenocytes are harvested 5 days later and incubated with complement and the immunizing antigen (Cunningham 1965). The plaque forming cell (PFC) assay was the first TDAR and is performed by visually enumerating hemolytic plaques formed when complement is activated by antibody secreted by plasma cells. Alternatively, specific antibody concentrations may be measured in serum samples by ELISA, a method that is readily automated and less tedious to perform (Temple et al. 1993). The PFC assay has a strong (78%) correlation with immunotoxicity when performed in the mouse (Luster et al. 1992). When this assay is performed in combination with another assay, the concordance with immunotoxicity is increased to 80–100% depending on the paired assay.

1.7.1.3 Natural Killer Cell Assay

Because NK cells play a central role in immune surveillance, assaying NK cell activity is a commonly recommended practice to identify immunostimulants or immunosuppressants. Typically, this assay is performed with cells harvested from the peripheral blood. The cells are incubated for several hours with a labeled target and the percentage of dead cells is determined (Cederbrant et al. 2003). The assay lends itself to clinical applications since it can be conducted ex vivo with NK cells from chemically exposed subjects or from naïve individuals. These qualities allow for the direct monitoring of human subjects. However, NK cells tend to be overly sensitive to chemical exposure, which does not correlate well with immune pathology (Luster et al. 1992).

1.7.1.4 Host Resistance Assays

Host resistance assays are the most useful method to evaluate the potential of a xenobiotic on host susceptibility to a disease in the general population and validate the efficacy of a functional assay to detect immunotoxicity. These models are useful for identifying both immunostimulation and immunosuppression. The most common experimental models in rodents include challenge with the pathogens *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Trichinella spiralis*, or *Plasmodium yoelli* and tumor challenge models such as PYB6 sarcoma and B16F10 melanoma (Germolec 2004). Immune function is measured by bacterial/parasite load and/or mortality in the pathogen models. However, more recent data have favored pathogen load over morbidity/mortality as a more sensitive endpoint since it allows the host to mount a more complete adaptive immune response and gives a dose-related estimate of immunocompetence. Tumor challenge models are assessed by recording the tumor frequency or the number of tumor nodules (Burleson and Burleson 2010). Models are chosen based on the anticipated effect on a particular immune response. For example, if defects in $CD8^+$ T cells or NK cells are

suspected, tumor challenges provide an assessment of immune surveillance and tumor cell elimination (Luster et al. 1993). Likewise, bacterial models matched to the suspected defect are useful for assessing a chemical's effect on the humoral response (*S. pneumoniae*) or the cell-mediated immune response (*L. monocytogenes*) (Luster et al. 1993).

Host resistance assays are not practical as a screening assay. Studies have demonstrated that regardless of the arm of the immune system that drives the response to a resistance model, altered host resistance is not the result of a change in one parameter or cell type (Germolec 2004). This has impeded attempts to extrapolate the results from a functional assay or changes in the population of one cell type to predict the outcomes in a host resistance model. In addition, host resistance models require considerable cost and technical skill. Regardless, host resistance models still remain the most relevant models for extrapolating animal data to human populations (Germolec 2004).

1.7.2 Hypersensitivity

1.7.2.1 Guinea Pig Skin Sensitization Model

The guinea pig is a well-characterized skin sensitization model for assessing hypersensitivity (van Loveren et al. 2008). The Magnusson and Kligman guinea pig maximization test (GPMT) and the Buehler test are two of the most common protocols. The GPMT involves an induction period over 1 week utilizing an intradermal injection of an adjuvant in combination with a test substance followed by a topical application challenge 2 weeks later (Magnusson and Kligman 1969). The Buehler test is exclusively performed with topical applications where animals are induced over the course of 2 weeks followed by a challenge after a 1-week rest period (Buehler 1965). The assays are evaluated by a visual inspection of the challenged area that note signs of erythema. Although both are used globally for regulatory studies, numerous examples indicate that these two assays are not equally sensitive (Basketter and Kimber 2010). It has generally been observed that the Buehler test is not as sensitive as the GPMT; however, the increased sensitivity of the GPMT results in greater false positive results.

1.7.2.2 Local Lymph Node Assay

The local lymph node assay (LLNA) was developed as an alternative to the guinea pig test because fewer animals are required, the induction and assessment phases are shorter, and the test has an objective endpoint, rather than subjective scoring of erythema. It has been extensively validated and is frequently used for regulatory studies. The principle of the assay is that sensitizers trigger proliferation of lymphocytes in lymph nodes draining the site of chemical application. The sensitizing

potential of a compound is proportional to the magnitude of lymphocyte proliferation. The test is conducted by topical application of the test article or vehicle to the ears of mice, harvesting the lymph nodes approximately 1 week later and quantifying cell proliferation. Results are useful for linear interpolations from dose–response data known as EC3 values, which are defined as the concentration of a chemical that generates a threefold increase in lymph node proliferation compared to the vehicle. Studies have shown these values to be accurate for ranking sensitization potential of chemicals (Basketter et al. 1999).

One drawback of the assay is that it is solely conducted by topical exposure, thereby making it susceptible to artifactual results induced by the vehicle (McGarry 2007). Vehicles such as propylene glycol and dimethyl sulfoxide are known to induce lymph node cell proliferation either individually or in formulations with nonsensitizing chemicals. Moreover, it is difficult to determine if these effects are artifacts or are accurate representations of the skin sensitization potential. Given these observations, no one assay is completely accurate for identifying sensitizers and researchers must be aware of the limitations of each assay before drawing conclusions.

1.7.3 Autoimmunity

1.7.3.1 Popliteal Lymph Node Assay

The PLNA the only assay extensively tested for its efficacy in predicting autoimmune effects of xenobiotics. The assay has been validated in small interlaboratory studies and tested in over 100 chemicals and drugs (Vial et al. 1997; Ravel and Descotes 2005). The standard method involves injecting the hind footpad with the test chemical and measuring nonspecific proliferation in the draining popliteal lymph nodes 1 week later by weighing and counting cells in the nodes (Ravel and Descotes 2005). However, the utility of the model is diminished by a general lack of understanding of the mechanisms regulating autoimmunity as well as a paucity of validation studies. As long as gaps in mechanistic understanding of autoimmune disease remain, this will preclude the selection of more specific endpoints, exposure routes, and study duration. Presently, the assay is not recommended for widespread implementation as a screen of autoimmunity. Other autoimmune animal models have been employed in immunotoxicity studies but their utility as a screening tool is diminished due to the diversity of autoimmune diseases.

1.8 Tiered Testing

Tier testing is a common risk assessment strategy employed across the subdisciplines of toxicology to efficiently screen chemicals and drugs for toxicity. Tier testing can include 2 or 3 steps of testing depending on the agency's or program's guidelines. Typically each subsequent step of testing is performed to obtain more

specific data on the nature of the effect. Thus, the choice of the initial test is critically important since it must be able to detect a large number of immunotoxicants. Many experts have presented evidence that functional assays such as the PFC assay are the most sensitive assays available and should be used in the first tier of testing (Luster and Gerberick 2010). However, due to the disadvantages of performing functional assays such as added cost and dedicated experimental animals, others have proposed utilizing histological approaches for initial testing (Snodin 2004).

The first tiered testing for immunotoxicity was proposed by Dean et al. (1979) and expanded upon by a workgroup organized by the National Toxicology Program (NTP) (Luster et al. 1988). This work established the basis for modern tier testing that is currently practiced (Table 1.1). The first tier generally includes assays that can be obtained during routine 28-day toxicity studies. Depending on the organization, the first tier can also include a functional test such as TDAR. If a significant effect is found, the second tier employs assays such as host resistance assays and quantifying lymphocyte populations to more specifically assess the populations of cells affected. In addition, tier 2 tests are conducted with doses that are relevant to human exposure. The implementation of a tiered testing approach provided a framework for determining the relative immunotoxicity of chemicals and drugs. Tiered testing protocols also became a foundation for regulatory guidelines.

1.9 Immunotoxicity Testing and Risk Assessment Guidelines

1.9.1 Organization for Economic Cooperation and Development

In 1995, Organization for Economic Cooperation and Development (OECD) became the first major international body to implement immunotoxicity screening in the Guideline 407. Adopted in 1995, this guideline required histopathology of local and distal lymph nodes in 28-day rat toxicity studies (OECD 2008). Although noteworthy for being the first immunotoxicity testing recommendation, the lack of a functional test limits its sensitivity for detecting immunotoxicants at low doses (Institoris et al. 1998). Despite a recent harmonized update in 2008, the guideline still does not include a functional assay in its recommended routine screening of chemicals.

1.9.2 US Environmental Protection Agency

The Office of Prevention, Pesticides, and Toxic Substances (OPPTS) of the US Environmental Protection Agency (US EPA) was one of the first government

Table 1.1 Tier testing battery proposed by NTP (reproduced from Luster et al. 1988)

Parameter	Procedure
Screen (tier 1)	
Immunopathology	Hematology: complete blood count and differential
	Weights: body, spleen, thymus, kidney, liver
	Cellularity: spleen
	Histology: spleen, thymus, lymph node
Humoral-mediated immunity	Enumerate IgM antibody plague-forming cells to T-dependent antigen (SRBC). LPS mitogen response
Cell-mediated immunity	Lymphocytes blastogenesis to mitogens (Con A) and mixed leukocyte response against allogeneic leukocytes (MLR)
Nonspecific immunity	Natural killer (NK) cell activity
Comprehensive (tier 2)	
Immunopathology	Quantitation of splenic B and T cell numbers
Humoral-mediated immunity	Enumeration of IgG antibody response to SRBCs
Cell-mediated immunity	Cytotoxic T lymphocyte (CTL) cytolysis. Delayed hypersensitivity response (DHR)
Nonspecific immunity	Macrophage function–quantitation of resident peritoneal cells and phagocytic ability (basal and activated by MAF)
Host resistance challenge models (endpoints)	Syngeneic tumor cells
	PYB6 sarcoma (tumor incidence)
	B16F10 melanoma (lung burden)
	Bacterial models
	Listeria monocytogenes (mortality)
	Streptococcus species (mortality)
	Viral models
	Influenza (mortality)
	Parasite models
	Plasmodium yoelii (parasitemia)

agencies to institute immunotoxicity testing guidelines (EPA 1998). Implemented in 1998, the 870.7800 guideline applies to pesticides and chemicals used in the United States that are regulated under the Federal Insecticide, Fungicide, and Rodenticide Act and the Toxic Substances Control Act. Citing several earlier studies demonstrating the advantages of including functional assays, the guideline requires a PFC assay to be performed in addition to routine toxicity testing such as hematology and histopathology. In cases of immunosuppression, the guideline also recommends more extensive analysis of NK cell activity and assessments of lymphocyte populations. Although this guideline expanded immunotoxicity testing requirements to include functional data, it falls short of requiring a test to detect potential sensitizers. In 2002, the EPA adopted the guideline 870.2600, which expanded testing to identify skin sensitizers (EPA 2003). Citing animal welfare issues, the guideline recommends the use of LLNA over the GPMT and Buehler Test, but considers all three models acceptable.

1.9.3 European Medicines Evaluation Agency

In 2000, the European Medicines Evaluation Agency (EMEA) Committee for Proprietary Medicinal Products (CPMP) produced the first immunotoxicity guidelines regulating medicines for human application in the European Union (EMEA 2000). The guidelines were adapted from older toxicity testing guidelines to include a tiered approach to immunotoxicity testing. The EMEA recommends an extensive initial immunotoxicity screen using primarily nonfunctional assays such as hematology, histopathology of lymphoid tissue, and lymphocyte population analysis, but also includes measuring NK cell activity. If NK cell activity and lymphocyte populations cannot be obtained, the EMEA recommends employing TDAR during the initial screen. Data that suggest immunotoxicity from tier 1 studies are followed with additional functional assays that are chosen based on the nature of the changes observed in tier 1. Most importantly, the guidelines do not specify which test to use for the second tier and instead rely on a weight of evidence approach, which allows flexibility in the toxicity testing.

1.9.4 US Food and Drug Administration

In 2002, the US Food and Drug Administration (FDA) Center for Drug Evaluation and Research issued a guidance document that is one of the most substantial to date. Applying to the evaluation of drug products and not biological products, the guidelines describe the potential immunotoxic adverse events due to pharmaceuticals. Similar to the EMEA guidelines, the FDA employs tiered testing strategies and a weight of evidence approach. The document provides a decision tree along with several validated models and methodologies to evaluate potential scenarios. For example, the document recommends performing a PFC assay when nonclinical or clinical data indicate immunosuppressive effects in histopathological examinations (FDA 2002). However, significant differences between FDA and EMEA guidelines exist. In contrast to the EMEA, the FDA recommends obtaining immunoglobulin levels in the first tier of testing but does not recommend measuring NK cell activity. In addition, FDA guidelines specify the need for specialized assays to evaluate medicinal products. These differences between the FDA and EMEA were problematic for drug manufacturers who attempted to meet both requirements for US and EU markets, which lead to a harmonization project to reconcile these differences as described below.

1.9.5 International Council on Harmonization

In 2005, the International Council on Harmonization (ICH) Immunotoxicity Studies for Human Pharmaceuticals released its final version of immunotoxicity guidelines

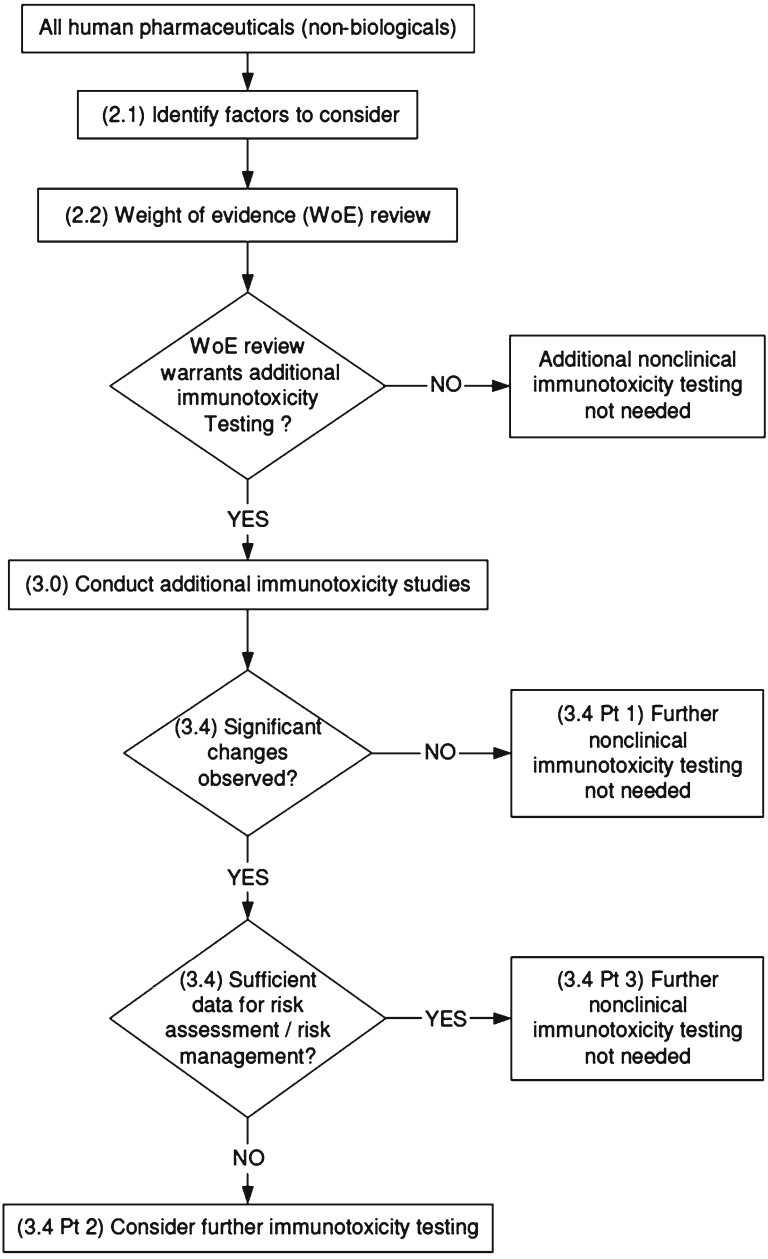

Fig. 1.1 ICH decision tree for pharmaceutical testing

(ICH 2005). The guideline adopts similar testing strategies recommended by both the FDA and the EMEA and focuses primarily on immunosuppression. Similar to the FDA, the ICH guideline presents a decision tree for outlining testing strategies

(Fig. 1.1). Histological examination of lymphoid organs, immunoglobulin changes, leukocyte counts via hematology, and lymphoid organ weights are all listed as standard evaluations to conduct for first tier screening. Other factors that could prompt additional immunotoxicology testing include the pharmacological properties of the drug, the intended patient population, homologous structure compared to other known immunotoxicants, the disposition of the drug, and clinical data. However, the guideline does not mandate functional testing as stated in the EMEA report. Both the strength and potential weakness of the guideline rests on the weight of evidence approach. The ICH states that one finding of sufficient magnitude or multiple smaller changes require further testing. However, the sponsor must provide rationale when more extensive testing is not performed. This places the responsibility of a prudent approach on both the regulator and the sponsor. Once finalized, the guideline was adopted by the EMEA in 2005 and published in the federal register of the FDA in 2006.

1.9.6 International Program on Chemical Safety Guidance for Immunotoxicity Risk Assessment for Chemicals

The International Program on Chemical Safety (IPCS) of the World Health Organization (WHO) is currently drafting a harmonized risk assessment guidance document that is slated for completion in 2012. The harmonization document is designed for adoption by various international, national, and private regulatory bodies. The document uses decision trees (immunosuppression, immunostimulation, hypersensitivity, and autoimmunity) to judge the quality and organize the available evidence of immunotoxicity in support of a weight of evidence-based risk assessment. The guidance assigns the greatest weight to human data followed by functional data from animal models. Although these guidelines are still in a draft state, the IPCS document promises to represent the most thorough approach to immunotoxicity risk assessment since evidence for all types of immunotoxicity is considered simultaneously.

1.10 Emerging Issues in Immunotoxicology

The field of immunotoxicology is rapidly evolving to incorporate both technological advancements and address the constant demand of improving the efficacy and efficiency of toxicity testing. At present, the main challenges of developing alternative models will be increasing the capacity of each test while reducing the need for animals, and thus, the cost associated with expanded testing. In addition, understanding how adult exposure predicts immunotoxicity in the developing animal will be important criteria to incorporate in future testing guidelines. The following sections describe two key issues likely to be at the forefront of immunotoxicology.

1.10.1 Developmental Immunotoxicology

Evidence accumulated over the past 30-plus years suggests that immunotoxicity occurs in the offspring at maternal doses that do not modulate immune function in adults, and that adverse effects are more persistent following developmental exposure (Dietert and Piepenbrink 2006; Burns-Naas et al. 2008). As shown in Table 1.2, the immune system undergoes a number of developmental events to achieve full competency beginning with the differentiation and dispersion of macrophages around 6–24 weeks of gestation and continuing throughout early adulthood (Dietert and Piepenbrink 2006). Immunotoxicants have been shown to target a variety of these events that are critical to normal immune system development.

Developmental immunotoxicology (DIT) is often defined in terms of greater susceptibility than the adult and is manifested in one of three ways: (1) quantitative differences, (2) qualitative differences, or (3) or more persistent effects. Quantitative differences are shifts in the dose–response where the developing immune system is susceptible to lower concentrations of toxicants than the adult. Qualitative differences are instances where a xenobiotic affects different immune parameters in the developing immune system than the adult. Finally, delayed onset or more prolonged effects have been observed in the developing immune system compared to the adult (Dietert and Piepenbrink 2006).

Developing standardized tests for measuring DIT has proven difficult. Prenatal or even prepubertal assessment of immune function in rodent models has met with limited success since immune system maturation is delayed in rodents. However, if assessment is delayed until puberty, significant transient effects (e.g., a delay in immune maturation) may be missed or underappreciated if chemical exposure is exclusively via maternal dosing. A number of exposure schemes have been proposed to address issues of recovery and windows of increased susceptibility (Holsapple et al. 2005); one protocol spans all windows of vulnerability by dosing dams until pups are weaned, followed by direct dosing until pups are immunologically mature (approximately 42–46 days of age). If pharmacokinetic data are lacking such as when lactational transfer does not occur, direct dosing of newborns may be necessary. The existing evidence indicates that DIT testing is a vital component of an immunotoxicity assessment and will likely be incorporated into future guidelines as a result of new data.

1.10.2 In Vitro Immunotoxicity Testing

The development of in vitro assays to study immunotoxicity will likely be one of the major efforts in the next several years in the field of immunotoxicology. The demand for validated in vitro assays will be driven by such programs as the EU's Registration, Evaluation, and Authorization of Chemicals (REACH) and simultaneous increased public demand for a better understanding of the potential risk posed by chemicals in the environment and reduced animal use in basic and applied research.

Table 1.2 Seven key perinatal events in the developing immune system (reproduced from Dietert and Piepenbrink 2006)

Key perinatal immune events	Timing in humans	Benefit to host	Examples of concern	Health ramifications
Differentiation and seeding of macrophages (Mφ) to tissues	6–24 WG	Self-renewing populations of microglia, Kupffer cells, alveolar Mφ; resident Mφ function in tissues	Lead, LPS, ozone, cyclophosphamide	Inflammation of lung, brain, or liver tissue dysfunction (e.g., male infertility)
Seeding of thymus by pro-T cells and thymopoiesis to expand populations	Seeding 8–12 WG; massive expansion of populations 14–26 WG	Production of T cell clones necessary to establish peripheral T-lymphocyte populations	PAHs, T-2 toxin, tributyltins, TCDD	Thymic atrophy, decreased postnatal T cells and T-dependent function, increased risk of cancer and infectious diseases
Negative selection and apoptosis of autoreactive thymocytes	15–26 WG	Elimination of most peripheral T-lymphocyte clones	TCDD promotes unnecessary negative selection increasing apoptotic cell death	If promoted, deceased numbers of thymocytes. If impaired, increased risk of later-life self-reactivity
Treg cell ($CD4^+CD25^+$high) population generation in thymus, seeding and activation in periphery	Thymus appearance 12–13 WG; periphery 14–16 WG	Active suppression of postnatal autoreactive T cell clones	Possible low-dose cyclophosphamide, selected doses of cyclosporin A	If promoted, possible immune suppression. If impaired, risk of autoimmunity or allergy (e.g., breaking tolerance to nickel)
Perinatal dendritic cell maturations to support T_H1 responses	Birth-juvenile	Increase in dendritic cell maturation and T_H1-promoting capacity after birth to achieve necessary T_H1 balance	Dexamethasone, nicotine	Increased risk of allergy and some forms of autoimmunity (e.g., type 1 diabetes)

(continued)

Table 1.2 (contined)

Key perinatal immune events	Timing in humans	Benefit to host	Examples of concern	Health ramifications
Increase in T_H1 response capacity among peripheral T lymphocytes after birth	Birth-juvenile	Prevent life-long T_H2 skewing	Pb, Hg, kynurenines selectively impair T_H1 cells, 1-methyl-tryptophan may promote T_H1	With depressed T_H1, increased risk of T_H2 associated diseases such as atopy and asthma
Maturation and regulation of fetal macrophages via interactions with surfactants A and D and glutathione sources	16 WG- (SP-D) 19 WG (SP-A) through neonatal	Prevent oxidative lung damage and increased risk of respiratory disease; facilitate parturition, regulate Mφ	Ethanol	Increased risk of childhood respiratory disease; potential problems with labor, increased risk of autoimmune disease

In 2003, a workshop assembled by the European Center for the Validation of Alternative Methods assessed the current state of in vitro immunotoxicity testing and provided recommendations on prevalidation and validation studies (Gennari et al. 2005). The report identified in vitro assays for detecting immunosuppression and hypersensitivity as a critical need, and it recommended that human cell models were preferred to avoid interspecies comparisons and to facilitate validation using available human data. The participants acknowledged that in vitro assays for autoimmunity, DIT, and immunostimulatory effects were important, but concluded that in vitro models for accurately assessing these effects were not available. Similar to guidance documents for in vivo methods, the group suggested the use of a decision tree for evaluating immunotoxicity in vitro.

One testing scheme proposes first evaluating a chemical's myelotoxicity effects since bone marrow toxicity has many downstream effects on immune function. Substances found to be myelotoxic would not need further testing (Gennari et al. 2005). The colony forming unit granulocyte/macrophage (CFU-GM) assay has been well characterized in this regard (Pessina et al. 2001). The main disadvantage of the assay is that it is low throughput and laborious. Recent efforts have been made to develop high throughput alternatives, but these still require extensive validation. Following a negative myelotoxic result, a compound's lymphotoxicity should be addressed (Gennari et al. 2005). The whole blood cytokine release assay is a useful in vitro model for identifying immunosuppression and the only in vitro assay to date that has been prevalidated for immunotoxicity testing (Langezaal et al. 2001). The primary advantages of this assay are that it is conducted in human cells and is well characterized; however, primary human cells limit its use in high throughput screening (HTS).

The development of in vitro allergic hypersensitivity models is another area yielding encouraging results. Several promising keratinocyte and dendritic cell models have emerged in the last decade. For example, the human keratinocyte cell line NCTC 2544 and KeratinoSens assay are two promising models for detecting contact sensitizers. Early results have yielded an accuracy of 85% in tests on 43 contact sensitizers (Emter et al. 2010). These models are also advantageous because of their potential application in HTS.

In vitro testing is limited by a lack of adequate assays to replace such cornerstone assays as the PFC assay and histopathology. The most pressing need is for HTS of immunosuppressants. Current efforts are focused on developing these assays to serve as screens so that chemicals can be prioritized for further testing with traditional methods. These reports suggest promising beginnings for in vitro immunotoxicity testing but a clear need for new method development.

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Chapter 2
The Environment-Immune Route to Chronic Disease

Rodney R. Dietert and Robert W. Luebke

Abstract Specific environmental factors including chemicals, drugs, microbes, and both physical and psychological factors can affect the immune system producing dysfunction and, ultimately, an increased risk of chronic disease. Several different types of immune alterations can result from environmentally induced immunotoxicity and these connect immune insult to chronic disease. We illustrate how many immune-based chronic diseases arising in nonlymphoid tissues may be categorized based on the target tissue (e.g., skin, lung) rather than on the probable cause: a dysfunctional immune system. Finally, we discuss various factors that affect: (1) susceptibility to immunotoxicity and (2) the specific spectrum of disease outcomes following exposure.

Key Points

- Chronic diseases represent a global health threat.
- Inflammation appears to be the root of many, if not most, chronic diseases.
- Gender-based differences in disease manifestations are likely following environmentally induced immune insult.

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- Many immunotoxicants are capable of producing more than one type of adverse immune outcome (e.g., immunosuppression and immunostimulation).
- Exposure to more than one environmental risk factor can significantly alter the risk of chronic disease.

2.1 Introduction

The direct linkage of environmental immune insult from chemicals, drugs, and physical agents to immunotoxicity, immune dysfunction, and subsequent disease has been known for decades; however, the scope, impact, and economic ramifications of this linkage on a lifetime of health risks are only beginning to be fully appreciated. From the standpoint of immunotoxicity-associated environmental health risk, the focus of prior decades has been on immunosuppression that produces increased susceptibility to infection and a potential for reduced cancer immunosurveillance. An additional historic concern has involved chemical-associated hypersensitivity. These focal points continue to represent legitimate adverse outcomes of immunotoxicity.

The original landscape of immunotoxicity and immune dysfunction-associated adverse outcomes represents a relatively narrow focus and barely scratches the surface of present-day immune dysfunction-based health risks. Furthermore, as is common in most approaches to toxicological screening, previous immunotoxicity concerns placed an emphasis on acute health risks while the importance of immunotoxicity with risk of chronic disease went largely underappreciated, understudied, and potentially undetected. We now know that immune dysfunction-driven chronic disease is among the most significant present-day societal concerns. One only has to examine the extent to which existing drugs are currently prescribed and new drugs are being developed for the sole purpose of treating allergic, autoimmune, and inflammatory-driven conditions. The proportion of drugs directed toward these conditions is one measure of the extent of immune-based health problems.

In this introductory chapter, we provide a foundation for the array of adverse health outcomes firmly established and/or proposed to be promoted by environmentally driven insult of the immune system. The traditional approach for reviews considering immunotoxicity and/or immune dysfunction and disease is to present material organized by (1) specific types of immune assays (e.g., T-dependent antibody response [TDAR]), (2) broader categories of altered immune surveillance (e.g., defective cell-mediated immunity), or (3) broad defects in immune function (e.g., immunosuppression or hypersensitivity). In contrast, this book is organized by specific diseases or conditions under broader categories of adverse immune outcomes. This is a purposeful change from most prior treatments of immunotoxicity and immune dysfunction. It is designed to better align the most meaningful outcomes of immunotoxicity and immune dysfunction with medical diagnoses, disease prevalence information, therapeutic treatments, national and international research priorities, as well as with numerous disease-specific advocacy and support groups. As such, this can contribute toward more useful strategies for risk reduction of immune dysfunction-based disease.

2.2 Chronic Diseases and Conditions

Chronic diseases and conditions are among the most significant human health challenges that are faced today and represent a leading cause of death in much of the world (Magnusson 2009). Yet, until recently, these diseases have been largely unappreciated globally (Beaglehole et al. 2007; WHO 2011). The advent of effective vaccination against many infectious diseases that used to cause significant childhood and early adulthood mortality has meant that populations are now living longer and, as a result, experiencing a greater array of chronic health challenges. It also means that diseases that persist across decades and/or emerge later in life are more prevalent than were experienced by previous generations.

Chronic diseases can lead to premature death, but even when managed, place a significant burden on individuals, families, communities, and healthcare systems. This is not only a burden in long-standing developed nations, but also has become an emerging crisis in rapidly developing countries (Yang et al. 2011; Patel et al. 2011). Chronic diseases can affect the entire trajectory of a person's life by causing a significant reduction in quality of life. While chronic diseases may arise in and/or affect virtually every system of the body, recent evidence suggests that the status of the immune system and inflammatory regulation are significant in determining the risk of many, if not most, chronic diseases (Huang and Glass 2010; Agrawal et al. 2011; Brusselle et al. 2011; Harrison and Maloy 2011; Rocha and Folco 2011; Selmi et al. 2012; Shankar et al. 2011). One obvious question might be: how can a physiological system responsible for protecting against external assault of the host play such a pivotal role in the internal malfunction associated with chronic disease?

The answer appears to reside in the fact that the immune system is distributed among virtually every tissue and organ where it both surveys the integrity of, and influences the ongoing processes of, that host tissue. This is highlighted by the information given in Table 2.1, which shows examples of resident macrophage populations (often assigned specialized names) located in nonlymphoid tissues. The existence and functional importance of these cell populations greatly expand the impact of immune dysfunction from the classical views held only a few decades ago. Resident macrophages not only remove the dead and dying cells from the tissues, they also play a pivotal role in orchestrating the development, function, and eventual senescence of tissues. Research into the status of these resident immune cells (e.g., brain microglia) and the health of tissues is one of the more active areas of research. Dysfunction among resident immune cells in nonlymphoid tissues does have ramifications for risk of chronic disease.

For this expanded view, dysfunction in the immune system goes beyond host defense against infectious agents and includes the status of tissues and organs under immune surveillance and homeostatic regulation. A dysfunctional immune system has a high probability for causing tissue malfunction and chronic disease given sufficient time, diversity, and number of infectious agent challenges and normal changes associated with development and aging.

Immune dysfunction-based chronic diseases are found in virtually all tissues and physiological systems including the neurological, respiratory, immune, dermal,

Table 2.1 Examples of specialized populations of resident macrophages in different tissues

Organ or tissue	Population(s)
Liver	Kupffer cells
Lung	Alveolar macrophages
Brain	Microglia
	Astrocytes
Fat	Preadipocytes
Gut	Intestinal macrophages
Kidney	Mesangial phagocytes
Cardiovascular	Monocytes
	Perivascular macrophages
Reproductive organs	Testicular macrophages
	Uterine macrophages
Placenta	Placental macrophages (Hofbauer cells)
Bone	Osteoclasts

gastrointestinal, reproductive, endocrine, hepatic, skeletal-muscular, and urinary systems. They often arise in portals of entry for environmental agents such as the lungs, skin, and gut and may manifest as either highly local conditions or alternatively, disseminated or systemic diseases (e.g., atopic dermatitis, systemic lupus erythematosus).

The resulting conditions may involve relatively simple symptomatic endpoints (e.g., allergic rhinitis) or, alternatively, present with a menu of potentially complex age- and/or gender-related symptoms (e.g., myalgic encephalomyelitis, cardiovascular disease, celiac disease). In fact, many immune-based chronic diseases are likely to exhibit sexual dimorphism in prevalence, cause–effect relationships, and presenting symptoms (Doherty et al. 2009; Collins et al. 2011; Dong et al. 2011; Ghazeeri et al. 2011). For this reason, a sex-specific approach to the immunotoxicity–immune dysfunction–chronic disease triad is important.

2.3 Multiple Roles of the Immune System in Risk of Chronic Diseases

Historically, the adaptive immune system, including both antibody and T cell effector responses, has been seen as playing a predominant role in the risk of many chronic diseases (Amrani et al. 2000; Bridges 2004). A significant focus was placed on both T cell function and regulation by specialized T cell subpopulations (Georas et al. 2005). This focus was not misplaced given the importance of tissue-directed adaptive responses that contribute to the tissue pathogenesis of most allergic, autoimmune, and inflammatory diseases. This can be seen with the role of T helper (Th) cell status and IgE production in allergic disease (de Vries 1994; de Vries and Yssel 1996; Maezawa et al. 2004), the relationship between T regulatory cells and other cell populations (e.g., Tregs and Th17 cells), and autoimmune activity (Mai et al.

2010) such as has been seen in multiple sclerosis (Dalla Libera et al. 2011) and psoriasis (Bovenschen et al. 2011).

During the past few years, there has been a significant increase in both our understanding and appreciation of the critical link between innate immune status and risk of chronic diseases. Additionally, the status of pattern recognition receptors (PRRs), such as the toll-like receptors (TLRs) and nucleotide oligomerization domain (NOD) proteins (Schertzer et al. 2011; Werts et al. 2011) and the TLR signaling molecule myeloid differentiation factor 88 (MyD88) (Schröder 2009; Denkers 2010) in innate immune cells appears to be particularly important when it comes to both infectious agent-driven and sterile tissue inflammation such as that triggered by dying cells (Chen et al. 2007; Ionita et al. 2010; Lin et al. 2011; Leifer and Dietert 2011). Additionally, it has been suggested that changes in TLR status in early life can reprogram later-life innate immune responses (Mouihate et al. 2010). The status of PRRs and their signaling molecules is not only critical in determining the risk of inflammatory-driven diseases but these receptors and pathways also represent promising therapeutic targets for the treatment of many chronic diseases (Cole et al. 2010; Hennessy et al. 2010; Keogh and Parker 2011).

2.4 Breadth of Immune Dysfunction-Based Diseases

Immune dysfunction-based diseases are likely to be underestimated given the fact that a majority of these diseases occur in nonlymphoid tissues and are often medically coded based more on the malfunctioning target organ (Dietert 2011a). For example, many respiratory (asthma, allergic rhinitis), neurological (multiple sclerosis, Alzheimer's disease), cardiovascular (atherosclerosis, some forms of acute myocardial infarction), pancreatic (type 1 diabetes), dermal (atopic dermatitis, psoriasis, alopecia areata), gastrointestinal (celiac disease, inflammatory bowel disease), endocrine (Grave's disease), and hepatic (hepatic steatosis) diseases have immune and/or inflammatory dysfunction as a basis, yet they are likely to be viewed as target system-based diseases rather than as immune-based diseases. One of the goals of the present book is to raise the visibility of immune dysfunction as a causal basis for some of the most significant chronic diseases we face today. Obviously, if immune dysfunction is a basis for these diseases, then protection against immune dysfunction is the key strategy for preventing such diseases. In turn, correction of immune dysfunction, such as the correction of improper inflammatory responses, might be expected to reduce the risk of later-life-onset inflammation-driven conditions.

2.5 Impact of Immune-Based Diseases on Populations

Immune dysfunction-based diseases represent a formidable target for prevention. Based on recent disease prevalences, it was estimated that as many as a quarter of all children in some developed countries exhibited at least one immune

dysfunction-based disease (Dietert and Zelikoff 2009). The implications are that early life environmental conditions, when combined with genetic background, can result in immune alterations that have an immediate impact during childhood that manifest in serious illnesses. Furthermore, several additional immune dysfunction-based diseases (e.g., systemic lupus erythematosus, atherosclerosis) arise more often in adulthood.

If one considers only autoimmune diseases, the societal impact is staggering. For example, a recent report released by the American Autoimmune Related Diseases Association (AARDA) in a briefing for the U.S. Congress suggested that (1) approximately 50 million Americans suffer from at least one autoimmune condition, (2) the total direct cost to patients, insurance companies, and the federal government is estimated at more than 100 billion dollars, (3) there are more than 100 known autoimmune diseases, (4) more than 75% of those with autoimmune conditions are women, and (5) autoimmune diseases are among the top ten causes of death in women under the age of 65 (American Autoimmune Related Disease Association 2010, 2011).

2.6 Differential Vulnerability for Environmentally Induced Adverse Immune Outcomes

It is clear that not all individuals are at the same risk for immunotoxicity-associated immune dysfunction given the same environmental exposure. This differential risk is associated with differences in age, genotype, sex, and prior environmental exposures. These variables can determine not only whether immunotoxicity and immune dysfunction result from a given environmental exposure, but also the nature and duration of specific immune alterations and the specific risks of disease that are affected via the exposure. Therefore, the age, genotype, and sex of exposed individuals are critical factors in evaluating actual immune-based health risks, and data need to be distinguished based on these variables.

For example, in an age-based comparison of the immunotoxicity of five well-studied chemicals and drugs, Luebke et al. (2006) reported that the developing immune system was more sensitive than that of the adult. Across the different compounds, the increased sensitivity of the young was seen in a greater persistence of effects, lower doses required to induce adverse immune outcomes, and a broader spectrum of adverse effects. This is not surprising since discrete immune maturational processes occur in early development that are not replicated in the adult. Hence, an adult lacks many of the targets of immunotoxicity that occur in the young (Dietert 2009). For this reason, widely applied and effective developmental immunotoxicity (DIT) testing is an emerging issue in safety testing (Dietert et al. 2010; DeWitt et al. 2011).

In addition to age, genotype can affect the relative risk of environmentally induced immune dysfunction. For instance, allergic variants associated with cytokine and immune cell receptor expression can influence the immune vulnerability of a given subpopulation following environmental exposures to specific toxicants. As examples, the following gene–environment interactions pertain to the differences in

the risk of childhood asthma associated with specific environmental exposures. A common variant of the cytokine IL-13 has been reported to increase the risk for wheeze and persistent asthma following maternal smoking during pregnancy (Sadeghnejad et al. 2008). The G-308A allele of TNF-alpha was reported to increase the risk of childhood asthma associated with excessive home dampness (Tsai et al. 2011). Finally, alleles connected to TLR 2 and TLR 4 have been reported to increase the risk of asthma connected to traffic-related air pollution (Kerkhof et al. 2010). Exposure to environmental pollutants, including tobacco smoke and fine particulates, may increase the risk of autoimmune disease by altering patterns of DNA methylation (reviewed in Meda et al. 2011).

Sex is also connected to differential adverse immune outcomes even when the exposures occur during early life. Males and females can have different risks of immunotoxicity from a given environmental exposure. Even when immunotoxicity occurs in both sexes, the type of immune alterations can be quite different (e.g., impact of exposure on specific functional parameters, cytokine balance in males vs. females). Given the number of immunotoxicants that have been compared among the sexes, differences in immune outcome appear to be more the rule than the exception (reviewed in Dietert 2008). The sex of the individual has major implications for specific disease risk following environmental exposures. An immunotoxic exposure, such as cigarette smoking, increases the risk of immune and inflammatory dysfunction in both sexes. However, the spectrum of alterations in exposed males vs. females is not necessarily identical and this can result in differences in gender-based diseases manifestations. With the cigarette smoking example, concerns for women who are smokers include an elevated risk of lupus as well as cardiovascular disease while the focus in men remains largely on the risk of inflammation-related cardiovascular disease (Chen and Boreham 2002; Corwin et al. 2006; Kiyohara et al. 2009). While both men and women have an elevated risk of lung cancer associated with smoking, the risk per cigarette appears to be greater for women than men (Kiyohara and Ohno 2010). Smoking-related chronic obstructive pulmonary disease (COPD) has a classic later-age onset phenotype that was historically more predominant in men as compared with women (Fukuchi et al. 2004; Gershon et al. 2011), but that appears to be more a distinction in disease presentation among two sex-based phenotypes of this chronic disease (Ohar et al. 2011). In fact, women appear to preferentially experience a distinct early onset version of the disease (Foreman et al. 2011). Han et al. (2007) suggested that sexual dimorphism of the human immune response may be the basis of gender differences in this disease. For this reason, the risk of immunotoxicity, specific immune dysfunction, and specific chronic diseases needs to be considered by sex.

2.7 Environmental Risk Factors for the Immune System

Environmental risk factors (ERFs) for immunotoxicity and resulting immune dysfunction cover a wide range of chemicals as well as physical and behavioral environmental factors. Many of these factors have been the subject of prior reviews (Van

Loveren et al. 1995; Luebke et al. 2006; Dietert and Piepebrink 2006; Luebke et al. 2007) and will be highlighted in the context of specific chronic diseases in the subsequent chapters of this book. The categories of established ERFs include: (1) environmental chemicals/pollutants, (2) drugs, (3) physical and psychological conditions and stressors, (4) diet, and (5) the microbial environment including both the diversity of natural flora as well as pathological infections. ERFs can alter immune function in a variety of ways. They can act directly on established immune systems to produce immune dysfunction (Peden-Adams et al. 2008; Gardner et al. 2010). They can act as developmental immunotoxicants by exposure across the placenta and direct modulation of fetal developing immune cells (Heilmann et al. 2006, 2010). Additionally, they can produce later life immune alterations by producing epigenetically programmed alterations in the embryo that manifest as later life alterations in gene expression and immune programming (Martino et al. 2011). Toxicants, stress, infections, and dietary factors (e.g., methyl donors) have been implicated in fetal immune programming and/or epigenetic alterations of immune function (Wilson 2008; Pfefferle et al. 2010; Schaible et al. 2011). Epigenetic alterations have been suggested to promote immune-mediated diseases such as asthma (Pascual et al. 2011) and type 1 diabetes (Rakyan et al. 2011). Finally, recent evidence suggests that ERFs are likely to be able to exert effects subsequent generations across several subsequent generations (Skinner et al. 2010). While the study of immunotoxicity has a decades-long history (House and Selgrade 2010), the extent to which ERFs can produce unanticipated and undesirable immune outcomes is only beginning to be fully appreciated in the context of risk of chronic disease.

2.8 Multiple Adverse Immune Outcomes from Single Toxicant Sources

It is important to recognize that immune dysfunction-based diseases such as asthma can have multifactorial causes (reviewed in Dietert 2011b). But the converse is also true that single sources of toxicants can contribute to a myriad of immune dysfunction-based diseases. One of the most well-established examples is that of early life exposure to tobacco smoke via either maternal smoking during pregnancy and/or environmental tobacco smoke (ETS) (a source of numerous toxic chemicals). Individual susceptibilities vary based on dose, developmental timing of exposure, genetic background, and sex. But across the human population, tobacco smoke contributes to virtually every category of immune-based disease (Dietert and Zelikoff 2009). Immunosuppression-driven gaps in host defense are evident following exposure to tobacco smoke. Similarly, inflammation-driven later life diseases are a major outcome of these same exposures and include an elevated risk for diseases such as atherosclerosis (Oren et al. 2003) and COPD (Svanes et al. 2010; Forey et al. 2011). Allergic diseases are reported to be at elevated risk with maternal smoking and/or ETS exposure including not only the risk of respiratory conditions but also that of nonrespiratory allergic manifestations such as atopic dermatitis (Wang et al. 2008).

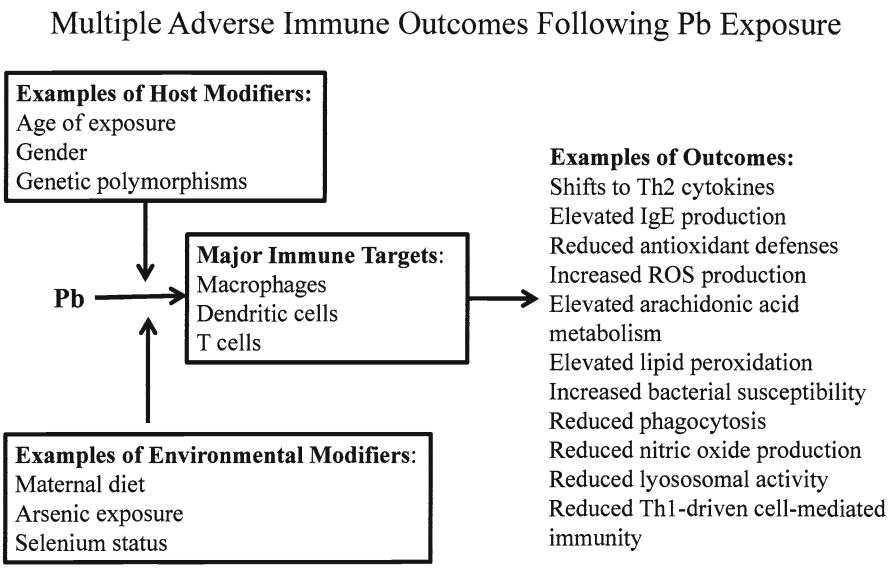

Fig. 2.1 The heavy metal lead (Pb) is able to target several different types of immune cells producing a wide array of immune dysfunction. This figure illustrates the major immune cell targets for Pb as well as both host and environmental modifiers that affect Pb-induced immunotoxicity. Adverse outcomes are shown on the right and include major shifts in T helper (Th) function and reactive oxygen species (ROS) production. These alterations affect not only the risk of immunosuppression-driven infectious diseases but also the risk of immunostimulation-driven allergic, autoimmune, and/or inflammatory diseases. Adapted from Dietert and Piepebrink (2006) and Leifer and Dietert (2011)

Tobacco smoke, as a single environmental source, represents a collection of numerous known immunotoxicants including heavy metals, polycyclic aromatic hydrocarbons, pesticides, and aromatic hydrocarbons. Therefore, an elevated risk across multiple categories of immune dysfunction-based diseases might be expected. A similar breadth of adverse immune outcomes has been reported for exposures involving only one toxicant from tobacco smoke, the heavy metal lead (Pb) (Dietert and Piepebrink 2006; Leifer and Dietert 2011). Figure 2.1 illustrates the multiple immunotoxic effects and/or elevated health risks that have been attributed to Pb.

In fact, it is clear that a majority, if not virtually all, immunotoxicants can contribute to more than one type of immune outcome and as a result, more than one category of immune-based disease (Fig. 2.1). What is observed as outcomes in a given exposed population depends upon the dose, age of exposure, sex of exposed populations, as well as the range of immune assessment and diagnostic tools employed. For example, neonatal gestational exposure to the herbicide atrazine suppresses immune function in rats (Rooney et al. 2003) yet stimulates function in mice (Rowe et al. 2006). Thus, blanket labeling of a chemical or drug as immunosuppressive or immune stimulating is tending to lose its utility. For example, cyclosporine A (CYP A) is a prototype immunosuppressive drug utilized in transplant patients.

Yet, even this drug is not necessarily a pure immunosuppressive agent. With gestational exposure to CYP A, there appears to be an elevated risk of later-life autoimmunity (Bucy et al. 1993). This is surprising only if one takes the quite narrow perspective of labeling immune-altering chemicals and drugs as only "immunosuppressant" or "immunostimulants," rather than immunomodulators that may be capable of producing suppression of some immune responses while stimulating others across the range of exposures and exposed populations. To avoid restrictive and possibly misleading classification, it is useful to define the specific immune dysfunctions that are observed based on different levels and conditions of exposure and the nature (e.g., age, sex, genotype) of the exposed populations.

2.9 Identifying and Partitioning Environmental Risk Factors by Functional Role

ERFs for immune dysfunction-based diseases are most often considered as one group particularly when it comes to public health strategies for risk reduction/avoidance. We may therefore discuss risk factors for childhood asthma or type 1 diabetes that span a variety of categories including environmental chemicals, drugs, and infectious agents. Grouping ERFs together, while convenient, has potential pitfalls when it comes to disease prevention, effective therapeutics, and even safety testing. The processes whereby immune dysfunction is created in an individual can be wholly distinct from the subsequent process in which the triggered response of the dysfunctional immune system contributes to pathogenesis of disease. Yet, the tendency is to blur the risk factors across these two distinct processes into one target for public health protection. However, it is important to recognize that ERFs often play distinctly different roles in the multistep process leading to immune dysfunction-based disease. Some are likely causative while others appear to simply trigger inappropriate responses. This is not a trivial distinction. Instead of contributing to disease by profoundly altering the course of immune development and causing immune dysfunction, some ERFs appear to act in the child in such a way that they reveal an already-existing immune dysfunction.

The example of childhood asthma can be used to illustrate these differences among EFRs. ERFs for childhood asthma would include an ever increasing list of factors such as maternal smoking, paracetamol, early antibiotic use, traffic pollution, respiratory syncytial virus (RSV), influenza virus, and cockroach allergen (reviewed in Selgrade et al. 2006; Dietert 2011b). However, factors such as maternal smoking during pregnancy are causative for childhood immune dysfunction. In contrast, there is little evidence that RSV infection or exposure to cockroach allergen actually causes immune dysfunction. A majority of children can encounter RSV without an outcome of childhood asthma. Instead, these EFRs appear to contribute to the steps toward childhood asthma by triggering a problematic response from an insufficiently balanced and/or already dysfunctional immune system. An inappropriate immune response to a respiratory viral infection caused by earlier-life

environmental exposures and genetic background can result in a diagnosis of childhood asthma shortly after the episode of infection.

This relationship extends beyond a single disease outcome. For example, the same respiratory viruses that can trigger immune dysfunctional responses and the onset of asthma in children can also produce a significant risk of secondary bacterial infections including pneumonitis in some individuals and require antibiotic treatment. Again, it is the status of the host immune system and the nature and progression of the host response to viral infection in the airways that largely determines the risk of additional airway disease.

From the standpoint of risk reduction, it may be impractical to think that children can be protected *en masse* from exposure to the various respiratory viruses that may trigger immune dysfunction-based childhood asthma. However, it is quite practical to think that we can better: (1) identify causative immunotoxicants that can produce childhood immune dysfunction and (2) reduce the risk of exposures that are likely to result in asthma and other chronic diseases.

2.10 Interactions Among Immune Risk Factors

Risk factors affecting immune function are most often studied individually. However, real-life environmental exposures are likely to involve interactions among multiple environmental factors and/or environmental and genetic factors. This can occur either from mixtures of potential toxicants (e.g., tobacco smoke), concomitant exposures from different sources (e.g., a child living in a lead-contaminated house and playing on an arsenic-contaminated playground), or sequential exposures during different stages of development (e.g., prenatal exposure to estrogenic chemicals followed by hormone replacement therapy as an adult). These interactions among both protective and detrimental factors can result in a variety of patterns of determining whether immune function is well balanced or significantly compromised.

2.11 The Importance of Improper Inflammation in Chronic Disease

In the following chapters in the book, a consistent theme will become apparent: inflammatory dysfunction is the form of excessive, inappropriately prolonged and/or misdirected inflammation that is at the root of many, if not most, chronic diseases. The association of inflammatory dysfunction and chronic diseases is becoming widely recognized (D'Mello and Swain 2011; Rocha and Folco 2011; Chang et al. 2012), and the diseases and conditions affected involve virtually every organ, physiological system, and medical category of disease. At issue is the precise role that inflammatory dysfunction plays. In some cases, this role appears to be causative while in others it appears necessary to maintain the chronic disease state. In other

Table 2.2 Examples of inflammatory diseases by system, organ, or tissue[a]

Target system or organ	Inflammation-associated disease or condition
Cardiovascular	Atherosclerosis; coronary heart disease
Dermal	Psoriasis
Dental	Dental caries/periodontal disease
Gastrointestinal	Celiac disease; colon cancer; inflammatory bowel disease
Hepatic	Nonalcohol fatty liver disease
Nephrotic	Chronic kidney disease
Neurological	Alzheimer's disease; multiple sclerosis; Parkinson's disease; depression
Otological	Otitis media
Pancreatic	Type 1 diabetes; Type 2 diabetes
Skeletal	Juvenile idiopathic arthritis; osteoporosis/risk of bone fractures
Systemic connective tissue disease	Systemic sclerosis

[a]Information adapted from Leifer and Dietert (2011)

Table 2.3 Examples of cancers reported to be associated with immune-inflammatory dysfunction in tissues[a]

Type of cancer(s)	Associated immune-inflammatory-related condition
Colon, small intestine, and rectal cancer	Ulcerative colitis
Lung cancer	Asthma, chronic obstructive pulmonary disease (COPD)
Skin cancer	Psoriasis
Liver cancer	Primary biliary cirrhosis
Stomach cancer	Pernicious anemia
Esophageal cancer	Systemic sclerosis
Thyroid cancer	Autoimmune thyroiditis
Various lymphoid malignancies	Rheumatoid arthritis, Sjogren's syndrome, celiac disease, psoriasis, hemolytic anemia, pernicious anemia

[a]Adapted from Dietert (2011a, b)

cases, inflammatory dysfunction may be a significant part of the spectrum of outcomes rather than playing a causative role. In fact, the topic of inflammation in chronic disease is now considered so pivotal that it is the subject of scientific forums, some of which seem likely to affect the direction of future health-related research. Among these is a 2011 internal U.S. National Institutes of Health conference titled "Inflammation: The Route and Root of all Chronic Disease?" with the sole purpose of educating NIH's extramural research officers and staff to the potential significance of the inflammation-chronic diseased linkage. Table 2.2 lists just a few chronic diseases and conditions where inflammation is thought to be key to the disease state.

There are further ramifications of dysregulated inflammation occurring in tissues. A surprising number of immune inflammation-related chronic diseases are associated with an elevated risk of specific cancers in later life. These fall into two categories: cancers involving immune cells (e.g., leukemias, lymphomas) and cancers involving the target tissue for the initial chronic disease. Table 2.3 provides examples of cancers that are associated with specific immune dysfunction-based conditions.

2.12 Conclusions

Chronic diseases represent a serious epidemic in developed countries and a growing threat in most developing nations. The impact is felt both economically and in terms of lost human productivity and quality of life. The shift in attention away from risk of acute disease that is necessary to address the global toll of chronic diseases requires a better recognition of the extent to which environmentally mediated immune dysfunction contributes to chronic disease. In turn, this requires an appreciation of the full range of adverse outcomes associated with immunotoxicity and the full spectrum of chronic diseases that are connected to immune dysfunction. Part of the solution for risk reduction of chronic disease resides with (1) an improved ability to evaluate immunotoxicity connected to immune dysfunction and (2) the ability to address immune dysfunction in children in such a way as to reduce the risk of later life emerging disease. The information contributed in the following chapters is intended to help increase the recognition of the role of immunotoxicity and immune dysfunction in chronic disease and fill the current information void that could help to address the increasing prevalence of global chronic disease.

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Part II
Allergic Diseases

Chapter 3
Asthma and Respiratory Allergic Disease

David M. Lehmann and Marc A. Williams

Abstract The pathogenesis of noncommunicable diseases such as allergy is complex and poorly understood. The causes of chronic allergic diseases including asthma involve to a large extent, immunomodulation of the adaptive and particularly the innate immune systems and are markedly influenced by distinct environmental and genetic interactions. In mammals, the existence of both innate and adaptive immune systems further complicates our ability to understand the mechanisms responsible for allergy and asthma. This chapter discusses the current state of the art in our understanding of allergic immunity and particularly the role of innate immunity as the major conduit linking the host response to the respirable environment. It also provides a conceptual appreciation of the complex interplay between host specific factors and those environmental triggers that challenge the immune system and provoke allergic conditions in susceptible individuals.

Key Points

- Chronic allergic diseases such as asthma have increased dramatically over the last 30 years and post a significant cost and health burden to society. Although

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such diseases can be clearly managed, there is currently no cure. A leading contributor of respiratory morbidity and mortality in the Western World are airborne pollutant particles and these are known to be associated with exacerbations of chronic allergic diseases like asthma.

- Allergic diseases encompass a spectrum of disorders in addition to allergic asthma that are typically characterized by the development of an overactive and inadvertent immune response to an otherwise harmless allergen, resulting in a CD4+ T helper type-2 (Th2) polarized or pro-allergic cytokine response to the allergen and the production of an IgE antibody response.
- The segregation of the immune response to innate and adaptive immunity permits an appreciation of the role that both arms of the immune response play in promoting allergic disease. The allergic innate immune response and particularly the role played by antigen sensing and presenting cells such as dendritic cells are particularly important.
- In the lung, respirable environmental danger signals including aeroallergens and particulate pollutants activate the pro-allergic response to dendritic cells that serve a crucial link between exposure to "environmental danger" and the adaptive response.
- The discovery of cell surface and intracellular pattern recognition receptors (PRRs) such as Toll-like receptors and the recently identified NALP3 inflammasome that sense respirable environmental triggers of allergic immunity have provided important information that may permit novel therapeutic targeting in allergic asthma.
- Predisposition of certain individuals to environmental triggers of allergic innate and adaptive immunity is as much dependent on genetic and epigenetic factors as it is inducible and constitutive factors that lead to defective epithelial barrier function and regulatory arms of the immune response such as regulatory T cells (Tregs) that help maintain peripheral tolerance to otherwise harmless antigens.
- Our understanding of allergic asthma and other chronic allergic diseases has increased markedly over the past decade. Recent developments in understanding the role of the gut microbiota and microbiome have been pivotal in this regard since they can affect both innate and adaptive immune responses. The gut microbiota plays a key role in modulating and helping mature the immune system and is markedly influenced by environmental factors. The gut microbiome plays a key role in maintaining immune tolerance and in guarding against the development of allergic disease.
- Future work will attempt to understand the immune networks involved in the breakdown of immune tolerance and the role played by the local immunological milieu in the gut, lung, skin, and bone marrow environments. It is likely that future therapeutic and preventative strategies in allergic airways disease like asthma will target distinct sites such as the gut microbiota in addition to deliberate targeting of the symptoms in the lung.

3.1 Introduction: Scope of the Problem, Fundamentals, and At-Risk Populations

The incidence of allergic diseases and particularly allergic asthma has rapidly increased over the last several decades in Westernized countries, but also more recently in newly emerging and rapidly industrialized countries in Indo- and South East Asia. Moreover, the impact of allergic disease is far-reaching and accounts for a high level of morbidity and in the case of severe asthma or anaphylaxis, may even be fatal. The impact on society is also quite significant with increased healthcare costs and lost productivity at work and schools all impacting on national economies. Socioeconomic and urbanized living conditions also impact on the incidence and severity of allergic disease, and this is particularly relevant when one considers the most susceptible individuals in society such as children, older adults, and minority populations.

Allergic diseases encompass a spectrum of disorders typically characterized by the development of an overactive (and often inappropriate) immune response to an otherwise harmless allergen, resulting in a CD4+ T helper type-2 (Th2) polarized or pro-allergic cytokine response to the allergen and the production of an IgE antibody response. Various routes of exposure provoke allergic diseases including contact sensitization and food allergies, which are discussed elsewhere in this book. In the setting of allergic asthma, the factors responsible for triggering disease exacerbations are numerous and are found predominantly in the respirable ambient air that we are exposed to every day. A major focus of research has set out to understand why certain respirable pollutants behave as allergens including those associated with vehicular traffic (e.g., diesel exhaust particles or DEP), ambient particulate matter (APM), certain classes of immunotoxicological and chemical pollutants, house dust mite, and cockroach antigens drive a particular inflammatory signature that may lead to symptoms in asthmatics only but not necessarily in otherwise healthy individuals. This tells us that an immune synergy is necessary between those mechanisms involved in de novo asthma development and those asthmatic symptoms that are initiated by causative agents that may exacerbate preexisting disease. Current thinking is that central to this immune synergy are cooperative pathways that link innate and adaptive immunity making treatment of allergic diseases like asthma difficult.

The risk of air pollution to human health and specifically as a protagonist in the exacerbation of preexisting allergic asthma or in the nascent development of inflammatory airways disease is one that has received escalating interest over the last decade. In the USA at least, the debate over the regulation of air pollution particles and ozone and the “acceptable” ambient levels of these air pollutants across states continues. The balance is partly dependent on achieving safe or acceptable levels that reduce the risk to human health. This needs to take into account the impact of air pollutants on climate change. Mitigating that risk by lowering the levels of ambient air pollutants as well as reducing costs by implementing revisions to what are perceived as “acceptable levels” of air pollutants, are the overarching goals of these efforts.

Similarly, asthma, and particularly allergic asthma, presents an inordinate economic burden upon society, not least of which is the concern that there is currently no cure of asthma. In the USA alone, more than 60 million Americans suffer from one kind of allergy or another including asthma, and the incidence of these conditions has been steadily increasing for the last 30 years with no respect for age, sex, or ethnic/racial boundaries. Allergy as a whole is the fifth leading chronic disease in the USA among all age groups and the third most prevalent condition in children under the age of 18 years of age. Hospital admissions as a direct consequence of asthma are a major cause for concern with over one quarter of emergency department visits in the USA being associated with the exacerbations and/or complications of preexisting disease and translating to over two million emergency room admissions each year. The annual cost of asthma to the US economy is estimated at almost $20 billion and of that the cost of treating asthma in children under the age of 18 in the USA is estimated at $3.2 billion per year.

Children inhabiting urban environments are particularly at risk of developing asthma and in suffering frequent and potentially fatal exacerbations of their condition, in part because of the morbidity associated with air quality and vehicular traffic pollution. Asthma-associated morbidity has been increasing in the USA for decades and inner-city children and minority populations are especially vulnerable (Weiss et al. 1992). The US Centers for Disease Control and Prevention (CDC) reported that during the years 1980 through 2005, non-Hispanic black and Puerto Rican children presented with higher incidences of asthma as compared with their non-Hispanic white counterparts and that black children in particular were more than 260% more likely to make emergency department visits and a 250% higher hospitalization rate. Of most concern was that non-Hispanic black and Puerto Rican children had a 500% higher mortality rate as compared with white children.

Similarly, the National Institute of Allergy and infectious Diseases focused on inner-city asthma and in 1991 begun a program to assess children with asthma who resided in economically poor households and urban communities. In this first study called the National Cooperative Inner-City Asthma Study, more than 1,500 children ages 4–9 were assessed (Kattan et al. 1997). This group of children was shown to have frequent and repeated episodes of asthma and presented with a broad spectrum of aeroallergen sensitivities, both of which contributed to the high incidence of asthma-associated morbidity in inner-city children. This work also indicated that an important environmental determinant of allergen sensitization and exposure in asthma commonly seen in inner-city children was cockroach allergens. Several follow-up studies were completed as a result of the information obtained from this first report (Evans et al. 1999; Szefler et al. 2008). These studies focused mainly on improving the quality of life and asthma care provided to inner-city children suffering from asthma as well as focusing efforts on reducing exposures to indoor allergens and pollutants that were known to contribute to the exacerbations of allergic asthma (Morgan et al. 2004).

Genetic and familial factors that may contribute to the incidence and severity of allergic asthma are poorly defined but extremely complex. Familial factors provide insight to our understanding of the incidence and heritability of asthma.

For example, there is an approximate one in three chance that each child will go on to present with asthma should one of the parents suffer from this condition. Moreover, in those rare instances where both parents suffer from asthma, then the risk that each child will go on to develop asthma increases to more than 70%. Of course, there are environmental factors too that all contribute to the overall risk of developing asthma. Such environmental factors include anthropogenic atmospheric or airborne pollutants such as ozone, DEP, and APM that collectively contrive to promote chronic allergic airways diseases (Chow et al. 2006; Pope et al. 1995; Dockery et al. 1993; Katsouyanni et al. 2009; Eftim et al. 2008; Abbey et al. 1999; Filleul et al. 2005; Ostro et al. 2010).

With these familial and gene–environment interactions in mind it is sobering to appreciate that so far, the only effective treatments for chronic allergic disease have been those that attempt to dampen the symptoms once they have become established. Despite some remarkable advances in clinical and therapeutic management, the global increase in allergic disease shows little signs of abating and there is currently no cure for many allergic diseases. This is especially true in the case of allergic asthma where curative therapy for this debilitating disease is at least 10 years away. Although standard clinical care for treating diseases like asthma has been available for some time, those therapies shown to be effective have been developed and incorporated into standard clinical care of allergic disease over the last 25 years and has significantly improved clinical management beyond what was available at that time. These pharmacological therapies control the disease largely by either limiting inflammation (e.g., inhaled steroids) or relaxing bronchial smooth muscle cells (e.g., long-acting and short-acting β-2 agonists). In the case of refractory asthma, current pharmacological interventions are mostly ineffective in controlling the disease (Humbert et al. 2005; Leckie et al. 2000; Brightling et al. 2008). Indeed even some of the more promising candidates used to date such as anti-IgE monoclonal antibodies have proved to be efficient in only some patients (Humbert et al. 2005), while others such as anti-IL-5 (Leckie et al. 2000) and anti-TNF-α (Brightling et al. 2008) have been found largely ineffective in the majority of individuals with asthma. The major reason for this is paucity in our understanding of the pathogenesis of allergic diseases like asthma. It also underpins the need for continued and considerable investment in basic and applied research efforts in understanding the mechanisms of action of environmental cues that promote asthma as well as the complex immunological pathways that are involved in the development of allergic diseases and in the exacerbations of preexisting disease.

The focus of this chapter is to understand the immunological mechanisms involved in allergic airways disease and particularly allergic asthma. We will survey the cells and mediators involved, with the aim of identifying important immunomodulatory pathways that are involved in allergic airways disease. We critically survey recent developments that have led us to consider environmental exposures to airborne and environmental pollutants as well as aeroallergens as novel pro-allergic "danger signals" or immune adjuvants in the development and exacerbations of allergic asthma.

3.2 What Is Asthma?

Asthma is a chronic respiratory disease of the conducting airways and is characterized by airway hyperresponsiveness, airway inflammation, transient airways narrowing (bronchoconstriction), secretion and deposition of mucus in the airways, and repeated episodes of airway obstruction, wheezing, and repetitive cough. These episodes occur both spontaneously and in response to a broad range of stimuli (Cohn and Westenberg 2004; Holgate 2011). In the severest form of the disease, episodic asthma can result in coma and death. The prevalence of asthma is growing worldwide and has increased markedly in Western populations over the past several decades (von Mutius 2009) achieving incidences of more than 10% in childhood and adolescence in many industrialized countries. Additionally, we have come to appreciate that asthma is a highly complex disease and despite some major advances, we still do not fully understand the cross-talk that is involved between the external environment, the pulmonary immune system, and gene–environment interactions that predispose certain susceptible individuals to the allergic asthmatic condition. Two major phenotypes of asthma have been proposed to exist. One variant is the atopic or allergic asthmatic phenotype. This is the most common variant found in infants and adolescents and will be the major focus of this chapter. By contrast, the nonatopic or "intrinsic" phenotypic variant of asthma becomes more prevalent in later life (Hollams et al. 2009; Sly et al. 2008; Stern et al. 2008). The relevance of nonallergic asthma will be discussed briefly below (see Fig. 3.1).

3.3 Nonallergic (Intrinsic) Asthma

Although less common than allergic asthma, intrinsic asthma is triggered by nonallergens foremost among which are irritants (e.g., dust, smoke, paint fumes, cleaning agents); sudden inhalation of cold, dry air, exercise, respiratory infections, and anxiety (Humbert et al. 1999; Novak and Bieber 2003). The most common agents to elicit irritant-induced asthma are chlorine, sulfur dioxide, combustion products, and ammonia (Mapp et al. 2005).

The cytokine profiles of the bronchial mucosa in allergic and intrinsic asthma are distinctly different. Expression of the IL-4 receptor is lower in intrinsic asthmatics and is associated with decreased expression of the transcription factor STAT6 (Christodoulopoulos et al. 2001). Also, BAL fluid collected from intrinsic asthmatics contains higher levels of RANTES (i.e., CCL5) than BAL fluid collected from allergic asthmatics (Ying et al. 1999; Humbert et al. 1997a, b; Folkard et al. 1997). RANTES is produced by macrophage subtypes (Ying et al. 1999) and may be involved in eosinophilia (Novak and Bieber 2003).

Intrinsic asthma is also associated with an increase in the abundance of macrophages in the bronchial mucosa expressing the alpha subunit of the GM-CSF receptor (Kotsimbos et al. 1997). The elevated number of macrophages has been hypothesized to be due to (1) recruitment of a subset of activated macrophages into

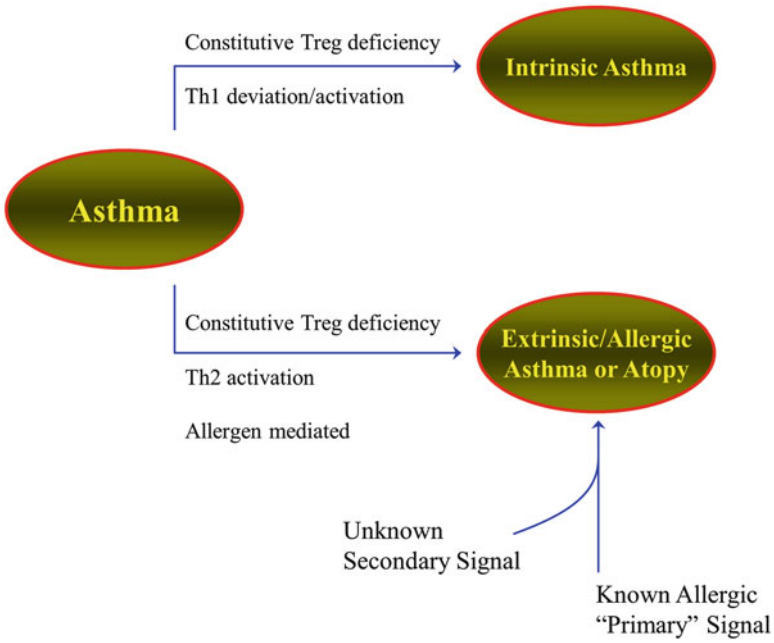

Fig. 3.1 Extrinsic/allergic and intrinsic nonallergic asthma. The immunopathology of allergy and asthma is complex. Allergy is distinguished by a Th2-dominance of pro-allergic inflammatory cytokines, IgE isotype class switching, and an underlying and inducible Treg deficiency following activation by an allergen. Asthma by contrast is characterized by a constitutive defect or deficiency in Treg activation and is associated with an underlying Th1 deviation. Allergic asthma is complicated more by a coupled Th1 deviation and Th2-mediated pro-allergic response that cannot be suppressed or regulated by Tregs under such conditions

the airway, (2) macrophage dysfunction, or (3) an ongoing infectious or autoimmune disease (Humbert et al. 1999).

Despite the clinical differences between allergic and intrinsic asthma, the basic similarities have led some to argue that these are not distinct entities immunopathologically (Humbert et al. 1999). Though nonatopic intrinsic asthma is an important variant of the disease, this chapter will focus almost entirely on the atopic or allergic phenotype of asthma since interactions between the adaptive and innate arms of the immune response play crucial roles in the pathogenesis of this form of the disease and it is by far the most prevalent.

3.4 The Concept of Atopy and Allergic Disease

The term atopy (originating from the Greek meaning for "out of place" or "atypical disease") was introduced some time ago (Coca and Cooke 1923) and in clinical immunology is typically associated with allergic diseases like asthma and dermatitis.

Atopic individuals often develop classic allergic diseases including atopic dermatitis, allergic asthma, and allergic rhinitis (Pearce et al. 1999). These diseases arise from an inherited predilection to develop inappropriate type I (immediate) hypersensitivity reactions to commonly encountered, normally innocuous, protein antigens (Stone 2003).

Pollen, pet dander, and excreta from house dust mites and cockroaches are common atopy-inducing allergens. The classic signs of atopy are elevated levels of allergen-specific IgE responses and positive skin prick tests to common allergens (Willart and Lambrecht 2009). Antigens that provoke atopy are typically soluble proteins often complexed with indoor or outdoor airborne particulate matter that is both respirable and migratory to the upper and lower airways of those individuals that come into contact with these materials. Thus, atopy is a predisposition of an individual to develop an IgE-mediated disease that is largely characterized by an eosinophilic infiltration.

Atopic diseases also represent a growing public health problem. The prevalence of atopy has been steadily increasing since the 1960s (von Mutius 2009). Some have estimated that the prevalence has increased by 400% during this time (McFadden et al. 2011). The steady rise in the number of affected individuals has been attributed to increased exposure to allergens and Th2/Th1 cellular imbalance leading to abnormally high production of IgE (Novak and Bieber 2003). However, no single causal factor has been identified.

Although the underlying mechanisms for the upward trend in atopy incidence remain elusive, it is clear that genetics combined with environmental factors contribute to disease etiology. In many cases, atopic individuals are simultaneously afflicted with three diseases including atopic dermatitis, allergic asthma, and allergic rhino-conjuctivitis. These are collectively referred to as the atopic triad (Oettgen and Geha 1999). These conditions tend to occur in sequence, a phenomenon referred to as the atopic march, beginning in childhood with eczema progressing to asthma and upper airway allergies (Demehri et al. 2009).

The steady increase in atopic dermatitis has been mirrored by a similar increase in allergic asthma. Asthma develops in up to 70% of patients with severe atopic dermatitis (Spergel and Paller 2003) and atopy has been proposed to be a significant risk factor associated with asthma. However, asthma also occurs in the absence of atopy (London and Romieu 2009). Given the co-occurrence of these diseases, it seems likely that both asthma and atopy may have common underlying mechanisms. Presentation of allergic asthma in susceptible individuals is highly dependent on interactions of host genetic factors and exposure to the external environment—the "gene–environment interaction" (Xiao et al. 2011; see Sect. 3.18).

3.5 Hypoxia-Inducible Factor-1 and Allergic Inflammation

Many advances have been made recently in our understanding of allergic inflammation, atopy, and asthma. One of the most intriguing is the concept of hypoxia-inducible transcription factors in allergic airway inflammation and asthma.

Recent evidence points to the hypoxia-inducible factor (HIF) family of transcription factors as key modulators of innate immune function and allergic asthma (Nizet and Johnson 2009; Huerta-Yepez et al. 2011). The HIF family of transcription factors enables cells to sense and respond to changes in oxygen tension—a crucial function of all nucleated cells. Under hypoxic conditions, gene expression is either switched on or off by HIF family members.

The HIF transcription factors are heterodimeric and are comprised of different alpha subunits and a common HIF-1β subunit (referred to as the aryl hydrocarbon receptor nuclear translocator or ARNT). HIF-1β is constitutively present in the nucleus and dimerizes to form a functional transcription complex. By contrast, the functional expression of HIF-1α is inducible and regulated by oxygen-dependent hydroxylation (Semenza 2007). Under normal oxygen levels, regulation of HIF-1α expression is tightly regulated and degraded following the binding of von Hippel-Landau (VHL) protein. HIF-1α is targeted for proteasomal degradation following the recognition of bound VHL by the E3 ubiquitin-protein ligase pathway (Maxwell et al. 1999). Moreover, activation of HIF-1 is seen in conditions such as cancer and in the general settings of immunity and inflammation. However, a recent study implicated the role of HIF-1α in allergic airway inflammation (Huerta-Yepez et al. 2011). It was shown that mice lacking functional expression of HIF-1β were protected from allergen-mediated inflammation of the airways in an ovalbumin (OVA) sensitization and challenge model. The authors also showed that in endobronchial biopsies taken from human subjects following allergen challenge that both HIF-1α and HIF-1β were upregulated, providing new information that supported the role of HIF in allergic airways disease. In mouse models of hypoxia of the lung, it was shown that the "HIF-1-regulated transcriptome" is complex with many Th2-associated gene products altered by hypoxic conditions such as arginase, the chemokine receptor CXCR4, Muc5AC, and the highly pleiotropic chitinase/Fizz family members that have been implicated in a number of Th2-mediated inflammatory conditions in the lung including fibrosis and in the draining lymph nodes (Nair et al. 2004; Fajardo et al. 2004; Hoshino et al. 2005). The growing appreciation that HIF-1 is an important transcription factor in the pathogenesis of allergic airway inflammation will contribute to our understanding of chronic allergic inflammatory diseases like asthma and may pave the way for novel therapeutic targeting of this condition.

3.6 Cell Types and Mediators in Allergic Inflammation and Asthma: A Brief Survey

Asthma is a heterogeneous inflammatory disease of the conducting airways accompanied by declining airway function and tissue remodeling. The order in which chronic inflammation and airway remodeling occur is an area of controversy (Murdoch and Lloyd 2010). The following are hallmarks of lung remodeling: increased thickness of the reticular basement membrane, deposition of extracellular matrix protein, neovascularization, increased airway smooth muscle mass (hyperplasia

and hypertrophy), decreased epithelial barrier function, and mucus gland hyperplasia (Siddiqui et al. 2007; Boulet et al. 1997; Woodruff et al. 2004; Ebina et al. 1993). It is generally believed that airway remodeling is the consequence of Th2-mediated chronic inflammation (Murdoch and Lloyd 2010). Th2 cells that secrete IL-4, IL-5, and IL-13 infiltrate the asthmatic airway leading to eosinophilia, leukocytosis, and subsequent activation of mast cells and IgE secretion from B cells (Kabesch et al. 2006; Loza and Chang 2007).

Eosinophils are drawn to the bronchial submucosa by local secretion of pro-eosinophilic cytokines like IL-5 and GM-CSF (Humbert et al. 1999; Esnault and Malter 2002, reviewed in Wardlaw et al. 1995). Eosinophils play a vital role in the inflammatory response in asthma and they are often used as a biomarker for the disease (Holgate 2011). Eosinophils damage the airway epithelium by release of cytotoxic granule-derived basic proteins and generation of reactive oxygen species (ROS) (reviewed in Wardlaw et al. 1995, discussed in Corrigan 2004). Th2 cytokines IL-4 and IL-13 are also found in the bronchial mucosa of asthmatics (Humbert et al. 1997a, b; Ying et al. 1997). These promote immunoglobulin switching in B cells resulting in IgE production (Del Prete et al. 1988; Minty et al. 1993).

Activation of mucosal mast cells is another characteristic of asthma. Degranulation of mast cells releases histamine, prostaglandin D2, and leukotriene C4. These induce characteristic asthma features: bronchoconstriction, mucus secretion, and mucus edema (expansion of mucus volume). Mast cells also release pro-inflammatory cytokines (i.e., IL-4, IL-5, and IL-13) involved in IgE immunoglobulin production, eosinophilia, and airway smooth muscle hyperresponsiveness. Binding of antigen to FcεR1-bound antigen-specific IgE typically activates mast cells. However, mast cells can also be activated by a number of IgE-independent factors relevant to asthma including proteases, cytokines (e.g., TNF-α, IFN-γ), complement, toll-like receptors, and monomeric IgE (discussed in Bradding 2008). An important consequence of innate immune activation in the airways and elsewhere is the recruitment and activation of dendritic cells and likely basophils too (see Fig. 3.2). Given the prominence of these cell types in allergic inflammation, the contributing roles of these cells in allergic asthma will be discussed in more detail in the relevant sections below.

3.7 Atopy, Allergy, and IgE

There are essentially two types of atopic reactivity, which are segregated into systemic and local reactions. Systemic atopic responses are called anaphylactic reactions and are disseminated throughout the entire body and will not be discussed further here. In localized atopic responses, the allergic reactions are largely dependent on the route of entry of an allergen and anatomical site of the cell type affected. In asthma, for example, there is inflammation of the upper and lower airways and lungs, and in allergic/atopic asthma, the inflammation of the airways is a consequence of an IgE-mediated response to respirable allergens.

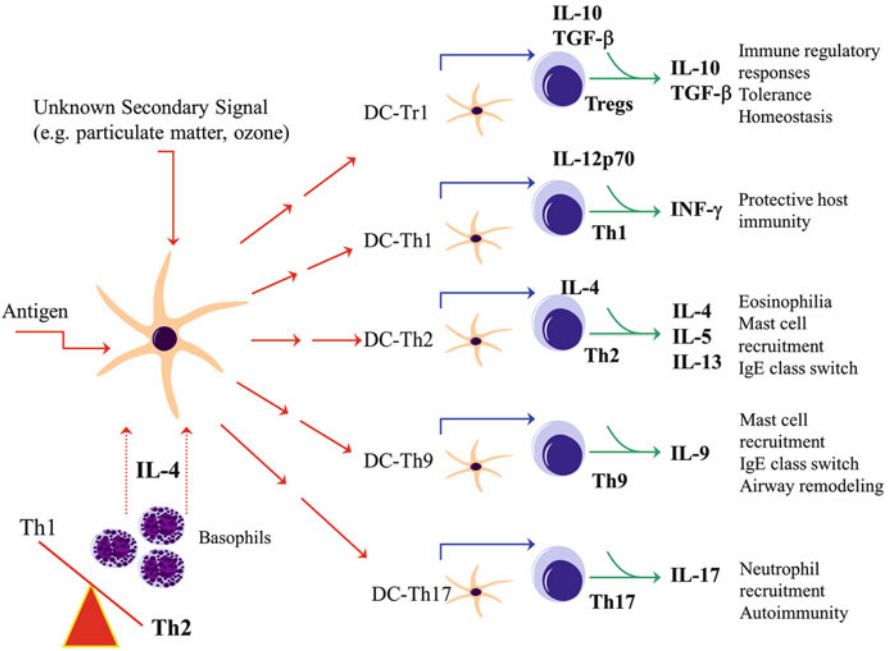

Fig. 3.2 Dendritic cell-mediated regulation of T cell differentiation. The nature of the signal that instructs dendritic cell functional activation also largely underpins the subsequent development of T cell subpopulation differentiation including those responsible for the allergic inflammation. In this schema, regulatory T cells (Tregs) differentiate from naïve T cells in the periphery that can dampen both Th1 and Th2 type immune responses. Tregs can use a number of feedback pathways to regulate immunity including secretion of IL-10, TGF-β, and other pathways such as CTLA-4 and PD1. Tregs can suppress Th2 cells directly or regulate the functions of "pro-Th2" DC to dampen the potential for a pro-allergic immune response. Under conditions of optimal or strong antigenic stimulation of TLRs, DCs secrete high levels of IL-12p70 that promote Th1 differentiation and functional activation. Under conditions of allergic stimulation or weak antigenic engagement of TLRs, secretion of bioactive IL-12p70 is downregulated and the production of IL-4 by differentiating T cells tips the balance from a Th1 response to that of a pro-allergic Th2 response. It is thought that inflammatory basophils that secrete high levels of IL-4 may promote the development of pro-Th2 DC further pushing the pro-allergic response. More recently, Th9 cells that secrete high levels of IL-9 and like Th2 cells promote IgE class switching and mast cell recruitment as well as Th17 cells that secrete high levels of IL-17 and promote inflammatory neutrophil recruitment have been identified and these T cell subsets too may be under the distinct immune regulation of DC subpopulations

Similarly, atopic dermatitis or allergic eczema is an allergic inflammatory response in the skin and is also IgE mediated to an allergen that triggers mast cell or basophil activation and degranulation. Some common allergens that are known to trigger both skin and respiratory symptoms include the allergens Der p1 (house dust mite derived from *Dermatophagoides pternonyssinus*), Fel d1 (cat dander derived from *Felis domesticus*), Amb a2 (or Ragweed, *Ambrosia artemisifolia*), Asp f1 (mold spores derived from *Aspergillus fumigatus*), Phl p5

(Timothy grass or *Prunum avium*), and more recent respiratory inflammatory agonists associated with anthropogenic activities including diesel exhaust particulate matter (DEP), ambient airborne particulate matter (APM), ozone, and other xenobiotics that may behave as immune adjuvants in promoting allergic inflammation.

Antibodies of the IgE isotype are classically associated with atopy and allergic diseases such as asthma. IgE circulates in the peripheral blood as monomers of 190 kDa and is present in the serum at an extremely low level and in the order of some 50 pg/mL or 0.000003% due to the infrequency of class switching to this antibody isotype. IgE is thus the rarest of all antibody isotypes. While in the circulation, IgE has a short half-life of some 1–5 days. Additionally, human serum IgE, although low at birth, increases steadily to a peak shortly after puberty and declines steadily thereafter. Unlike other immunoglobulins, IgE is quite different in that it fails to fix complement, does not behave as an opsonin, nor does it ever cross the placenta.

In atopic individuals, IgE concentrations can be up to 1,000 times greater than in normal individuals but remain at levels that are still far lower than the normal baseline concentrations of other immunoglobulin isotypes. CD4+ Th2 cells play a crucial part in this isotype class switching to IgE and enhanced IgE production. Th2 cells are the primary sources of cytokines such as IL-4 and IL-13 required for switching immunoglobulin synthesis to the IgE isotype. However, natural killer T (NKT) cells, mast cells, or basophils and eosinophils can also secrete IL-4 and IL-13 though NK cells can only produce IL-13.

Despite their very low abundance in the serum of normal individuals, the physiological and clinical functions of IgE are important and remarkable. In conditions of parasitic worm infections, for example, the levels of IgE rise steadily and dramatically. This encourages the migration of pro-allergic CD4+ Th2 cells to the local area following the uptake of worm antigens by phagocytosis and endocytosis and the subsequent processing and presentation of those taken up antigens to Th2 cells by antigen-presenting cells like dendritic cells. The secretion of Th2 cytokines, such as IL-4 and IL-13, promote immunoglobulin isotype switching in local memory B cells to IgE, thereby amplifying the response. Additionally, eosinophils express a high-affinity receptor for IgE FcεR1 and are the only lytic cell type capable of destroying invading parasitic worms. Local secretion of IgE as a consequence of the parasitic worm infection coats the invading worm that is then recognized and eliminated by locally infiltrating eosinophils in an FcεR1-mediated mechanism.

However, in allergic or type I (immediate) hypersensitivity conditions, secreted IgE promotes many of the disease manifestations associated with allergic asthma as well as wheeze, urticaria (hives), hay fever (allergic rhinitis), and anaphylactic shock. Mast cells and IL-4 secreting basophils are pivotal in this setting and are already in a primed state of activation whereby high affinity FcεRI receptors bind secreted IgE prior to binding antigen. This serves to focus the concentration of IgE to the surface of mast cells, which can then display the IgE molecules such that they behave as antigen-binding receptors. Shortly after a

critical threshold density of allergic antigens bind to and cross-link FcεRI receptor-bound IgE molecules, the "primed" mast cells are activated to degranulate and release their armory of inflammatory mediators that both induce the characteristics of a pro-allergic immune response and the typical symptoms associated with allergic reactions.

These mediators include histamine which induces vasodilation, increased blood vessel permeability, mucus deposition, bronchial smooth muscle contraction, and sensory nerve excitation that is responsible for the sneezing and itching responses of the allergic sufferer. Other mediators produced by degranulation of mast cells and basophils include: chemokines that promote the chemotaxis of neutrophils and eosinophils; inflammatory cytokines that activate cells of the innate and adaptive immune response; proteases that promote mucus deposition, cytokine production, and basement membrane digestion.

Upon the arrival of infiltrating eosinophils, for example to the lung, mediators such as major basic protein contribute to the ability of mast cells to degranulate, promotes smooth muscle contraction, and apoptotic cell death of respiratory epithelial cells—a feature also shared by eosinophilic-derived cationic protein. Other factors largely produced by mast cells or present on the cell membranes of mast cells include platelet activating factor (PAF) and prostaglandins that contribute to platelet aggregation and degranulation and pulmonary smooth muscle contraction. Leukotrienes produced by mast cells and degranulation of eosinophils can also promote vasodilation, pulmonary smooth muscle contraction, mucus secretion, and enhanced blood vessel permeability.

Many of these factors collectively contribute to the tissue remodeling process in which the uppermost layers of the bronchial airways die and are stripped away following which the submucosal layers thicken as a consequence of increased deposition of collagen underneath the basement membrane. Contributing to this process of an inflamed and morphologically altered airway are chronically pro-inflammatory cells, inflamed cells and tissues and newly arriving pro-allergic cells of the adaptive and innate immune systems typically characterized by infiltrating eosinophils, mast cells, basophils, T cells, B cells, and neutrophils. FcεRI receptors recognize all IgE molecules (i.e., total IgE) irrespective of antigenic or allergen specificity. Thus, all IgE-primed mast cells are fully capable of efficiently responding to many different allergens in a highly efficient and robust allergic or antigen response. It should also be noted that following stimulation of the high-affinity FcεRI receptors on mast cells, there is no lag phase; the degranulation response is immediate and there is thus a continuum of histamine and pro-allergic mediators secreted.

In the setting of intrinsic asthma, total serum IgE may be elevated and it correlates with both asthma prevalence and severity (Beeh et al. 2000). There is also evidence of local IgE production in the airways of those with intrinsic asthma. The IgE receptor FcεRI, which is regulated by IgE, is expressed in the bronchial mucosa of nonatopics (Humbert et al. 1996a, b). Furthermore, IgE heavy chain and Cε transcripts that are both characteristic of IgE switching are present in the bronchial mucosa of nonatopics (Ying et al. 2001). However, production of mature IgE has

not yet been demonstrated. The significance of local IgE production in intrinsic asthma is unclear, but it may relate to unidentified allergens (discussed in Humbert et al. 1999; Corrigan 2004).

3.8 Allergy and the Curious Functions of IgG4

In the human system, IgG4 is also associated with allergic reactions although the functions of this immunoglobulin remain poorly understood. Like IgE, IgG4 is present in the serum at a very low abundance and is the least abundant of the IgG immunoglobulin isotypes (nominally less than 5% of the total). There is also some Th2-dependent similarity between murine IgG1 and human IgG4. It was found that there was an association between different immunoglobulin isotypes and disease specificity. For example, allergens were found to be associated with an IgG4 response, at least in patients that were receiving allergen-specific immunotherapy.

The interaction of IgG4 and either Fc gamma receptors or the complement component C1q is also weaker than for other immunoglobulin isotypes. However, a unique feature of IgG4 is a predilection to interact with other immunoglobulin molecules. Using IgG1 as the binding target, IgG4 was found to have binding activities that were mediated by its constant (and not variable) domains. Additionally, like IgE, the class switching to IgG4 requires IL-4 or IL-13 (Punnonen et al. 1993; Vercelli et al. 1998) and both IgE and IgG4 are now considered an integral component of the pro-allergic Th2 immune response (Meiler et al. 2008; Avery et al. 2008).

Additionally, there is a situation referred to as the modified Th2-mediated response (Platts-Mills et al. 2001) whereby certain individuals mount an IgG4 response in the absence of measurable IgE, and subsequent studies have shown this is quite common. Indeed, the profile of Ig4 in the absence of IgE is usually seen following occupational exposures to protein antigens (e.g., rodent allergens) and the modified Th2 immune response is considered the typical or physiologically normal response to an innocuous antigen (Akdis 2006). However, it is unusual for some typical allergen exposures not to elicit an IgE response and in these situations the secretion of IgG4 is essentially absent, for example following exposure to grass pollens or house dust mite allergens (Thomas and Hales 2007). This disparity suggests that not all allergens provoke the same type of response and are unique in the type of Th2 response induced. That said however, both IgE and IgG4 require Th2 cytokines for synthesis, and in those allergens that provoke IgE secretion, they are usually found to also promote IgG4 production. Nonetheless, in terms of allergic diseases, in general, IgG4 has a rather ambiguous association with allergic disease symptoms since it is unclear to what extent (if any) IgG4 contributes to the observed symptoms. High levels of IgG4 are usually found in symptomless individuals and IgG4 in this setting is considered a marker of tolerance induction. Although the mechanisms are poorly understood, in the context of IgE-facilitated allergen presentation, IgG4 may attenuate this process because of competition between IgG4 and IgE. IgE-facilitated allergen

presentation is a process whereby in the presence of high-affinity IgE receptor bearing antigen-presenting cells, the receptor bound IgE antibody substantially lowers the dose of allergen required to activate T cells.

3.9 The Susceptible Airway in Allergic Asthma

The airway epithelium is the largest surface in the human body that is in continuous contact with the environment (Fahy and Dickey 2010). The lung epithelium is also, along with the mucociliary escalator, the first physical barrier to inhaled insults (Xiao et al. 2011). About 11,000 L of air go through the respiratory system daily. Thus, the intricate surface of the airway epithelium is constantly exposed to aeroallergens and other noxious substances (Seibold and Schwartz 2010, see also Fig. 3.3). Defects in the epithelial barrier may lead to increased penetration of agents that might promote asthma and contribute to bronchial hyperresponsiveness in response to environmental stimuli (Xiao et al. 2011).

The epithelial barrier is a complex network of cell–cell and cell–extracellular matrix interactions that physically link adjacent cells and seal off the intercellular space (Groschwitz and Hogan 2009). Loss of epithelial barrier function has been implicated in the development of atopic dermatitis and food allergy (De Benedetto et al. 2011; Palmer et al. 2006; Heyman 2005). Likewise, there is evidence of abnormal bronchial epithelial barrier function in asthma. Previous studies have shown that damaged epithelium is present in adults and children with asthma (Puddicombe et al. 2000; de Boer et al. 2008).

Tight junctions are key players in maintaining the integrity of the physical barrier (Xiao et al. 2011). Located in a band just beneath the apical surface of polarized epithelial cells, tight junctions control permeation through the paracellular route (Cereijido et al. 1988). As shown by immunohistochemistry of whole airway tissue mounts, epithelial tight junctions are severely disrupted in asthmatics (Fahy and Dickey 2010; de Boer et al. 2008). Tight junctions do not fully form in asthmatic epithelium cultured at the air–liquid interface in vitro (Holgate et al. 2009). Furthermore, even when fully differentiated, the physical epithelial barrier formed by these cultures is leaky (Holgate et al. 2009; Xiao et al. 2011).

In addition to the decreased physical integrity of the epithelial barrier, the ability of the epithelium to limit the damaging effects of ROS may also be hindered in asthma due to impaired antioxidant pathways (e.g., superoxide dismutase and glutathione peroxidase) (Sackesen et al. 2008; Yang et al. 2009; Schultz et al. 2010). Cell stress and injury markers are found in adults and children with a range of asthma severities. Thus, epithelial injury may not only result from the disease, but also play a role in its development (Holgate et al. 2009; Puddicombe et al. 2000; Bertorelli et al. 1998). Normally, ROS-induced injury triggers repair mechanisms. However, in asthma, the repair process is incomplete, which leads to tissue remodeling and structural changes to the airways (Holgate 2011).

Structural changes associated with asthma include myofibroblast hyperplasia, myocyte hyperplasia and hypertrophy, epithelial hypertrophy, mucus gland

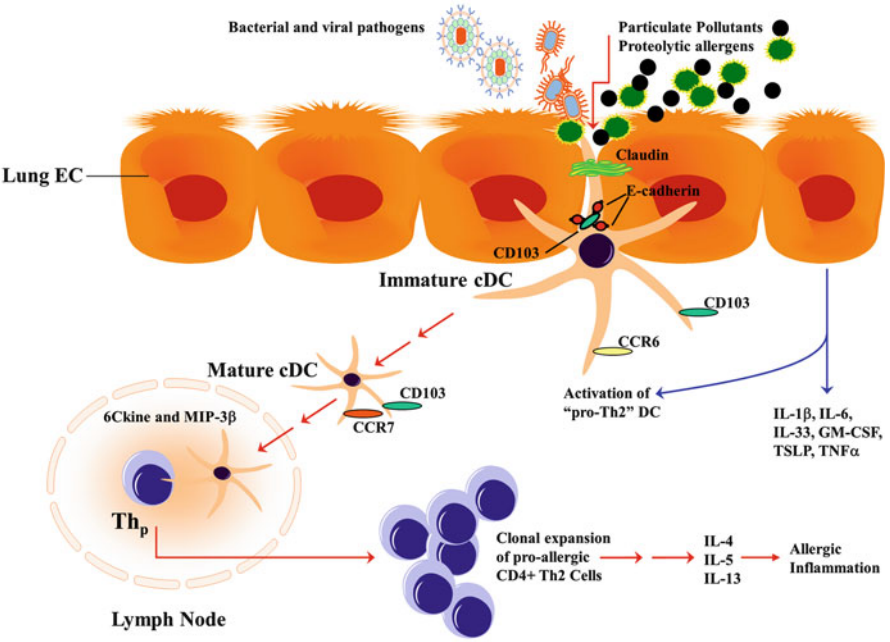

Fig. 3.3 Pro-allergic inflammation and the communication of lung epithelial cells with dendritic cells. The relationship between a Th2-directed immune response and organ (lung)-specific allergic inflammatory diseases (atopy) is complex. Inadvertent immune dysfunction partly characterized by genetic predisposition and partly characterized by unique environmental triggers promotes allergic inflammation and asthma. In this model, CD103+ lung dendritic cells sample their environment via the airway lumen by extending their dendrites between the tight junctions of adjacent lung epithelial cells (EC). By extending their processes to the lumen, DCs form remodeled tight junctions by expressing claudin and occluding adhesion proteins, and firmly attaching to the lung EC by expression of E-cadherin. Proteolytic allergens and respirable ambient pollutants (e.g., diesel exhaust particles or ambient particulate matter) can gain access to DC and stimulate both pro-inflammatory and pro-oxidative activation of DC partly by engaging cell surface expressed or intracellular toll-like receptors or other pattern-recognition receptors (PRRs) such as C-type lectins or protease-activated receptors (PPARs) in addition to activation of DC following endocytic or phagocytic uptake. Following activation of immature pro-Th2 DC, loss in expression of the chemokine receptor CCR6, and gain in expression of the chemokine receptor CCR7 directs the migration of what are now mature lung DC to the regional peribronchial or mediastinal lymph nodes in response to the chemokine gradients put down by CCL19 (also known as macrophage inflammatory protein-3-beta [MIP-3-β]), and the chemokine CCL21 (also known as 6Ckine, exodus-2, and secondary lymphoid-tissue chemokine or SLC). It is in the lymph nodes that DCs form immunological synapses with naïve CD4+ T cells and instruct their clonal differentiation to pro-allergic Th2 cells that is characterized by secretion of IL-4, IL-5, ands IL-13 in the lung

hyperplasia, angiogenesis, and subepithelial fibrosis (Busse et al. 1999, reviewed in Lemanske and Busse 2003). Once these changes occur, the effects are long lasting and lead to reduced lung function. Interestingly, these structural changes are present not only in severe asthma, but also in mild to moderate asthma (Busse et al. 1999; Jarjour and Kelly 2002). Although remodeling is viewed to be the result of chronic

inflammation, restructuring of the airways has been reported prior to symptomatic asthma in young children (reviewed in Bush 2008). This observation coupled with the prominent role of the airway epithelium in asthma susceptibility prompted Holgate et al. (2004) to propose a role for the epithelial–mesenchymal trophic unit in asthma pathogenesis (Holgate et al. 2004). In this model, perpetual activation of fibroblasts, smooth muscle cells, neurons, and the epithelium generates a chronic wound healing state, which sets the stage for chronic Th2 cell-mediated inflammation and consequent airway remodeling (Holgate et al. 2004).

3.10 Innate Immunity and the Recognition of Danger in Allergy

Perhaps one of the most important advances in immunology was the categorization of the immune system to innate and adaptive responsiveness. The adaptive immune system is largely derived from the functional contributions of B and T lymphocytes in protective host immunity (Medzhitov and Janeway 1997). By contrast, innate immunity is driven by many other cell types of myeloid and nonmyeloid lineage that respond to infectious agents, damaged cells and tissues. The adaptive immune system is also slow to respond to infectious agents, taking days to weeks to fully mount an adequate immune response to an infection while the innate immune system responds more rapidly taking minutes to hours to provide the initial immunological strike against an invading pathogen.

Additionally, the adaptive or specific arm of host immunity enables the host to prepare for secondary immunological challenges. By contrast, the innate immune system responds to such infectious agents or other exogenous "nonself" challenges in a less specific though much broader manner and there is quite complex interplay between the strictly antigen-specific adaptive and antigen nonspecific innate immune responses. While this cooperation operates well, it is important to realize that distinct microbial or molecular patterns as well as the vast array of protein antigens recognized differ significantly between the innate and adaptive arms of the immune system.

Cells that constitute innate immunity are quite unique in that they exhibit a highly restricted ability for prolonged immunological memory and respond to an equally restricted set of antigenic targets. This is achieved partly by the expression of an evolutionary conserved and phylogenetically "ancient" class of PRRs encoded by the genomic DNA. Innate immunity includes both sensor and effector components that participate in both humoral and cell-mediated immune defense (Wright 2005; Antal-Szalmas 2000). One of the major functions of the innate immune system is to communicate with and direct the functional responsiveness of adaptive immunity, which in the case of vertebrates is critical for protective host immunity. The innate immune system, encoded in the germline DNA, is phylogenetically ancient and better established than the evolutionary more "recent" adaptive immune system. The innate immune system is thus evolutionary conserved in all complex vertebrates and invertebrates whereas the adaptive system evolved only in mammals and other vertebrates.

It is pertinent at this point to survey the role of innate immunity in recognizing the external environment and the manner by which the immune system is able to distinguish potential immunological agonists (or danger) from those environmental cues (both exogenous and endogenous) that are otherwise innocuous under normal physiological conditions and otherwise ignored or tolerated by the host immune system. A hypothesis termed "the danger theory" has captured the imagination of many investigators and is a widely accepted tool to explain the ways by which the immune system distinguishes noninfectious self (i.e., those environmental signals that are innocuous and otherwise ignored or tolerated by immunity) from infectious nonself (i.e., infectious danger signals that trigger an inflammatory primary immune response).

Polly Matzinger and co-workers proposed the "danger theory" in 1994, and in follow-up studies by others, to help our understanding of how the immune system is able to distinguish between dangerous and innocuous signaling patterns (Matzinger 1994; Seong and Matzinger 2004; Zitvogel et al. 2010). The noninfectious or innocuous signals were found to be derived from molecular structures termed damage-associated molecular matters (DAMPs) or cell death-associated molecules (CDAMs). PRRs recognize such molecular DAMPs and CDAMs and on occupation of distinct PRRs that have highly specific and almost exclusive binding affinities for a particular type of DAMP or CDAM. The expression and function of PRRs is gaining increasing importance in our understanding of allergic innate and allergic adaptive immune responses.

PRRs facilitate the recognition of DAMPs and so-called pathogen-associated molecular patterns (PAMPs), both of which are recognized by cells of the innate immune system as "danger signals." The PRRs that recognize DAMPs include complement receptors, prostanoid receptors (including DP1, EPA4, and IP), neuropeptide receptors (including NK1 and CGRPR), high-mobility-group box 1 (HMGB-1) receptor (e.g., RAGE), purinergic receptors (including P2X and P2Y) as well as the receptors for heat shock proteins. The PRRs that recognize PAMPs are similarly numerous and include Toll-like receptors 1 through 13, intracellular receptors such as TLR7, TLR9, and NOD-like receptors (NLRs), C-type lectin receptors (including the macrophage mannose receptor or CD206, dectin, DEC-205, and BDCA-1), and the RIG-like receptors. Occupation of cell surface or intracellular PRRs by DAMPs, PAMPs, or CDAMs provokes inflammatory and oxidative-stress-mediated immune responses.

The functional expression of PRRs serves to mediate biological responses to quite disparate environmental cues including invading pathogens, commonly encountered environmental and air pollutants such as DEP and oxidative stress. Unlike PAMPs that are derived from pathogens, CDAMs and DAMPs are largely derived from the plasma membranes, the nucleus, cytoplasm, cytoplasmic organelles, and mitochondria. However, both DAMPs and PAMPs are immunomodulatory and provoke an immune response when seen by antigen-presenting cells like dendritic cells or macrophages. Since PRRs recognize DAMPs (or CDAMs) and PAMPs, there is very likely a similar signaling pathway and cellular responsiveness in the context of pathogen-mediated and nonpathogen or innocuous inflammatory triggers.

In mammals, the functional importance of PRRs in innate immune recognition of infectious nonself and distinguishing this from noninfectious self was worked out

by seminal studies pioneered by Charles Janeway (and reviewed in Janeway 1992). The reader is also guided to Chap. 1 that details the identification and function of PRRs in immunity. The first PRRs identified were scavenger receptors found to be highly expressed on macrophages (Kodama et al. 1990). During this time, studies in the fruit fly Drosophila had identified Toll as an important factor in cellular development and embryogenesis (Anderson et al. 2011).

Additional work showed that Drosophila Toll played a crucial role in mediating host defense to fungal pathogens (Lemaitre et al. 1996). Studies in human and animal models subsequently identified a PRR called Toll-like receptor 4 (TLR4) as a homologue of Drosophila Toll which was found not only to play important roles in protective immunity but also served as a co-receptor for gram negative bacterial endotoxin or lipopolysaccharide (LPS) that along with the glycosylphosphatidylinositol (GPI)-anchored cell surface receptor CD14 permitted innate and adaptive immune responses to be mounted against bacterial infections (Lemaitre et al. 1996; Medzhitov et al. 1997; Poltorak et al. 1998a). The expression of CD14 had since been shown to be a component of the endotoxin receptor complex (Vercelli 2008)—more on this below.

Several of the TLRs are cell surface expressed (e.g., TLR1, TLR2, TLR4, TLR5, and TLR6) while others are cytosolic associated with early and late endosomal units (e.g., TLR3, TLR7, TLR8, and TLR9). The repertoire of ligands recognized by TLRs is not just restricted to microbial pathogens but also to molecular structures or patterns that are indicative of cell or tissue damage in the inflammatory lung environment. Both cell surface TLRs and endosomal-expressed TLRs associate with each other as homo- or hetero-dimers and it is this structural feature of TLRs that enables ligand specificity by virtue of extracellular or transmembrane-spanning leucine rich repeats. Although there are now thought to be up to 13 TLRs in the human system recognizing diverse PAMPs and DAMPs with similarly broad cellular and tissue distribution, the functional expression, complex signaling pathways, and the role of TLR4 in environmental pulmonary inflammation and allergic asthma is particularly relevant.

3.11 The Hygiene Hypothesis: Relevance to Allergy

An interesting concept proposed in 1989 and termed the “Hygiene Hypothesis” (Strachan 1989) was put forward to assist our understanding of how allergic asthma has increased in prevalence at the same time that our quality of life has improved. At the time that this hypothesis was first published, it had already been largely acknowledged that early life exposures to viral (and likely bacterial) infections dampened the likelihood of the onset of an allergic disease like asthma later on in life. In this novel yet controversial hypothesis, Strachan proposed that it was a lack of early life exposure to microbial infections, in developed and industrialized countries, that contrived to impair the normal development of host protective immunity leading to an enhanced risk of allergic diseases in later life. Moreover, it was the first hypothesis to suggest that common allergic diseases found in children were less frequent in those that had many other siblings where presumably they were exposed

to a "less sterile" environment than those children that were the only child. Presumably, exposure to viral and bacterial infections in early childhood provided protection from developing the pro-allergic immune responses that may develop in children from families with only one child since the repeated challenge of the immune system by disease-causing microbes would provoke a protective cell-mediated immune response.

This is an attractive (yet over-simplistic) hypothesis since pathogenic microorganisms usually elicit a protective Th1-mediated immune response and the Hygiene Hypothesis postulated that dampened or insufficient Th1-mediated inflammatory responses may actually subvert host immunity to a more dominant and inappropriately hyperactive Th2-mediated and pro-allergic immune response. Thus, the bias would switch from one of a predominantly cell-mediated immune response to one of an antibody-mediated response coupled with immunoglobulin isotype switching, for example, enhanced production of IgE.

Additionally, a direct association between microbial infection and allergy was not directly shown and to this day, there remains doubt regarding the validity of this hypothesis (Flohr et al. 2005). Further confounding or confusing the issue with regard the "hygiene hypothesis" is evidence from epidemiological and experimental work that has actually shown a reduced incidence of allergic and autoimmune diseases following exposure to helminthic parasitic infections (Maizels 2009). Additionally, any imbalance in the Th1/Th2 cytokine paradigm would of course mean that under circumstances of a Th2-prevalence, Th1-mediated autoimmune diseases should also be on the decline. This is clearly not the case and autoimmune diseases such as insulin-dependent (Type I) diabetes and gastrointestinal disorders such as Crohn's disease are steadily increasing. The incidence of these diseases is also more prevalent in developed and heavily urbanized countries, in much the same way as those communities that see dramatic incidences of atopic allergic diseases. However, in an interesting extension to the hygiene hypothesis, the gut microbiota was proposed to play an important role in the development of allergic disease (Wold 1998). This article proposed that perturbations in the gut microbiota early in life provoked by alternations in lifestyle, diet, and so on affect the normal development of immune tolerance of the mucosal system. The net result of this altered mucosal tolerance is the development of a pro-allergic immune response (Wold 1998), see Section 3.21.

3.12 Toll-Like Receptor Signaling: Relevance to Allergic Immunity

Numerous epidemiological studies support the notion that exposure to TLR ligands can protect against subsequent development of atopic asthma. Indeed, a diverse repertoire of common respirable environmental challenges including free ambient endotoxin or endotoxin complexed with particulate matter, diesel particulate matter, ozone, and a host of other environmental allergic inflammatory agonists may interact with TLR4 and stimulates the innate immune response. The function of TLR4 was initially recognized in studies of the interaction of LPS with the innate immune

system and its function was initially realized in animal models of environmental airways disease (Poltorak et al. 1998b; Arbour et al. 2000). Since then, several studies have now indicated that TLR4 exhibits an extensive role in enabling the innate immune system to sense and respond to the external respirable environment. Although TLR4 and indeed all of the TLRs are membrane associated (either at the cell surface or in the cytosol associated with endosomes), none of them to our knowledge have been shown to transduce a cytoplasmic-domain-mediated signal. The complex architectural association of soluble co-receptors and intracellular signaling as well as adaptor proteins to the cytoplasmic TLR and interleukin-1 receptor (TIR) domain cooperates to permit TLR-mediated signal transduction. These signaling molecules and adaptor proteins are mobilized on occupation of the TLR by a particular ligand.

In the specific case of TLR4, recognition of LPS is made possible by the association of LPS with a soluble LPS binding protein (LBP) which occupies membrane-associated CD14, which although does not transmit an intracellular signal per se, functions to aggregate LPS at the cell surface, and allows it to interact with TLR4 and its adaptor protein MD-2 (Shimazu et al. 1999; Perera et al. 1997). Subsequently, there are two possible outcomes during the occupation of TLR4 by LPS (Fig. 3.4).

In the first outcome, LPS-induced TLR4 signaling mobilizes the rapid MyD88-dependent pathways whereby the cytosolic TIR domain of TLR4 activates the TIR domain-containing adaptor protein (TIRAP) that assists in the association of MyD88 and TLR4 and subsequent signal transduction via IL-1 receptor-associated protein kinases (IRAKs) and TNF-receptor-associated factor 6 (TRAF6). Mobilization of TRAF6 activates NF-κB by p38-mitogen activated protein kinase (MAPK) dependent or independent mechanisms. In the second outcome, LPS-induced TLR4 signaling may mobilize TRIF-dependent signal transduction pathways. Like MyD88-mediated signaling, TRIF-mediated signaling is NF-κB-dependent. Unlike MyD88, activation of TRIF-mediated signaling is a delayed pathway and relies on the TRIF-related adaptor molecule (TRAM) to associate TRIF with TLR4 (Fig. 3.4).

In macrophages and dendritic cells, TRIF-mediated signaling activates the transcription factor interferon regulatory factor 3 (IRF3) via TRAF3, inducing IFN-α and IFN-β secretion as well as type I IFN-mediated enhanced expression of the co-stimulatory molecules CD40, CD80, and CD86 (Hoebe and Beutler 2006; Hoebe et al. 2003). Thus, TLRs including TLR4 play crucial roles in mediating host immunity to bacterial, fungal, or viral infections, including amplification of the pro-inflammatory cytokine and oxidative stress responses, innate and adaptive immune cross-talk, and in mounting innate immune responses to cellular stress or tissue damage.

However, respirable endotoxin or LPS in the environment can contribute to occupational lung diseases and many environmental airborne pollutants provoke inflammation and allergic responses in the lung in part because of their interaction with TLRs and other PRRs. Many ligands of TLRs are ubiquitous in the rural and urban environment and are readily inhaled. There is no standard protocol for measuring LPS or endotoxin levels in the environment but we know that high levels of endotoxin have been reported on sampling airborne particulates in many different environments including cattle ranches and pig farms (Schierl et al. 2007; Portengen et al. 2005), biofuel manufacturing facilities (Madsen 2006a, b), as well as other occupation environments such as

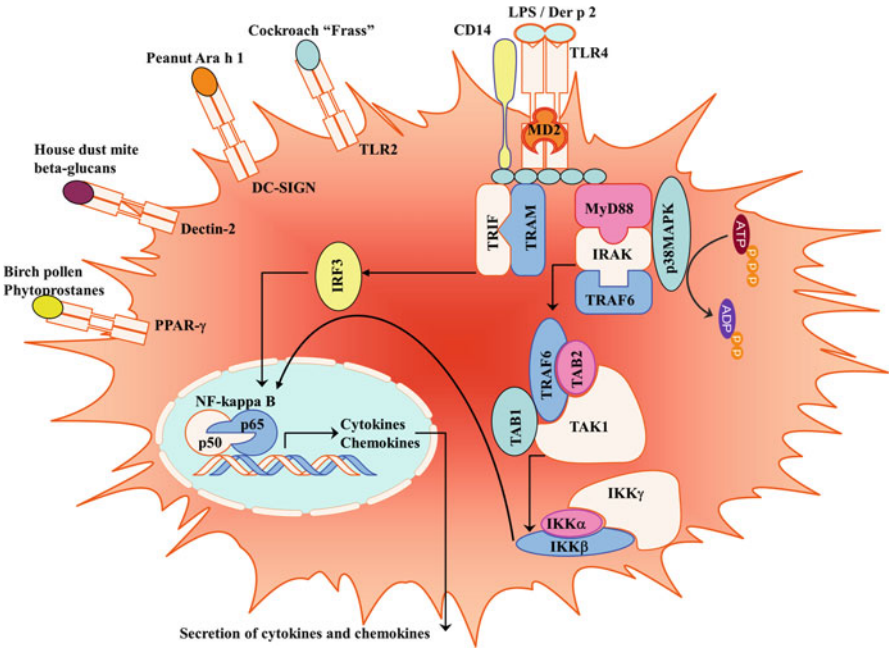

Fig. 3.4 Dendritic cells and Toll-like receptor pathway signaling. Toll-like receptors are PRRs that are expressed on the plasma membrane and intracellularly of most cells that constitute the allergic and adaptive arms of immunity. Here is shown an example of the diverse array of allergens that are thought to occupy particulate TLRs and instruct signal transduction. The signal transduction pathway for LPS/Der p2 (house dust mite allergen) and TLR4 is shown. Conformational alterations in the structure of TLR4 allow it to associate with the adaptor proteins MyD88, TRIF, and TRAM. Here, mobilization and phosphorylation of p38 MAP kinase drives a signaling cascade that switches on the transcription factor NF-κB (a heterodimer of p50 and p65 subunits), and the expression and secretion of inflammatory cytokines and chemokines

composting facilities, grain silos, and cotton mills (Bunger et al. 2007; Schwartz et al. 1995; George et al. 2001; Castellan et al. 1984; Christiani et al. 2001). Exposure of human subjects to respirable endotoxin has been widely studied in the settings of occupational lung diseases where repeated exposure to endotoxin can promote occupational asthma and other diseases such as emphysema. In the lung, TLR-mediated signal transduction plays crucial roles in the immune pathogenesis of many pulmonary diseases including asthma. Some major advances in the field as well as work from our own groups have shed light on the complex interactions between common respirable environmental exposures and TLR signaling. There is substantial evidence that PRRs can recognize many ligands beyond recognition of microbial endotoxins, nucleic acids, and fragments. There is evidence that cell surface expressed PRRs like TLR2 and TLR4 as well as cytoplasmic transcriptional signaling receptors such as the aryl hydrocarbon receptor (AhR) and the NOD-like-receptor-protein-3 (NLRP3) inflammasome complex (see Sect. 3.14) can contribute to the pulmonary responses following exposure to ozone, APM, diesel particulate matter, hyperoxia, and bleomycin.

However, whether or not endotoxin contributes to allergic immunity is rather controversial. Endotoxin has been reported to both exacerbate and dampen the incidence of allergic asthma. Clearly, considerations such as frequency and the dose or level of exposure are important to help understand this apparent contradiction. However, a vast number of studies clearly implicate the importance of innate immunity and the role of TLRs in allergic airways disease. Indeed one of the fundamental observations that supported the hygiene hypothesis was that exposure to TLR ligands can be protective and block the development of allergic asthma. For example, episodes of microbial diseases and fever in early life can affect the natural history of asthma by preventing the development of atopy (Calvani et al. 2002).

Mouse models of asthma have been studied extensively for a long while and have proved useful in understanding the mechanisms involved in asthma immune pathogenesis, physiological effects, and for the identification or preclinical testing of potential therapeutics. The C57BL/6J mouse has been used most often in studies of the sensitization to and subsequent challenge with chicken OVA. The model is extremely useful as it demonstrates both early and late phase type I hypersensitivity reactions including airway eosinophilia and airway hyperresponsiveness. Mouse models have been used to show that in normal and IL-4 gene knockout mice treated with OVA that IL-4 was crucial for the development of OVA-specific IgE secretion and airway hyperresponsiveness and that the transcription factor STAT6 was also necessary for OVA-specific atopic responses. Mouse models also confirmed the importance of antigen-presenting cells, particularly dendritic cells (DC) in presenting MHC Class II-restricted antigens to T cells and confirmed the importance of CD4+ Th2 cells in the allergic atopic response. Studies in mice have also confirmed the importance of genetic background in the development and maintenance of atopy. In such studies, C57BL/6 mice lacking IL-5 and subjected to a standard sensitization and challenge model of OVA exposure did not go on to develop airway hyperresponsiveness while IL-5 gene knockout BALB/c mice do. This observation and the early and late phase type I hypersensitivity reactions seen in C57Bl/6 mice has favored this model by the vast majority of investigators in the field of allergy, atopy, and allergic asthma.

However, one should apply caution in applying observations made in mouse models of asthma to human asthma since mouse models of asthma differ from their human counterpart in many crucial ways that make extrapolation problematic. There is the issue of wide variability in the protocols used for sensitization and allergen challenge models in mice, and importantly in contrast to measures done in humans, there is no universally accepted standard for measuring lung function in mice and this remains a controversial area of investigation. Nonetheless, animal models used to study extended periods of model antigen (e.g., OVA) challenge have shown that low doses of LPS or endotoxin can attenuate allergic inflammation in both mouse and rat models (Watanabe et al. 2003; Rodriguez et al. 2003; Hollingsworth et al. 2006; Tulic et al. 2000, 2002, 2004). The dampening of experimental allergic airways disease by chronic lower doses of LPS or endotoxin supports the hygiene hypothesis. This and other observations support the view that exposure to very low levels of TLR ligands can protect against the development of allergic asthma.

Some studies support the hypothesis that expression of the Asp299Gly minor allele of TLR4 in asthmatic individuals reduced functional signaling and is associated with increased severity of atopy as well as increased incidence of atopic or allergic asthma (Yang et al. 2004; Fageras Bottcher et al. 2004). Additionally, in a study of asthma specifically associated with endotoxin contamination in house dust, it was found that those individuals with the Asp299Gly minor allele of TLR4 had an attenuated risk of airway hyperreactivity (Werner et al. 2003). By contrast, some studies have not shown any effect of the Asp299Gly minor allele of TLR4 on the overall incidence of asthma (Werner et al. 2003; Raby et al. 2002; Noguchi et al. 2004). The relevance of genetic polymorphisms and gene–environment interactions in the context of the incidence of and susceptibility to allergic asthma will be considered in more detail later in this chapter.

3.13 Sensing of the External Environment in the Lung and the Role of the Dendritic Cell

Circulating monocytes have recently been shown to play a prominent role as pre-DC precursors that when cultured in vitro with the recombinant human cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) differentiate to immature DC that take up, process, and present antigen to naïve CD4+ T cells in lymphoid organs, thereby activating a primary immune response to the MHC-class II presented antigen (Cheong et al. 2010; Sallusto and Lanzavecchia 1994). The striking aspect of dendritic cell immune function is their highly efficient and prolific ability to capture soluble as well as particulate antigens, to efficiently respond to exogenous or endogenous microbial and inflammatory stimuli, and to present antigen to helper CD4+ or cytotoxic CD8+ T cells (Melman and Steinman 2001). For DC to potently stimulate and shape an appropriate response by CD4+ helper T cells, a unique series of receptor-mediated, contact-dependent and soluble signals needs to be in place at the immunological synapse between the DC and CD4+ T cell.

As a consequence of innate immune activation, affected tissues such as lung are rapidly infiltrated by newly recruited and activated dendritic cells. Dendritic cells are prolific professional antigen-presenting cells endowed with highly efficient immune stimulatory functions in the settings of infectious disease (Soloff and Barratt-Boyes 2010; Grainger et al. 2010; McGill et al. 2009; Heath and Carbone 2009), human cancer (Palucka et al. 2010; Melief 2008; Alvarez et al. 2008), autoimmune disease (Jensen and Gad 2010; Jahrsdörfer et al. 2010), and allergic inflammatory conditions like allergic asthma (Porter et al. 2007; Williams et al. 2007a, b, 2008; Bezemer et al. 2011; de Haar et al. 2005; van Rijt et al. 2005; Lambrecht and Hammad 2010; Hammad et al. 2009, 2010; Yerkovich et al. 2009). Dendritic cells are ideally positioned to sense and respond to the exogenous "respirable" environment. Airway DC have been identified throughout the epithelium of the nose, conducting airways, the lung, as well as the peribronchial and

mediastinal lymph nodes, pulmonary vascular system, pleural fluid, and pulmonary interstitial cells where DC are interdigitated to rapidly respond to, sense, take up, and process environmental aeroallergens, particulate antigens/agonists, and microbial pathogens.

It is in these zones that DC are poised to respond to infection or tissue injury and instruct the adaptive immune system of the presence of "danger" (Lambrecht and Hammad 2010). On activation by environmental danger signals, DC undergo a rapid and programmed pattern of functional maturation that is conventionally characterized by a downregulation in antigen uptake pathways, augmented antigen processing, and presentation and secretion of pro-inflammatory cytokines and chemokines that assist in defining the polarization of the immune response to that of a Th1-type or Th2-type adaptive immune response.

As we discussed earlier, activation of antigen-presenting cells such as DC is mediated in part by engagement of TLRs and other PRRs including the newly discovered intracellular NLR protein complex or NLRP3 inflammasome. The nature of the environmental "danger" triggers seen by immature DC defines the nature of T helper cell differentiation and response.

During the maturation of DC, an altered pattern of cell surface chemokine receptors (for example, loss of CCR6 expression and gain of CCR7 expression) influences the migration of DC and helps direct their migration to the draining or regional lymph nodes where they educate the lineage commitment of CD4+ Th cellular differentiation. Although the mechanisms responsible for DC-mediated instruction of T lineage commitment are poorly understood in allergic immunology, some recent studies, as well as those from our own groups have provided novel insights. Studies in mice, for example, have shown that specific signals imprinted in functionally activated DC induce the expression of organ-specific homing receptors in responding T cells (Mora and von Andrian 2006). This typically involves altered expression of chemokine receptors and adhesion molecules on T cells that play important roles in diapedesis and transmigration of those activated T cells. For example, in the context of the gut microbiome (see Sect. 3.21), gut mucosal DC help instruct enhanced expression of CCR9 and the α4β7 integrin on activated T cells that associated with their respective cognate ligands CCL25 and MadCAM-1 in inflamed areas of the skin (Mora et al. 2006).

We and others have shown that ambient pollutants, including diesel emission-source particulate matter do not drive Th1-mediated immune responses as one might expect from their pro-oxidative and pro-inflammatory properties, but instead promote a Th2-like pattern of allergic inflammation in many animal and human model studies. This has led to the proposal that such respirable particulate pollutants behave as immune adjuvants capable of augmenting the host response to allergen exposure or exacerbating preexisting allergic airways diseases (Williams et al. 2008; Bezemer et al. 2011; de Haar et al. 2005; Blanchet et al. 2004; Rumelhard et al. 2007; Norwood et al. 2001; Whitekus et al. 2002; Sharkhuu et al. 2010). These studies inform us that exposure to outdoor air pollutants can enhance the incidence of asthma, exacerbate preexisting disease, and compromise lung function.

In the case of common allergens such as house dust mite allergens and plant pollens, we know that sensitization occurs very early in life and that sensitization in susceptible children likely occurs via the epidermal route (Redlich 2010). This is not always the case, however, since some aeroallergens are more likely to traffic to and reside in the mucosal membranes of the lung and nose. It is worth recalling that in children with asthma, more than 90% of them will have some type of identified reaction to a known allergen too.

In the context of pro-allergic inflammation in asthma and the proposed association of exposure to outdoor pollutant particles and the development of allergic asthma, there are several features of ambient pollutant particles that are seen as "dangerous" by the innate immune system. This is particularly relevant in terms of the responsiveness of pulmonary DC to APM—a complex bioorganic and biophysical mixture of heavy and transition elements, semivolatile and nonvolatile organic and inorganic compounds, allergens, endotoxins, and other biologically active components that collectively provoke a pro-inflammatory and pro-oxidative response in the lung following exposure.

Since DC are ideally positioned in the lungs, interdigitated below the substrata and between or within the tight junctions of the airway epithelium as well as throughout the parenchyma of the lung, they are uniquely positioned in space and time for the efficient sensing and uptake of particulate pollutants that traffic to the lung from the external environment. As such DCs reside in this tissue (as well as other mucosal immune sites) in an immature state of "primed" functional activation. Dendritic cells in this state of readiness are extremely efficient at taking up exogenous soluble and particulate materials by endocytic, phagocytic, and macropinocytic antigen uptake mechanisms. Additionally, since DC display a repertoire of TLRs on their cell surface as well as those in the cytoplasm associated with early and late endosomal compartments, they are armed with an impressive array of information management and rapid response pathways. Such rapid antigen sensing and processing pathways endow the DC with an impressive ability to process and present nonself antigen to naïve CD4+ T cells.

We have shown that the interaction of APM and diesel PM with both murine and human DC is complex and the ultimate response is likely dependent, at least in part, on the coordinate activation of PRR signaling including TLRs and the nucleotide binding and oligomerization domain (NOD) like receptors (NLRs). On exposure to various PM species, we have shown dependence on both the TLR4 and TLR2 pathways of cellular activation and the adaptor protein MyD88 using targeted gene knockout mouse models (Williams et al. 2007a). The adaptor protein MyD88 is important for cell membrane-associated TLR signaling and transduces signals via the p38MAPK and p40/p42MAPK (ERK I/II) group of mitogen-activated protein kinase-dependent signaling pathways. We have previously studied the effects of APM on MyD88 gene knockout DC derived from mouse bone-marrow (BM-DC) as compared with their wild-type counterparts (Williams et al. 2007a). We found that deletion of MyD88 almost completely abolished the APM stimulated and dose-dependent secretion of IL-12p70 and IL-6 while the secretion of TNF-α was only marginally dampened in MyD88 knockout DC on stimulation by APM (Williams

et al. 2007a). These data were interesting as they implied that there was a MyD88-independent pathway of enhanced TNF-α secretion in response to APM. By contrast, the cell surface expression of the co-stimulatory molecule CD80 as well as MHC class II expression was actually enhanced in MyD88 knockouts on activation by APM (Williams et al. 2007a). This suggested an MyD88-independent pathway link was involved in the expression of those function-associated molecules and strengthens the notion that APM as well as LPS from a separate series of experiments activate DC via distinct mechanisms (Williams et al. 2007a).

Curiously, we also found that in human CD14+ monocyte-derived and CD34+ peripheral blood progenitor cell-derived DC exposure to ambient and diesel-enriched environmental PM directed a dramatic loss in TLR2 and TLR4 expression (Williams et al. 2007b). In subsequent work, we found that this phenomenon occurred within 4 h and was still evident 24 h later after the initial PM exposure. The transmembrane expression of both TLR2 (CD282) and TLR4 (CD284) serves important functions in linking innate and adaptive immunity. TLR2 is important in the recognition of microbial products including lipoproteins, lipoteichoic acids, lipomannans, beta-glucans, as well as the endogenous danger agonist Hsp60 while TLR4 serves equally crucial roles in the recognition of microbial products and predominantly transduced signals on activation by LPS. Since exacerbations of allergic asthma are often driven by airway viral and/or bacterial infections, our studies (Williams et al. 2007b) suggest that one link between environmental PM exposure and the exacerbations of pulmonary allergic conditions is a relative immune suppression and predisposition to infections as a consequence of downregulated TLR2 and TLR4 cell surface expression.

One could also postulate that the diminution in TLR expression in response to PM could represent an inherent mechanism to dampen the potential for pro-inflammatory airway hyperactivity and that dampened TLR expression would compromise the function of DC and render them hyporesponsive upon subsequent exposure to infectious agents. We can thus appreciate that on each inspiration of air, the immune system, and particularly antigen-sensing sentinels of innate immunity such as DC, is constantly challenged by both harmless and potentially "dangerous" antigens such as those provided by pathogenic microorganisms.

Pulmonary DC therefore are licensed to direct a protective immune response to pathogenic microbes or otherwise ignore harmless exposures—a system that is compromised and ultimately fails in those individuals presenting with allergic asthma. In very elegant in vitro culture models, it was shown by confocal laser scanning and conventional transmission electron microscopy that DC come into intimate contact with deposited particles in the lung (Blank et al. 2007; Gehr et al. 2006; Rothen-Rutishauser et al. 2005).

Although the conducting airways and alveoli provide an otherwise intact physiological, structural, and functional barrier through which micro- and nano-sized particulate matter cannot usually breach, it has been shown that such deposited particles are displaced and directly sampled by DC that gain access to the apical side of the epithelium by two separate mechanisms. The first was by extending their dendritic processes between the tight junctions of epithelial cells and the second

was by transmigration through the epithelium to the luminal space where DC could then take up the microparticles (Gehr et al. 2006; Rothen-Rutishauser et al. 2005). These studies nicely demonstrated that DC and macrophages communicated to form what the authors of this work termed "a trans-epithelial interacting cellular network" (Gehr et al. 2006). In this network, DC interacted with microparticle-loaded alveolar macrophages such that they sampled the particles directly from them or took up microparticles directly from the apical as well as luminal side of a three-cell culture matrix as described earlier (Gehr et al. 2006; Rothen-Rutishauser et al. 2005).

Our observations partly support these important studies in that we have shown exquisite sensitivity of DC to direct stimulation by APM, source-specific environmental diesel particulate matter, and engineered nano-particulate matter. In addition, in differential cellular cytospin preparations and visual inspection by light microscopy, we have found particles to be present in the cytoplasm of macrophages present in bronchoalveolar fluid (BALF) samples taken from mice following acute exposure to various PM species (Porter et al. 2007; Williams et al. 2007a, 2008; Bezemer et al. 2011). Additionally, we expanded on previously published studies where the direct in vitro effects of ambient PM were further explored by studying the in vivo effects of PM in a naïve mouse model of a single acute exposure to APM, diesel-enriched particulate matter, and silver nanoparticles (Bezemer et al. 2011). We found that APM and diesel PM activated myeloid as well as plasmacytoid subsets of lung DC in vivo (Bezemer et al. 2011). Of particular importance was our observation that on acute exposure to ambient PM (but not any of the other particles tested including diesel PM, carbon black particles, or silver nanoparticles) was marked infiltration of the lung by eosinophils. Thus, APM and diesel PM has the potential to promote many of the hallmarks that would one would associate with allergic asthma.

Some recent studies have informed us that protease-dependent pro-Th2 allergens like Der p have distinct enzymatic activities including the class 1 cysteine protease found for Der p1 and the class 3 serine protease found for Der p3 (Platts-Mills et al. 1997). It turns out that the cysteine protease activity of Der p1 contributes markedly to its allergic and immune modulating activities in promoting the pro-allergic biasing functions of DC including the dampening of the secretion of endotoxin-stimulated functional IL-12p70 by DC and the cleaving of important cell surface expression of functional molecules including CD40, the alpha-chain of the IL-2 receptor (CD25) and CD23 which is the low affinity receptor for IgE expressed by DC (Herbert et al. 1995; Kikuchi et al. 2006). Importantly though, protease-dependent allergens like Der p can alert the cell membrane-expressed G-protein coupled protease-activated receptors (PARs) including PAR1 and PAR2 and PAR2 activation may be especially relevant to Th2-mediated allergic inflammation and PAR2 gene knockout mice do not go on to develop allergic airways inflammation reminiscent of human asthma (Kauffman et al. 2006; Henry 2006; Schmidlin et al. 2002; Ramelli et al. 2010).

In the case of environmental APM and diesel-enriched PM, it is likely that these agonists promote both protease-dependent and protease-independent

signals given their complex constitution and likely trigger both PAR-dependent and TLR-dependent signaling in a cell type-specific manner. Protease-dependent mechanisms include house dust mite allergens as discussed earlier. Non-protease-dependent mechanisms might include such disparate observations as Der p2 behaving as molecularly structural mimic of the TLR4 adaptor protein MD2 that could supplant the natural signaling activities of MD2 and activate target cells like DC. Additionally others have shown that the dominant peanut allergen Ara h1 occupies the adhesion receptor DC-SIGN (Shreffler et al. 2006), whereas birch-pollen phytoprostanes dampened IL-12 secretion by DC and helped promote a pro-allergic biasing of the birch-pollen stimulated DC (Traidl-Hoffmann et al. 2005). It is formally possible that any one of these protease and non-protease-dependent activities may be complexed with APM or environmental diesel PM and contribute to the biological functions of PM in airway inflammation.

In addition to the known associations of endotoxin or other microbial contaminants of APM and diesel PM, heavy metals and transition elements as well as aryl hydrocarbons may all be intricately involved in stimulating DC, and other cells with a multitude of signals that trigger a nonclassic or noncanonical program of cellular differentiation that can collectively reprogram DC to behave as pro-Th2 innate immune effectors in allergic asthma as we have previously indicated (Porter et al. 2007; Williams et al. 2007a, b, 2008). Additionally, we have shown that short ragweed extract (RWE) modulates the functional activation of DC in a mouse model and found that RWE did so in a pro-inflammatory and pro-oxidative manner and was similar to those observations we made for the pro-oxidant effects of APM on murine bone-marrow derived DC (Rangasamy et al. 2010; Williams et al. 2008). Others have postulated therefore that ROS or the pathway of oxidative stress behaves as a "danger signal" and is interpreted as such by cells of the innate immune system including DC (Bianchi 2007).

3.14 Sensing of Respirable Danger and the Inflammasome

Other PRRs that serve important functions in innate immunity include the Dectin family of cell-surface C-type lectins that are important in recognizing the cell wall fragments of various fungal species (Brown 2006) and the intracellular NLR protein or NLRP3. Activation of PRRs and especially the NLRs leads to the assembly of high-molecular-mass complexes called inflammasomes that in turn leads to the generation of active caspase 1 and to the production of mature IL-1β. In some recent exciting experiments, an intracellular danger sensing protein complex termed the NOD-like-receptor-protein-3 (NLRP3) inflammasome that includes the NLRP3 protein has been identified which forms a complex with the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD [caspase-recruitment domain]) and an inactive pro-caspase-1 which collectively is termed the inflammasome (Martinon et al. 2002, 2009).

The NLRP3 inflammasome responds to diverse intracellular inflammatory signals including reactive oxygen intermediates (Dostert et al. 2008), uric acid crystals (Martinon et al. 2006), adenosine triphosphate (ATP), and a host of toxins (Mariathasan et al. 2006) and does so by inducing caspase-1 autocleavage (Martinon et al. 2009) such that the activation of caspase-1 promotes cleavage of pro-IL-1β to a mature active form of secreted IL-1β (Thornberry et al. 1992). The secretion of mature IL-1β allows it to be used as a surrogate immunological marker of NLRP3 inflammasome activation in a host of innate immune cells including dendritic cells (Li et al. 2008, 2009; Kool et al. 2008; Couillin et al. 2009). The discovery that NLRP3 can recognize host-derived materials such as uric acid and cholesterol crystals has led to the NLRP3 inflammasome being implicated in a number of inflammatory diseases such as atherosclerosis, gout, and type 2 diabetes as well as bacterial infections (Dunne 2011).

Activation of the NLRP3 inflammasome has been associated with exposure to a host of toxins, pathogens, and inflammatory agonists and these interactions are not only linked to Caspase-1 dependent IL-1β expression but also to the expression of IL-18 and IL-33 (Martinon et al. 2002). The NLRP3 inflammasome is also activated by the pro-allergic immune adjuvant alum and may thus contribute to the generation of Th2-mediated adaptive immunological responses (Li et al. 2008; Kool et al. 2008; Eisenbarth et al. 2008). However, others report that alum can provoke its effects in an NLRP3 inflammasome-independent manner too (McKee et al. 2009).

Until recently, it was uncertain whether ambient particulate pollutants or DEP could activate the NLRP3 inflammasome. However, given that particulate matter is a complex mixture of polyaromatic hydrocarbons and transition metals that induce ROS production, it is likely that environmental pollutants such as APM and DEP would activate NLRP3 (Vincent et al. 1997; Chen and Lippmann 2009). Indeed, it was shown recently that the NLRP3 inflammasome was involved in DEP-induced lung inflammation in a mouse model (Provoost et al. 2011). In this model, IL-1 type I receptor gene knockout mice had reduced pulmonary inflammation. By contrast, in NLRP3 and caspase-1 gene knockout mice, showed similar secretion activities for IL-1β and comparable lung inflammation as compared with their wild-type counterparts. Thus, what this important study found was that, at least for DEP, inflammation was provoked independently of the NLRP3/caspase-1 pathway and that other proteases may instead be involved.

3.15 Dermal: Lung Sensitization

The skin is the largest organ in the human body; it forms the outer sheath that interfaces with the external environment. Generally speaking, the skin can be divided into two main layers: the inner dermis and the outer epidermis. These are separated by a basement membrane. The dermis supports the avascular epidermis by providing nourishment and removal of waste products (McGrath et al. 2008). The reader is reminded that as we mentioned earlier atopic conditions such as atopic dermatitis

and allergic asthma often afflict the same individual. Thus, it seems likely that these conditions may share common mechanisms. Indeed, a number of studies provide PM compelling evidence that skin barrier defects increase susceptibility to asthma and influence its severity. It has been postulated that skin barrier defects facilitate allergen absorption, which leads to systemic Th2-like sensitization and asthma (discussed in Redlich 2010).

The epidermis is a stratified squamous epithelium containing melanocytes, Merkel cells, Langerhans cells, and keratinocytes which are the predominant cell type in the epidermis and differentiate as they advance from the basal layer to the stratum corneum at the surface (McGrath et al. 2008). The stratum corneum is a cornified layer of lipids and proteins that provides the skin's vital barrier functions (Redlich 2010). Just beneath the stratum corneum, the apical intercellular spaces between the living stratified epidermal cells are sealed with tight junctions, which also contribute to maintaining the integrity of the physical barrier (Kubo et al. 2009).

Despite the dermal barrier function, it is possible for substances from the environment to be absorbed via this route. The degree of absorption through intact skin is impacted by factors such as lipophilicity, molecular weight, concentration, co-exposures, hair follicles, and clothing (reviewed in Redlich 2010). If the barrier function of the skin is compromised, its ability to protect us from environmental toxicants and infections is greatly diminished.

3.16 Skin Barrier Impairments: Link to Asthma

Human and animal studies reveal the importance of the dermal route of exposure and identify the skin as a locus for sensitization in the development of asthma (discussed in Redlich 2010). Filaggrin is a protein involved in the terminal differentiation of the epidermis and the formation of the skin barrier (Candi et al. 2005). Genetic mutations of filaggrin (R501X and 2282del4) impair its ability to support epithelial barrier formation. These mutations are strong predisposing factors for development of extrinsic atopic dermatitis, but not the intrinsic disease subtype (Palmer et al. 2006; Weidinger et al. 2006). Importantly, these two loss-of-function mutations were also associated with asthma in patients with co-existing atopic dermatitis. Furthermore, filaggrin mutations were significantly associated with increased asthma severity independent of concomitant eczema.

Claudin-1 is a tight junction protein expressed in skin epithelium, where it contributes to barrier function (discussed in Xiao et al. 2011). In human keratinocytes, in vitro knockdown of claudin-1 expression by RNA interference reduced transepithelial electrical resistance and increased permeability. The expression of claudin-1 is reduced in patients with atopic dermatitis compared to nonatopics. Additionally, in patients with reduced claudin-1 levels, total serum IgE levels and eosinophil numbers were increased. The authors postulated that low claudin-1 expression in patients with atopic dermatitis may lead to tight junction deficiencies and increased susceptibility to environmental allergen sensitization.

3.17 Genetic Susceptibility and Asthma

Heredity plays a key role in asthma susceptibility with current estimates of the incidence of heredity-linked asthma ranging from 40 to 60% (Adcock and Barnes 2011). Having a family history of asthma is one of the few strong, consistent risk factors for the disease (London and Romieu 2009). The risk of developing asthma increases twofold if one parent is affected, and the risk increases to approximately fourfold if both parents have the disease (London et al. 2001). More than 670 genes associated with asthma have been identified (Adcock and Barnes 2011). Many of these may play a role in the complex relationship between genetic and environmental components involved in the manifestation of asthma (von Mutius 2009).

Genome-wide association studies (GWAS) are promising for identifying genes associated with susceptibility to asthma (or gene–environment interactions). These studies compare single nucleotide polymorphisms (SNPs) between cases and controls (Akhabir and Sandford 2011). Typically, 100, 000 – 1, 000, 000 SNP markers are incorporated in GWAS (Saffron 2009). The major advantages of this method are that it (1) allows the researcher to evaluate the entire genome in an unbiased manner, and (2) enables the discovery of truly novel candidate genes (von Mutius 2009).

There have been 12 asthma-related GWAS studies published to date (reviewed in Akhabir and Sandford 2011). The first GWAS study evaluated 317,000 SNPs from 994 patients with childhood asthma and 1,243 subjects without asthma. Nearly 700 million genotypes were analyzed in this study. Several genetic markers associated with childhood asthma were identified on chromosome 17q21.1 associated with genes *ORMDL3* and gasdermin B (*GSDMB*) (Moffatt et al. 2007, 2010). The exact role of these genes in asthma is not clear. The *ORMDL3* gene appears to trigger an inflammatory response by regulating endoplasmic reticulum-mediated calcium signaling (Cantero-Recasens et al. 2010). *GSDMB* is expressed in dermal epithelial cells (Komiyama et al. 2010) and T cells (Sy et al. 2004). Discovery of these genes represented a significant advancement in the field of asthma research because none of them would have been targeted in gene candidate studies based on current knowledge of their function. Nonetheless, these findings have been replicated consistently in several independent studies (Galanter et al. 2008; Hirota et al. 2008; Tavendale et al. 2008). Thus, chromosome 17q21 is likely to be a true asthma susceptibility locus.

Additionally, *IL-33*, *IL1RL1*, *SMAD3*, *IL2RB*, *HLA-DQB1*, and *RAD50* have been identified as asthma susceptibility genes by multiple independent GWAS reports (Moffatt et al. 2010; Li et al. 2010; Gudbjartsson et al. 2009). The connections between some of these genes and asthma might easily have been predicted based on existing knowledge of their function. For example, IL-33 is constitutively expressed in endothelial and epithelial cells. It induces nuclear factor κB (NF-κB)-dependent production of Th2 cytokines (e.g., IL-4, -5, and -13) by binding to its receptor, the gene product of *IL1RL1* (Gudbjartsson et al. 2009; Schmitz et al. 2005; Carriere et al. 2007). Also, SMAD3 is involved in transforming growth factor β (TGF-β) signaling, which plays a role in both (1) regulatory T cell proliferation and

differentiation (Nouri-Aria and Durham 2008), and (2) airway remodeling (Makinde et al. 2007). IL2RB is the β chain of the IL-2 receptor, which is intimately involved in differentiation of T cell subtypes (Letourneau et al. 2009), and HLA-DQB1 is involved in antigen presentation (Li et al. 2010). On the other hand, the role of *RAD50* in asthma is not quite as obvious as its major function is currently understood to be involved in DNA double-strand break repair (Li et al. 2010). *DENND1B* and *IL2RB* have been identified as asthma susceptibility genes, but these findings have not been replicated (Sleiman et al. 2010; Moffatt et al. 2010). The protein encoded by *DENND1B* is believed to block signaling by tumor necrosis factor receptor type 1. Although no studies have been conducted to determine the influence of this gene on asthma, *DENND1B* is interesting because it is expressed by dendritic and T cells (Sleiman et al. 2010).

3.18 Gene–Environment Interactions and Asthma

Gene–environment interactions impacting asthma have been reported for air pollution (reviewed in London 2007), microbial exposures (reviewed in Koppelman 2006; von Mutius 2009), work place exposures (reviewed in Jones 2008; Christiani et al. 2008), and environmental tobacco smoke (ETS). Exposure to ETS in utero or in early childhood increases the risk of developing asthma and also impacts disease severity (Strachan and Cook 1998). Recently, *ADAM33* induction, in combination with cigarette smoke was shown to increase susceptibility to asthma in utero, but not postnatally (Reijmerink et al. 2009).

ETS exposure during infancy was factored into a genome-wide linkage study involving 144 white families from the Collaborative Study for the Genetics of Asthma. Three chromosome regions (1p, 5q, and 9q) were identified with nominal evidence for linkage to asthma on the basis of ETS exposure ($P < 0.01$) (Colilla et al. 2003). A second genome-wide linkage study in 200 Dutch families identified through a parent with asthma provided further evidence that genes on chromosome 5q interact with ETS (Meyers et al. 2005). In the French Epidemiological study on the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness and Atopy, six regions were identified for airway hyperresponsiveness to ETS: three by predivided sample test (PST) and mean interaction test (MIT) (1q43-q44, 4q34 and 17p11); two using only the MIT (14q32 and 17q21); and one using only the PST (5p15) (Dizier et al. 2007). The association of these regions with airway hyperresponsiveness was not previously found in other linkage studies that failed to account for gene–environment interactions. Specific genes involved in susceptibility to ETS may include the following: *CD14* (Choudhry et al. 2005), Th2 cytokines such as *IL-13* (Liu et al. 2003, 2004), *2AR* (Barr et al. 2001) on chromosome 5q; and glutathione *S*-transferase (*GST*) on chromosomes 1 and 22 (Gilliland et al. 2002; Kabesch et al. 2004).

Cigarette smoke contains high endotoxin levels and as noted earlier, the TLR4/CD14 signaling complex transduces signals very rapidly in response to LPS stimulation. A family-based cross-sectional cohort study of the effects of ETS was used

to determine the association between *CD14* gene polymorphisms and asthma-related phenotypes. In Puerto Ricans and Mexicans, asthma severity and total serum IgE were correlated with three SNPs (CD14/−810, −159, and +1437). In ETS-exposed individuals, SNP-1437 was associated with lower forced expiratory volume in 1 second (FEV1) and SNP-159 was associated with total serum IgE (Choudhry et al. 2005).

GST genes are involved in detoxification and protection from oxidative stress such as that associated with exposure to ETS. Deletions of *GSTM1* and *GSTT1* are prevalent in white populations. One study assessed the effect of GSTM1 genotype in 2,950 children in the USA. In this study, in utero exposure to ETS was associated with asthma in children with the *GSTM1* null genotype compared to children with the wild-type genotype (Gilliland et al. 2002).

Epigenetic mechanisms may also play a role in mediating the interaction between exposure to ETS and asthma susceptibility. For example, in utero exposure to cigarette smoke has been reported to alter gene expression through alterations in DNA methylation and consequent changes in chromatin structure (Bouzigon et al. 2008). Table 3.1 summarizes many of the putative genes that have been associated with allergic diseases in the lung as well as the skin.

3.19 Multiethnic Genome Wide Association Studies

Asthma is a global health problem. Disease prevalence varies based on ethnicity within and across countries. The prevalence of asthma ranges from 1 to 18% among nations. However, the prevalence of asthma is increasing at a faster rate in Africa, South America, and parts of Asia compared to North America and Western Europe (GINA 2008). In the USA, asthma prevalence, hospitalization, and mortality are higher in Puerto Ricans, African Americans, Filipinos, and Native Hawaiians and lowest in Mexicans and Koreans (Drake 2008). The influence of ethnicity on asthma prevalence is complicated and may be influenced by ethnic group, where an individual was born, and, if they migrated to a new country, the amount of time elapsed since migration (Davidson et al. 2010).

The complexity can be illustrated by considering asthma prevalence in Mexican Hispanics born inside the USA who were found to have a higher prevalence of asthma than those born outside the USA (Subramanian et al. 2009). Similarly, individuals born outside of the UK have a lower risk of asthma than individuals of the same ethnic group born in the UK (Subramanian et al. 2009). These findings point to the pivotal role of gene–environment interactions in asthma. Asthma is a complex disease; gaining a firm understanding of the impact of race and ethnicity on the etiology of this disease is crucial to public health. Despite these findings, racial and ethnic minorities have been largely understudied. Only a relative few genetic linkage studies included racial and ethnic minorities such as African American, Latino and Asian populations (reviewed by Drake et al. 2008).

Table 3.1 Asthma susceptibility genes

Tissue	Gene	Chromosome	Function	References
Skin	Filaggrin (FLG)	1q21.3	Barrier function	Palmer et al. (2006) and Weidinger et al. (2006)
	Claudin-1	11q3	Barrier function	De Benedetto et al. (2010)
	GSDMB	17	Role in asthma unknown	Moffatt et al. (2010)
Lung	HLA-DQB1	6p21	Antigen presentation	Moffatt et al. (2010)
	ADAM33	20p13	Cell–cell and cell–matrix interactions	Van Eerdewegh et al. (2002)
	ORMDL3	17q21	Inflammatory response	Moffatt et al. (2007)
	ADRA1B	5q33	Pro-inflammatory responses, role in asthma unknown	Mathias et al. (2010)
	DPP10	2q12.3-q14.2	Bronchial reactivity	Mathias et al. (2010)
	RAD50	5q31	Role in asthma unknown	Li et al. (2010)
	DENND1B	1q31	Role in asthma unknown	Sleiman et al. (2010) and Moffatt et al. (2010)
	PRNP	20pter-p12	Role in asthma unknown	Mathias et al. (2010)
	PYHIN1	1	Role in asthma unknown	Torgerson (2010)
	SMAD3	15	T cell differentiation and airway remodeling	Moffatt et al. (2010)
	IL2RB	22	T cell differentiation	Moffatt et al. (2010)
	IL-4, IL-5, and IL-13	5q31-q33	Th2 responses	Kauppi et al. 2001
	IL-13	5q31	Gene–environment interactions, Th2 response	Liu et al. (2003, 2004)
	IL-33	9	Th2 response	Moffatt et al. (2010)
	IL1RL1	2q12	IL-33 receptor	Moffatt et al. (2010)
	CD14	5q31	Gene–environment interactions, innate immunity	Choudhry et al. (2005)
	β2AR	5q31-32	Gene–environment interactions, bronchial smooth muscle relaxation	Barr et al. (2001)
	GSTM1	11p13.3	Gene–environment interactions, detoxification, protection from oxidative stress	Gilliland et al. (2002) and Kabesch et al. (2004)

Gene clusters have been segregated to those that may be associated with the skin and those with the lung. The gene name, chromosomal location, and function of the gene product are shown

Recently, GWAS studies have begun to shed some light on asthma-associated genes in specific racial and ethnic populations (Mathias et al. 2010; Hancock et al. 2009; Choudhry et al. 2008; Torgerson et al. 2011). Choudhry et al. (2008) conducted the first multiethnic GWAS study (Choudhry et al. 2008; Torgerson et al. 2011). In this study, 96 moderate-severe Puerto Rican asthmatics, 88 matched controls, and 109 samples representing Puerto Rico's founding population were genotyped. Analysis revealed that chromosomal region 5q23.3 harbors genes associated with asthma in Puerto Ricans (Choudhry et al. 2008; Torgerson et al. 2011). A second GWAS study including 492 Mexican children with asthma identified chromosome 9q21.31 as a susceptibility locus for childhood asthma in Mexicans (Hancock et al. 2009).

Two GWAS studies sought to identify asthma susceptibility loci in African American populations. In the first of these studies, two independent populations of African descent and matched controls were genotyped (Mathias et al. 2010). A meta-analysis of the two populations identified three asthma-associated loci: the -1B-adrenergic receptor (ADRA1B) gene on chromosome 5q33; the dipeptidyl peptidase 10 (DPP10) gene on chromosome 2q12.3-q14.2; and the prion-related protein (PRNP) gene on chromosome 20pter-p12 (Mathias et al. 2010). DPP10 is expressed in the bronchial epithelium of rats; it has been speculated to be involved in bronchial reactivity (Ren et al. 2005; Schade et al. 2008). PRNP is expressed in B cells, T cells, and dendritic cells, but its function in these cells is not well understood (Isaacs et al. 2008). More recently, a meta-analysis of European, African American, or African Caribbean and Latino descent identified a locus at pyrin and HIN domain family member 1 (PYHIN1) as being specific to African descent (Torgerson 2010). Little is known about the function of PYHIN1 and it has not been implicated in asthma pathogenesis.

Findings from other studies also suggest that genetic risk factors can vary based on race and ethnicity. For example, ADAM33, linked to bronchial hyperresponsiveness, was the first asthma gene identified by positional cloning (Van Eerdewegh et al. 2002). The function of ADAM33 is unknown. However, it is expressed in mesenchymal cells, which suggests that it may be involved in airway remodeling (Holgate et al. 2006). ADAM33 has been associated with asthma in European, one African American, and one Hispanic population but was not associated with asthma in Mexican, Puerto Rican, Korean, or Costa Rican populations in other studies (discussed by Drake et al. 2008). It is important to note that the association with ADAM33 involves different SNPs when evaluated in different populations (Lara et al. 1999).

3.20 Epigenetics and Asthma

Functional gene expression is dependent not only on genetics, but also on epigenetic factors. Epigenetics is a term used to describe heritable changes in phenotype or gene expression that are not encoded in the underlying DNA sequence. Cellular

functions such as gene expression, DNA repair, and cell proliferation are controlled in part by epigenetic mechanisms (Adcock et al. 2007). Epigenetic mechanisms include DNA methylation, noncoding RNAs, and modification of histones (Seibold and Schwartz 2011). Histones are proteins that package DNA into chromatin. These are subject to posttranslational modification by the addition of methyl, acetyl, phospho, SUMOylation, citrullination, and ubiquitin groups that influence the structure of the chromatin and ultimately gene expression (Li et al. 2007).

Histone modification and other epigenetic changes can occur in response to environmental stimuli, which may impact disease state (Seibold and Schwartz 2011). For example, it was demonstrated that mice are more likely to develop allergic airway disease when fed an in utero diet rich in methyl donors; exposure to this diet was associated with hypermethylation of Runt-related transcription factor 3 (Runx3) (Hollingsworth et al. 2008). Hypermethylation of the Runx3 gene led to reduced expression of the Runx3 protein, which negatively regulates allergic airway disease. Methylation of CpG islands associated with folic acid ingestion has been reported in humans (Jiang et al. 2004). Importantly, dietary supplementation with folic acid during the first 3 months of pregnancy was associated with an increased risk of lower respiratory tract infection and wheeze during the first 18 months of life (Haberg et al. 2009).

Like DNA methylation, histone deacetylation generally stops gene expression (Egger et al. 2004). Histone deacetylase (HDAC) 2 expression and activity are significantly reduced in bronchial biopsies and alveolar macrophages collected from both asthmatics (Barnes et al. 2005) and patients with chronic obstructive pulmonary disease (COPD) (Ito et al. 2005). In the patients with COPD, reduced HDAC2 expression and activity were also correlated with disease severity and the level of inflammation (Ito et al. 2005).

MicroRNAs (miRNAs) are another epigenetic regulatory mechanism for gene expression. miRNAs are noncoding RNAs approximately 17–24 nucleotides in length that are each believed to regulate hundreds of genes by binding to 3′-untranslated regions (UTR), which results in translational silencing and/or degradation of mRNA (Bartel 2004). The role of miRNAs in inflammation and asthma is currently under investigation. In one study, a microarray-based approach was applied to identify 21 miRNAs differentially expressed between doxycycline-induced lung-specific IL-13 transgenic mice with allergic airway inflammation and control mice. Of note, the investigators reported overexpression of miR-21 and underexpression of miR-1 in the transgenic mice. The investigators applied bioinformatics to identify IL-12p35 as a candidate target for miR-21. IL-12 is a cytokine derived from macrophages and dendritic cells involved in Th cell polarization. Importantly, mutation of miR-21 binding sites in the IL-12p35 3′ UTR abolished repression of the gene by miR-21. Thus, induction of miR-21 in experimental asthma is likely to lead to a decrease in mIL-12p35 that pushes Th cell polarization toward a Th2 response (Lu et al. 2009). In a second study, a small group of miRNAs were identified in a mouse model of acute allergic bronchopulmonary inflammation induced by intranasal challenge with house dust mite extract. This work demonstrated that inhibition of miR-126 suppressed Th2-driven airway inflammation, mucus hypersecretion, and airway hyperresponsiveness further linking miRNA to asthma pathogenesis (Mattes et al. 2009).

3.21 Novel Concepts in Allergic Asthma: The Gut Microbiome

In 1998, the hygiene hypothesis (see Sect. 3.11) was extended to the microbiota hypothesis which proposed that alterations of the sum collection of gut microbial flora in early life may affect the development or breaking of immune tolerance of the mucosal innate immune system thus promoting skewed allergic or pro-atopic immune responses (Wold 1998). Although allergic diseases like allergic asthma or dermatitis are mediated in part by dysregulation in the adaptive immune response with a bias towards a pro-Th2 cytokine milieu (especially IL-4, IL-5, IL-9, and IL-13) that maintains the allergic inflammatory condition (eosinophilia, mast cell recruitment and degranulation, IgE immunoglobulin class switching, etc.), the mechanisms responsible remain obscure despite intense efforts and some controversies exist. For example, it has been known for a while that in addition to the typical Th2-bias seen in allergic asthma that Th1 cells have also been implicated in the disease (Kero et al. 2001).

Consistent with this argument is the view that CD4+ Th17 T cells that secrete high levels of IL-17 and CD4+ CD25+ FoxP3+ regulatory T cells (Tregs) play crucial roles in allergic inflammation. Similarly, newly identified Th9 T cells that differentiate to this phenotype under the influence of TGF-β secrete high levels of IL-9 and IL-10 yet they do not exert any suppressive functional activity. Despite this, Th9 cells may play an important role in allergic immune diseases including allergic asthma (Veldhoen et al. 2008). Indeed, individuals with allergic asthma have the ability to secrete high levels of IL-17 against the Th2-allergic cytokine milieu informing us that diseases like allergic asthma are not strictly Th2 mediated and are instead under the influence of other cell types and cytokine-driven mechanisms that regulate complex effector T cell activities (Cheng et al. 2002; Wang et al. 2010; Karlsson et al. 2004).

With this in mind, the gut microenvironment serves a crucial role in immune homeostasis that provides an immunomodulatory environment to protect normal individuals from inflammation, allergic, and autoimmune disease states. Rather like the mucosal membranes of the lung, the protective epithelial cells and tight junctions that line the intestinal lumen are not impenetrable but are constantly breached so that the antigen-rich contents of the lumen can be taken up and sensed by cells of the innate immune system and specialized cells called microfold cells that do the bulk of the antigen sampling. This process takes place at specialized sites called GALT or gut-associated lymphoid tissues that includes lymphoid zones of the lamina propria and Peyer patches. The gut, like the lung and skin, is constantly exposed to and challenged by the environment including microbial pathogens, particulate pollutants, and other xenobiotic pollutants or toxins. It is no surprise that given the surface area of the gut, that GALT is the largest site of organized and more diffuse lymphoid cells in the human body.

It is in the GALT, including Peyer patches and lamina propria, that microfold cells transfer this preprocessed antigen (transcytosis) to the dendritic cells located in the subepithelial dome and lamina propria. Like mucosal DC, intestinal epithelial

cells express a broad spectrum of TLRs and NLRs. However, it is important to realize that under normal physiological situations the mucosal sites of the intestine are immune privileged sites whose primary function is to distinguish commensal bacteria from potentially pathogenic bacteria in the gut and thus provide an efficient platform for immune tolerance. The concept of immune tolerance allows the host's immune system to distinguish innocuous antigens that are either respirable or ingested from those that are clearly a "danger signal" and thus prevent an inappropriate immune response to that antigen. Any dysfunction or failure of the immune tolerance network would clearly provoke disease and such an unfortunate event is central to the pathology of many disease processes. A key question is how does the adaptive immune system not get activated by antigen-presenting cells given the massive antigenic burden of the intestinal microbiota? Such antigens derived from commensal bacteria or potentially pathogenic microbes and other foreign proteins or particulate antigen must be either tolerated or ignored by the adaptive or innate immune system. Whereas commensal species of bacteria have evolved to evade or suppress undesirable consequences of inadvertent immune activation and inflammation in the gut, in part by dampening innate immune cells, it is no surprise then that failure of the tolerant or immune privileged gut can impart quite dramatic and adverse effects on systemic immune tolerance and thus a breaking of immune tolerance to self or innocuous nonself that is central to the immune pathology and clinical sequelae of allergic diseases and autoimmunity. In the gut, immune tolerance is assisted significantly by the fact that intestinal epithelial cells express very low levels of TLR4 (the major PRR that recognizes bacterial endotoxins like LPS) and this endows them the ability to largely ignore the otherwise massive bacterial flora present in the intestinal tract.

Thus, the burden of the gut microbiota clearly has marked influence on the responsiveness and immune tolerizing functions of both innate and adaptive immunity. Indeed, immune tolerance and immune privilege in the gut evolves during the lifetime of a human and is set in motion during neonatal development at the outset of a growing burden of environmental challenge. Clearly TLR-dependent pathways, immune regulating cytokine networks, Tregs, and specialized antigen-presenting cells like DC all play key parts in immune tolerance. A breakdown in this tolerance jigsaw, or any immune insult on any piece that constitutes that jigsaw in the host, will have the undesirable potential to promote inadvertent immune dysregulation and a breaking of tolerance to otherwise innocuous nonself antigens—the net result of which could be allergic disease like asthma.

3.22 Concluding Remarks

The immunological landscape in chronic allergic immunity is complicated and simultaneously tightly regulated. The innate and adaptive immune systems of the lung, gut, and skin are intricately linked and their role in immune tolerance and protection from inadvertent allergic immune reactivity to innocuous nonself

antigens is beginning to be fully realized. The innate immune system present in the lung, skin, and gut are the conduits that bridge the host to the external environment. We are also beginning to appreciate that the environmental air, particularly highly polluted air found in urban environments as well as in developing and industrialized countries is highly immuno-stimulatory, pro-inflammatory, and pro-oxidative in potential. The interactions of the cells that constitute the pulmonary and peripheral innate immune system are highly sophisticated but fragile.

Work from our group and that of others supports the growing concept that environmental pollutants and other more innocuous challenges, such as pollens, particulates, and low levels of chemical irritants, may all drive allergic immunity and possibly either promote or exacerbate preexisting allergic asthma. One of the central tenets of allergic respiratory diseases is a dominant Th2-biased immune response. More focused research is needed to help combat allergic diseases like asthma because while these diseases can be clinically managed, there remains no cure for them. Previous work done by our group as well as others strongly suggest that the pulmonary DC is key in setting into play the allergic and pro-inflammatory dance in the lung that provokes all of the hallmarks and clinical manifestations of chronic inflammatory diseases such as asthma.

It is anticipated that future work will focus on the molecular and cellular mechanisms of action of those innocuous environmental challenges that cause immune tolerance to be broken in the lung, skin, and gut. We believe that targeting the affected cells that provoke the allergic inflammatory response could enable a targeted pharmacological or immunotherapeutic treatment of the disease. Additionally, future work in this area will likely enable a greater appreciation of the immune pathogenesis of allergic asthma and most importantly future research must strive to provide tools for the management of not only allergic asthma but other allergic or autoimmune diseases that show no respect for the boundaries of age, gender, or ethnicity.

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Chapter 4
Cutaneous Allergies

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Abstract Allergic contact dermatitis remains one of the most common occupational and environmental health issues. As with other forms of allergy the disease progresses in two stages: an initial "induction" phase during which sensitization is acquired, followed later (after subsequent exposure to the same chemical allergen) by "elicitation" of dermal inflammation. In recent years much has been learned about the characteristics of immune responses to skin sensitizing chemicals and the roles played by dendritic cells, cytokines and chemokines and real progress has been made. However, a current and significant challenge is how to replace in vivo methods with non-animal alternatives. This issue is given added significance via consideration of the clinical information, where the reality is that, despite useful toxicology methods, the burden of the disease remains substantial, with some contact allergies being at hyperendemic levels.

Key Points

- The great majority of cutaneous allergies are caused by chemicals (skin sensitizers) which covalently bind to skin protein and trigger a cell-mediated delayed hypersensitivity reaction (allergic contact dermatitis [ACD]).
- Although immunobiology is not perfectly characterized, all the basic steps are now known and most aspects are well understood, including many aspects of the reactive chemistry involved.
- Predictive toxicology assays which can identify potential skin sensitizers have been available for many decades; the initial guinea pig protocols have been superseded by

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a murine method, the local lymph node assay (LLNA), which not only identifies hazardous chemicals, but also gives a first characterization of their relative potency.
- Use of quantitative potency information (in the form of the LLNA EC3 value) has led to the development of a quantitative risk assessment approach which has made a small revolution in skin sensitization toxicology. In part this is not only because of the transparency of the process, but also because it helps to explain why we have seen epidemics of ACD to certain chemicals, e.g., preservatives.
- The development of in vitro methods now continues apace, with methods for hazard identification based on chemical reactivity and dendritic cell maturation being under active validation. However, the application of these methods, or the development of others, for potency measurement remains in its infancy. Here, a key gap remains that we do not yet have a gold standard database of chemicals whose relative human potency is fully characterized.

4.1 Introduction

Immunotoxicology is the study of adverse health effects that may result from the interaction of xenobiotics with the immune system. In this context, allergic contact dermatitis (ACD) can be regarded as being the most frequent manifestation of immunotoxicity in humans; it is a common occupational and environmental health issue and many hundreds of chemicals have been shown to cause skin sensitization (de Groot 2008; Johansen et al. 2011). In common with other forms of allergy, ACD develops in two phases which are defined as induction and elicitation. Induction of skin sensitization is initiated following topical exposure of a susceptible subject to an amount of the chemical allergen sufficient to induce a cutaneous immune response of the necessary strength. This immunological priming results in sensitization and if the now sensitized individual is exposed subsequently, at the same or a different skin site, to the inducing chemical allergen then a more vigorous secondary immune response will be provoked at the point of contact. This in turn initiates the cutaneous inflammatory reaction that is defined clinically as ACD.

In the material that follows, mechanistic aspects are considered and then an overview of the clinical disease that can arise from skin sensitizing chemicals is presented. Subsequently, the toxicological approach to hazard identification, characterization, risk assessment, and risk management is presented. Finally, we discuss how toxicology in this area might progress to eliminating animal use and the challenges that this presents.

4.2 Mechanisms of Skin Sensitization and Allergic Contact Dermatitis

Contact allergy is a form of delayed type hypersensitivity. The induction of sensitization and the elicitation of allergic contact reactions are dependent on T lymphocytes. The cellular and molecular mechanisms that initiate and regulate T lymphocyte

responses to the inducing chemical allergen have recently been considered in detail elsewhere as have the relevant immunological processes, an exhaustive survey of which is beyond the scope of this article (see Rustemeyer et al. 2011). The aim instead is to explore some features of special toxicological interest in the context of a brief overview of the sensitization and elicitation processes and the clinical disease, ACD.

4.2.1 The Chemical Allergen

Chemical allergens are haptens and as such are unable themselves to directly stimulate an adaptive immune response. Immunogenicity is induced by the stable association of the hapten with skin protein, thus forming hapten-protein conjugates (Divkovic et al. 2005). Many contact allergens are either pre- or pro-haptens requiring change such as oxidation to acquire an ability to conjugate to proteins. The skin can be viewed as a nucleophilic environment with electron-rich functional amino acids present in the proteins. Electrophilic haptens conjugate covalently with amino acids cysteine, leucine, and to a lesser extent histidine, methionine, and tyrosine (Karlberg et al. 2008). It is assumed that in many instances it is the hapten–protein conjugate that is recognized and processed for subsequent presentation to the immune system, in a manner analogous to the processing of foreign proteins (Weltzien et al. 1996). However, this may not always be the case and it is possible that haptens will also bind directly to peptides associated with major histocompatibility gene products. In either event it is clear that for sensitization to proceed a chemical must be inherently protein-reactive or must be metabolized to a protein-reactive species (Smith and Hotchkiss 2001). Intrinsic or inducible protein reactivity forms the basis for many approaches to define structure–activity relationships in skin sensitization (reviewed in Lepoittevin 2011). The most recent understanding in this area centers upon the definition of mechanistic applicability domains, which describe the various types of chemical reaction mechanisms associated with skin sensitizing chemicals in an attempt ultimately to permit their predictive identification (Roberts et al. 2007).

4.2.2 Bioavailability

For a cutaneous immune response to be induced, the chemical allergen must gain access to the viable epidermis. This requires that the chemical has the physicochemical properties necessary for passage across the *stratum corneum*. The issues associated with this have been reviewed (Basketter et al. 2007a). However, beyond the obvious that permeation into the skin is necessary, and thus that large molecular weight, extremes of hydrophobicity and hydrophilicity and the presence of ionized groups generally limit permeation, relatively little is known. Attempts to model hapten concentration at the presumed reaction sites are fraught with difficulty (Dancik et al. 2012). Nevertheless, after entry into the epidermis has been achieved,

and protein adducts have been formed, there may also be a need for antigen processing. This has long been thought to be primarily the responsibility of epidermal Langerhans cells (LC), although increasingly it is recognized that other cutaneous (dermal) dendritic cells (DC) play a significant role. There is even a proposal that DCs are primarily responsible for induction, whereas the LC may function to aid in tolerance/regulation of the response (Vocanson et al. 2009).

Haptenization and the presence of danger signals (see below) provide the dual triggers required for the initiation of migration of LC/DC from the skin to the draining lymph nodes (Toebak et al. 2009). At least for LC, it is now well understood that they are induced to differentiate from antigen processing cells to mature immunostimulatory DC that are able to present antigens effectively to responsive T lymphocytes in draining lymph nodes (Kimber et al. 1998, 2000; Cumberbatch et al. 2000). A similar process will occur for DCs. The processes of migration and maturation are initiated and regulated by epidermal cytokines, and the directed movement of LC from the skin and their subsequent localization within the draining nodes is facilitated further by changes in chemokine receptor expression and specific chemokine receptor–ligand interactions (Cumberbatch et al. 2000). The epidermal cytokines that play mandatory roles in LC mobilization are tumor necrosis factor α (TNF-α), interleukin (IL) 1β (IL-1β), and IL-18 (Cumberbatch et al. 1997, 2000, 2001; Toebak et al. 2009).

A certain degree of local trauma (with a consequential induction or upregulation of proinflammatory cytokines) may facilitate, or be required for, the effective induction of skin sensitization (Cumberbatch et al. 1993, 2000). This would, of course, be consistent with the "danger" hypothesis proposed by Matzinger (1994), wherein a certain degree of tissue damage or disruption is required for the normal development of immune responsiveness. Even if at some levels of exposure certain allergens, through a combination of allergenic and irritant properties, are able to provide a complete stimulus for sensitization (McFadden and Basketter 2000), it can be argued that in circumstances where dose levels are low and/or cause little inflammation, sensitization will be sub-optimal in the absence of a costimulus. There is some indirect experimental evidence to support this (Cumberbatch et al. 1993) and such an interpretation may serve to explain why Kligman concluded from studies in humans and guinea pigs that chemical or physical inflammation, if not too severe, increases the opportunity for skin sensitization (Kligman 1996; Magnusson and Kligman 1970). This being the case it comes as no surprise that the vehicle or formulation in which a chemical allergen is encountered at the skin surface may impact on the development of sensitization (e.g., Basketter 2000). Not only can the matrix in which a chemical is delivered to the skin influence percutaneous penetration, but also the degree of trauma provoked and the resultant cytokine microenvironment.

4.2.3 T Lymphocyte Activation

The pivotal event in the induction of skin sensitization is the stimulation of a specific clonal T lymphocyte response. DCs displaying the allergenic epitope activate

responsive T lymphocytes that are thereby induced to divide and differentiate. As a result, there is a clonal expansion and systemic recirculation of allergen-reactive T cells, such that if the inducing chemical allergen is encountered again, then an accelerated and more aggressive secondary immune response will be elicited. The immunobiological processes that result in skin sensitization are essentially the same as those which confer cell-mediated host resistance to pathogenic microorganisms. In the case of contact allergy, however, the response is directed at an innocuous antigen that in non-sensitized individuals would be tolerated without any ill effects.

4.2.4 Effector T Lymphocytes and the Elicitation of Contact Hypersensitivity

ACD reactions require that effector T lymphocytes are recruited into the sites of dermal exposure to a sensitizer in pre-sensitized individuals, which demand complex cell–matrix interactions regulated by adhesion molecule and integrin–receptor interactions with directional guidance supplied by relevant cytokines and chemokines (Rustemeyer et al. 2011). It was thought for some time that allergen-specific Th1 cells played the predominant role in ACD elicitation. However, other cell types may be of equal or greater importance (Cavani et al. 2001). Evidence has accumulated to suggest that in mice, cytotoxic CD8 T lymphocytes (Tc) are the major or sole effector cells in allergic contact reactions and that CD4 cells may instead have counter-regulatory activity. In humans also there are indications that CD8 T lymphocytes may represent the critical subpopulation. Cavani et al. (1998) examined nickel-specific T cell responses in nickel allergic and nickel non-allergic subjects. Although both groups possessed memory CD4 T lymphocytes that were able to respond to nickel in vitro, discernible CD8 nickel-specific T lymphocyte responses were found only in those with nickel allergy. In parallel, with a growing recognition of the important role played by Tc cells in contact hypersensitivity, evidence is emerging that cytotoxicity for skin cells mediated by allergen-specific CD8 (and CD4) T lymphocytes is a key pathogenetic feature of cutaneous allergic reactions (Trautmann et al. 2000). On the basis of these observations, a case can be made for CD8 Tc1-type cells being major mediators of allergic skin reactions, although for full development of contact hypersensitivity there may be a requirement for CD8 and CD4 type 1 effector cells to act in concert. However, it must be emphasized that in some circumstances, and with some chemical allergens, other cells (type 2 cells) may be important.

4.2.5 The Role of Keratinocytes and Extracellular Matrix

Thirty years ago keratinocytes were not thought to play a role in cutaneous immune mediated inflammation. However, work in succeeding years conclusively proved that keratinocytes produced pro-inflammatory cytokines in response to agents such as

contact irritants and allergens (Barker et al. 1991). It is now recognized that extracellular matrix protein expression can also have pro-inflammatory effects principally through stimulation of the main extracellular Toll-like receptors (TLR), TLR4, and TLR2 (McFadden et al. 2011a). Application of contact allergens/irritants onto the skin results in expression of these pro-inflammatory extracellular matrix proteins (McFadden et al. 2011a). Furthermore, Th1 cells recruited into the skin release extradomainA+ fibronectin, an extracellular matrix ligand for TLR4, thus forming a positive feedback loop that could potentially be operative in Th1 associated inflammatory disease such as ACD (McFadden et al. 2011a).

4.3 Clinical Considerations

In this section, an overview is given of the various clinical aspects of ACD. For more detailed information on the topic, the reader is referred to recent comprehensive textbooks (Rietschel and Fowler 2008; Johansen et al. 2011). For clarity, references have been limited to essential material; for the remainder of the text, the reader is again referred to the standard textbooks just mentioned.

As noted earlier, the causation of ACD is largely the result of dermal exposure to low-molecular weight chemicals below 1,000 Da. It is also the case that proteins can give rise to a similar condition, termed protein contact dermatitis (Hjorth and Roed-Petersen 1976); proteins also can cause immunologic contact urticaria (reviewed in Basketter and Lahti 2011). However, there are still no predictive toxicology assays in this latter area and so for clarity, both of these relatively minor topics have been excluded from this clinical overview.

4.3.1 The Major Contact Allergens

Although a list of major contact allergens would vary in different parts of the world (nickel dominates in many locations, whereas poison ivy leads in parts of the USA and parthenium in India, for example), those variations would be relatively modest and more a matter of detail. The practical reality is that the most important contact allergens are defined simply by an examination of the "standard battery," the first choice allergens that are applied during the diagnostic investigation of suspected ACD. An indicative list is given in Table 4.1 (which is based on the European series)—there are small variations around the world and even between suppliers and member states of the EU; the list changes according to experience as well. For example, the International series adds the preservative imidazolidinyl urea, but removes several others. The Japanese series has slightly different mixes and also includes ethylenediamine dihydrochloride, but again removes several allergens compared to the EU. The US series is larger, adding an extra 40 or so substances—see Table 4.2. Taken altogether though, these 70 substances/mixes represent the

Table 4.1 Major contact allergens of the European standard series

European baseline series (in alphabetical order)	
Balsam of Peru	Mercapto mix
Benzocaine	2-Mercaptobenzothiazole
Bisphenol A epoxy resin	Methylchloroisothizaolinone/methylisothiazolinone
Budesonide	Methyldibromo glutaronitrile
4-*Tert*-butylphenol formaldehyde resin	Neomycin sulfate
Clioquinol	Nickel sulfate
Cobalt chloride	Paraben mix
Colophony	*p*-Phenylenediamine
Formaldehyde	Potassium dichromate
Fragrance mix 1	Primin
Fragrance mix 2	Quaternium 15 (Dowicil 200)
Hydroxymethylpentyl cyclohexene carboxaldehyde	Sesquiterpenelactone mix
N-Isopropyl-*N*′-phenyl-*p*-phenylenediamine	Thiuram mix
Lanolin alcohol	Tixocortol pivalate
DMDM hydantoin	Stearamidopropyldimethylamine

Table 4.2 Additional contact allergens for the US standard series

Bacitracin	Ethyl acrylate
Benzyl alcohol	Ethylenediamine dihyrochloride
Black rubber mix	Glutaraldehyde
Bronopol	Glyceryl monothioglycolate
Carba mix	Hydrocortisone-17-butyrate
Carvone	2-Hydroxymethoxy benzophenone
p-Chloroxylenol	2-Hydroxyethylmethacrylate
Cinnamic aldehyde	Imidazolidinyl urea
Clobetasol proprionate	Iodopropynyl butyl carbamate
Cocoamidopropyl betaine	Jasmine oil
Coconut diethanolamide	Lavender oil
Compositae mix	Lidocaine
Desoximetasone	Majantol
Dialkyl thiourea mix	Methyl methacrylate
Diazolidinyl urea	Oleamidopropyl dimethylamine
Dibucaine	Propolis
Dimethylaminopropylamine	Propylene glycol
Dimethylol dihyroxyethyleneurea	Shellac
Disperse blue mix	Tea tree oil

body of contact allergens which, experience has taught dermatologists, are of greatest clinical importance (i.e., frequency).

As can be seen in Table 4.1, some of the most common contact allergens turn out to be metals, or their salts. Nickel is widely regarded as the most common contact allergen globally (see Sect. 4.3.8), but reactions to cobalt and to chromates also occur frequently. Other common allergens include many fragrances, preservatives,

rubber chemicals, emulsifiers, dyes, epoxy and acrylate monomers, plant substances, and medicaments. Quite obviously, some of these (e.g., acrylates, epoxy resins, rubber) are more associated with occupational allergy, whereas others are more widespread environmental allergens; fragrance and preservative allergies are most commonly caused by cosmetics. On the other hand, some allergens, e.g., *p*-phenylenediamine (PPD), may be both consumer and occupational allergens, in this case in association with hair dyeing.

Considering organic chemicals, the most common allergens are often found in plants. Pentadecylcatechol is the highly potent allergen found in poison ivy in North America and is responsible for sensitizing approximately 50% of the US population. *Evernia prunastri* (formerly known as oakmoss), a natural material extracted from lichens and used in perfumes, is regarded generally as the most common fragrance sensitizer. Other chemicals used in fragrances are relatively potent and common allergens; one example is isoeugenol where the increasing understanding of its potency and evidence of ACD has led to a renewed interest in its risk management (IFRA 2009). Preservative materials such as formaldehyde and certain isothiazolinones also have been relatively common causes of ACD; for these the balance between obtaining adequate preservation activity and sensitization is often very subtle. As such they frequently show a pattern of increasing ACD over a few years as they become more generally used, followed by a steady decline in the incidence of ACD as the fine degree of understanding needed to accurately manage the risk is developed (Dillarstone 1999). The most recent failure in this respect has been methyldibromo glutaronitrile, which prompted a new look at what safe limits might actually be for this preservative (Basketter 2010).

As mentioned above, other chemicals can cause ACD, including epoxy resin chemicals, acrylates, rubber chemicals, certain emulsifiers, and dyes. Of these, much of the ACD which occurs results from occupational exposure (see below). However, non-occupational exposure, for instance to allergens in cosmetics, in clothing and footwear, in medicaments and in plants, represent important causes of ACD.

4.3.2 Minor Contact Allergens

As a rule of thumb, only contact allergens that give positive reactions in over 1% of an eczema population are usually included in a baseline series. Nevertheless, "minor" contact allergens represent a large number of chemicals and as a disclaimer it must be stated that for any individual with allergy to one of these, their experience of ACD may nevertheless be very far from minor. The severity of ACD is in no sense a function of the clinical frequency with which the chemical is noted as a cause of allergy. Many hundreds of contact allergens appear in "special series," e.g., for particular occupations such as hairdressers or bakers, or in classes of chemicals, e.g., the epoxy series, the rubbers series. The encyclopedic efforts of Anton de Groot have resulted in the third edition of his standard work identifying potential contact allergens with recommendations for test concentration in vehicle to be used diagnostically

(de Groot 2008). The list contains over 4,000 entries, of which only approximately 10% are available commercially (and therefore not tested with any great frequency). What follows therefore is simply a very short selection, representing the authors' biases, but which serve to illustrate a number of key points concerning specific contact allergens that also have some general applicability.

4.3.3 Hair Dyes

Hair dye allergy is normally identified by the use of the aromatic amine PPD in the standard baseline series (see above). However, where the clinical picture is highly suggestive of hair dye allergy, but PPD was negative, it may be appropriate to test with a wider panel of potential allergens occurring in hair dye formulations, including resorcinol, *p*-toluene diamine, 2-hydroxyethyl-PPD, etc. In this way, less frequent causes of hair dye allergy can be identified (e.g., Winhoven et al. 2007; Sosted and Menne 2005). Nevertheless, whatever the skin sensitizer responsible, hair dyes represent a major source of ACD associated with the use of consumer products. A screening of more than 2,500 normal adults in Thailand revealed 2.7% were allergic to hair dye (White et al. 2007). They are also significantly problematic as occupational allergens in hairdressers. An additional source of skin sensitization to PPD in recent years has been exposure to temporary henna tattoos, which can often contain very high concentrations of PPD.

4.3.4 Preservatives

This family of chemicals, which includes biocides, is also a relatively common cause of ACD and therefore a number of them are included in the standard series. However, an extended preservative series is available as well. Testing with this can help identify individuals with less common preservatives allergies. It can also assist in detecting changing patterns of preservative use, a phenomenon which leads to the "Dillarstone effect," with repeating epidemics of preservative allergy (Dillarstone 1999). An example of an infrequent allergen of this type might be propyl gallate, which is a very strong sensitizer but where clinical experience suggests it is rarely seen, probably due to low levels of exposure. Another example would be phenoxyethanol, where exposure is much more common, but its very low allergenic potential means that ACD to it is very uncommon.

4.3.5 Fragrances

These represent a wide range of substances, a significant minority of which is known to be sensitizing in man. The eight most common, at least historically, are combined

into a patch test series called fragrance mix 1. A further six are assembled into fragrance mix 2. However, a number of other agents are recognized to be of clinical importance, such that a list of 26 fragrances chemicals much now also be labeled on cosmetic and household products, at least in the EU, although this list is expected to be updated (SCCS 2007). Concomitant reactions to Balsam of Peru and colophony are often found in fragrance allergic patients as well, making these useful additional markers for this significant cause of ACD in consumers. More than 100 fragrance chemicals have been reported as being allergenic. Fragrance exposure is difficult to avoid, being in toiletries, household goods, air fresheners, scented candles, etc.; in addition, exposure can also arise as a secondary consequence of the use of fragrances by other people.

4.3.6 Acrylates and Epoxys

Both of these families of chemicals have long been recognized as important in causing occupational ACD (Kanerva et al. 2004). It is the reactive monomeric epoxy and acrylic chemicals, not the polymers, which represent the major problem, although epoxy hardeners can occasionally be the source of ACD. However, the chemistry is complex: diglycidyl ether of bisphenol A was reported as the most common epoxy allergen, but many other substances contribute to the disease (Jolanki et al. 2004). Acrylates, like epoxys, see widespread industrial use, e.g., in plastics, colors, lacquers, coating, dental and orthopedic materials, and adhesives. However, they also cause cosmetic allergy via acrylic nails. Again, the chemistry is complex, but diagnostic patch testing with a range of acrylates has proven valuable in the identification of individuals with this type of allergic skin disease (Shmidt et al. 2010).

4.3.7 Isopropyl Myristate/Cetostearyl Alcohol

These chemicals have been selected as examples of a wider category of substances that, at first sight, seem highly unlikely to behave as skin sensitizers. They are not reactive and seem unlikely to be the subject of significant metabolic conversion to active agents. Nevertheless, they do give rise to ACD, most notably as medicament allergens, particularly in relation to the chronic treatment of stasis ulcers. They also represent examples of low-level skin sensitizers that are sufficiently weak that they would not be expected to be positive in regulatory predictive tests for skin sensitization hazards. Such weak sensitizers require extensive skin exposure, commonly to damaged and inflamed skin, to provoke a sensitization response. Consequently, they are rare, or even apparently non-sensitizing, when applied at lower concentrations to normal skin.

4.3.8 Epidemiology

While thinking about the epidemiology of dermal allergies, it is important to keep in mind these definitions:

Skin sensitization is the intrinsic property of a substance
Contact allergy is the disease-free state that a skin sensitizer can induce
Allergic contact dermatitis is the disease elicited in an individual with contact allergy

Clearly, identifying skin sensitizers does not inform about epidemiology, only about which substances to use in diagnostic procedures. The substances mentioned in Tables 4.1 and 4.2 are those most commonly patch tested and thus it is for some of these that we can glean information on the extent of contact allergy in both dermatology clinical populations and, infrequently, in the more general population. Sometimes, there can also be information available on the frequency of allergy in selected occupational populations.

Poison ivy allergy is often estimated to occur in a substantial minority of the US population, and parthenium allergy may be similarly common in rural India, but for hard data, it is necessary to revert to the more widely tested allergens, and to focus on the most common of these, nickel. The available data suggested that perhaps 10% of women in Western Europe had contact allergy to nickel, but the proportion with ACD was not identified (Thyssen 2009). Data from Thailand indicated an even higher incidence of contact allergy, but again with no indication of the frequency of ACD (Basketter et al. 2006). Estimates for other general allergens, such as fragrances, suggest that the frequency of contact allergy may be perhaps 1–3% (Thyssen et al. 2009).

Interesting work emanating from Germany carried out statistically robust extrapolations from clinical patch test data to the general population (Schnuch et al. 2002). This information showed a good deal of consistency with the evidence from patch testing on limited numbers of individuals from the general population. Thus it is fair to say that, aside from the very common environmental allergens (e.g., poison ivy), contact allergy is likely to occur in 5–10% of the individuals. Of course, of these, only a more modest proportion is, at any time, expressing eczema relevant to that contact allergy. Nevertheless, this does lead to the conclusion that it is probable that the point prevalence of the resultant skin disease, ACD, in a randomly selected population may well exceed 1%.

From the considerations above an obvious question: what determines whether an individual becomes sensitized? Is it gender, age, genetic makeup, atopic status, and/or other environmental factors? The available evidence points very strongly to exposure being the most important determinant of whether sensitization is induced in an individual (Schnuch et al. 2011). However, it is also evident from some types of exposure that other intrinsic factors come into play, but notably, atopicity is not of relevance (Buckley et al. 2005). For example, the allergens contained in hair dye do sensitize a minority of those exposed (Basketter et al. 2011), but the (largely anecdotal)

evidence also suggests that there are individuals who have been exposed for many years without effect relatively quickly succumb. Subjects may have a positive patch test to PPD without a history of allergic reactions. Elicitation may occur after many years of exposure and presumably gradually increase in PPD reactive T cell population, or through a change of habit, e.g., an aging population may tend to dye their hair more frequently.

Consistent with all of the above, it is common clinical experience that only a proportion of a workforce becomes sensitized in a specific workplace situation and where it is very hard to conclude that those sensitized were those who had the most exposure. Nevertheless, ACD is a significant problem in the occupational setting (Kanerva et al. 2004; Frosch et al. 2011).

4.3.9 Experimental Human Data

Of course, the most convincing evidence that humans are highly variable in their susceptibility to the induction of skin sensitization is derived from experiments completed during the second half of the twentieth century. A substantial body of work exists (Kligman 1996a–c; Marzulli and Maibach 1973, 1974, 1980a, b). Data for a range of skin sensitizing chemicals show that there is a dose response for the induction of sensitization, often encompassing a few orders of magnitude from those most easily sensitized to those most resistant. What is also clear is that 100% can be sensitized—it is merely a matter of dose. A similar body of evidence exists showing that dose responses occur for the elicitation of ACD.

Human experimental data have also provided conclusive evidence that it is dose per unit area that is at the core of ACD (Kimber et al. 2008). As early as 1966, Kligman had shown that 20-fold higher induction dose of hydroquinone given over the whole body was ineffective at sensitization, whereas the much lower total dose applied to a single arm (and therefore achieving a much higher dose per unit area) was an effective sensitizing stimulus (Kligman 1966).

The impact of concomitant irritation on elicitation thresholds for ACD has been studied (Basketter 2000), but except for general acknowledgement that irritation increases the likelihood that sensitization will be induced, little quantitative knowledge exists in this area. Furthermore, primary determinants of individual susceptibility are not well understood and thus it is very difficult to predict which individuals are most likely to become sensitized. Those who have a skin that is more easily irritated may be a little more susceptible (Smith et al. 2000), but those with atopic dermatitis clearly are not (Buckley et al. 2008). Certain genes, for example acetylator status (affecting detoxification of hair dye chemicals), and TNF promoter status (affecting potential "danger" signaling for hair dye chemicals) are associated with hair dye allergy (reviewed in McFadden et al. 2011b). Several studies of identical twins have demonstrated that genetic factors (so far) seem much less important than actual skin exposure (reviewed in Schnuch et al. 2011).

4.3.10 Diagnosis of Allergic Contact Dermatitis

Although this chapter is in no way intended as a practical guide to the diagnostician, it is appropriate to provide a short commentary. The existence of a potential allergic eczema may be suggested by the pattern and type of exposure, together with a patient history, work pattern, which is suggestive of causation by chemical skin contact. Any individual with such a suspect allergic eczema may be invited to undergo diagnostic patch testing, which will use some (or all) of the standard series of contact allergens, together with any specialized allergens deemed relevant from the clinical history/anamnesis. These allergens are applied for 48 h under occlusion and any reactions read approximately 1 and 48 h after patch removal. Positive reactions are best judged by experienced dermatologists and may be relevant to the current eczema and/or to a past eczema. They may also be of no past or present relevance, perhaps suggesting that skin exposure of sufficient scale to evince a clinical reaction is no longer likely to occur, a classic example being thiomersal allergy, where sensitization is commonly associated with infant vaccination programs. A more detailed overview of diagnostic procedures is available (Johansen et al. 2011).

4.4 Identification and Characterization of Skin Sensitizers

4.4.1 Hazard Identification

Historically, guinea pigs have been the species of choice for the assessment of skin sensitization, the approach being to examine the ability of test chemicals to elicit challenge-induced cutaneous reactions in previously exposed animals (reviewed in Andersen and Maibach 1985). After some years, guinea pig methods focused on just two methods: the Buehler (1965) test and the maximization test (Magnusson and Kligman 1970). The basic protocol details of these assays are shown in Fig. 4.1. Extensive lists of chemicals tested in these assays can be found in a number of publications (Wahlberg and Boman 1985; Cronin and Basketter 1994; Basketter and Gerberick 1996). Nevertheless, the methods have never been the subject of formal validation in terms of their sensitivity and specificity. Certainly, false-positive and false-negative results are known to occur (Kligman and Basketter 1995; Basketter and Kimber 2010).

In the last decade attention has focused on a murine method, the local lymph node assay (LLNA). In this method, the skin sensitizing potential of chemicals is measured by their ability to stimulate proliferative responses in lymph nodes draining the site of topical exposure (Kimber and Basketter 1992; Gerberick et al. 2000). An outline of the method is shown in Fig. 4.2. Chemicals which at one or more application doses are able to provoke a threefold or greater increase in draining LNC proliferative activity compared with concurrent vehicle controls are classified as skin sensitizers (Kimber et al. 1994). This model was subjected to formal validation

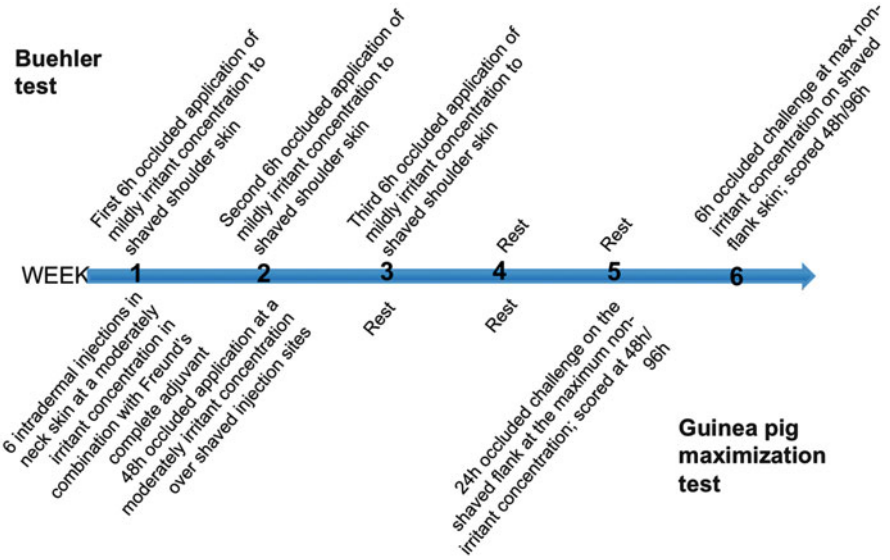

Fig. 4.1 The outline protocols for the two main guinea pig skin sensitization tests are shown as a 6 week timeline. Note that the challenge phase may be repeated if necessary

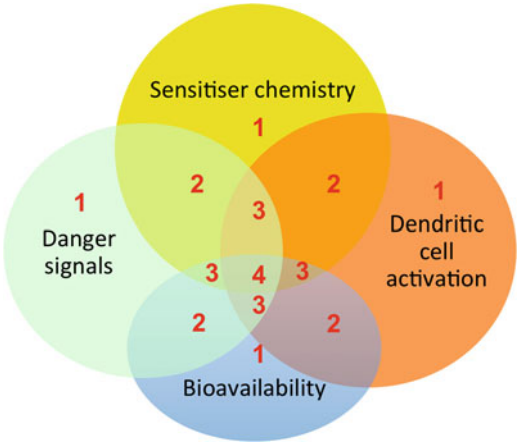

Fig. 4.2 The diagram shows the four main components of the induction of sensitization. Non-animal test data for each of these might be combined as shown, with increasing degrees of overlap indicative not only of confidence in the presence of hazard, but also in the likelihood of increasing potency (i.e., a 1 represents low confidence and weak sensitization, whereas a 4 suggests high confidence and strong sensitization)

via the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and appeared as an OECD Test Guideline in 2002 (OECD 2002). As with the guinea pig methods before it, during this validation the LLNA was shown to be approximately 85% accurate in the predictive identification of skin sensitizing hazards.

4.4.2 Hazard Characterization/Potency Assessment

In addition to conferring animal welfare benefits compared to the guinea pig models, one of the primary attractions of the LLNA was the objective, quantitative endpoint. Furthermore, it was known that there exists a close correlation between the vigor of proliferative responses induced in draining lymph nodes and the extent to which skin sensitization will be acquired (Kimber and Dearman 1991). Consequently, it has become a standard practice to interpolate LLNA dose response data for sensitizers to determine the concentration of chemicals required to cause a threefold increase in proliferation compared with concurrent vehicle control values. This is known as the EC3 value (Kimber and Basketter 1997; Basketter et al. 1999). This measure of potency has been demonstrated to be very robust (Basketter et al. 2007b). Importantly, it has been demonstrated that EC3 values calculated from LLNA studies correlate closely with what is known of the relative sensitizing potency of chemicals among human populations (Basketter et al. 2000, 2005a, b, 2008; Gerberick GF et al. 2001a, b), see Table 4.3 and Fig. 4.3. The table shows a comparison of LLNA EC3 values with expert judgment on human skin sensitization categories, produced by a combination of materials from two publications (Basketter et al. 2000; Gerberick GF et al. 2001a, b). The graph in Fig. 4.3 shows LLNA EC3 values compared to human predictive test thresholds for over 100 substances (with all thresholds expressed in $\mu g/cm^2$), produced by combining information from several publications (Griem et al. 2003; Schneider and Akkan 2004; Basketter et al. 2005a, b; Api et al. 2008). The use/merits of the LLNA EC3 value have been documented by both European expert groups (Basketter et al. 2005a, b) and subsequently, by the World Health Organization (Van Loveren et al. 2008).

4.4.3 Skin Sensitization Risk Assessment

The identification of chemicals with skin sensitization hazard may satisfy regulatory toxicology but is rarely adequate for the proper protection of human health, for example for those chemicals used in cosmetics and personal care products. The hazard tests will identify which chemicals are sensitizing, but may not provide critical information on potency, or of course do they integrate any information on human exposure. Therefore, they should be viewed as an essential prerequisite, but one on which it is necessary to build further analysis, particularly the measurement of the relative potency of a skin sensitizer. In combination with potency data, information on exposure permits a risk assessment to be undertaken. The measurement of the relative potency of skin sensitizing chemicals has been reviewed recently (Basketter et al. 2005a, b; van Loveren et al. 2008). Guinea pig tests were not designed for potency assessment and have not been proven to be particularly useful in this respect. In contrast, the LLNA has dose response assessment and the method used for potency measurement has been to estimate the

Table 4.3 Comparison of LLNA EC3 values and human sensitization potency category

Chemical	Human class	LLNA EC3 (%)
Methylchloroisothiazolinone/ methylisothiazolinone	1	0.05
Diphencyclopropenone	1	0.05
p-Phenylenediamine	1	0.06
Potassium dichromate	1	0.08
2,4-Dinitrochlorobenzene	1	0.08
Glutaraldehyde	2	0.2
Propyl gallate	2	0.3
Formaldehyde	2	0.4
Methyldibromo glutaronitrile	2	0.9
Isoeugenol	2	1.3
Cinnamal	2	3.0
Tetramethylthiuram disulfide	2	6.0
Citral	3	13
Eugenol	3	13
Hydroxycitronellal	3	20
Imidazolidinyl urea	3	24
5-Methyl-2,3-hexanedione	3	26
Ethyleneglycol dimethacrylate	3	35
p-Methylhydrocinnamic aldehyde	3	25
Hexylcinnamal	4	8.0
Benzocaine	4	22
Linalool	4	30
Penicillin G	4	46
Propylene glycol	4	NC
Isopropyl myristate	4	44
Propyl paraben	4	NC
Octanoic acid	5	NC
4′ Methoxyacetophenone (acetanisole)	5	NC
Isopropanol	5	NC
Glycerol	5	NC
Hexane	5	NC
Diethyl phthalate	5	NC
Tween 80	5	NC

concentration necessary to cause a threshold effect. In the LLNA, this is termed the EC3 value, the concentration necessary to cause a threefold simulation compared to concurrent vehicle-treated controls (Basketter et al. 1999). What is more important is that the EC3 values correlate well with human predictive test thresholds (Basketter et al. 2000, 2005a, b, 2008; Ryan et al. 2000; Gerberick GF et al. 2001a, b; Griem et al. 2003; Schneider and Akkan 2004). It is critical to note that this correlation uses the available threshold data from published human experiments, and does not relate to either induction or elicitation thresholds associated with consumer and occupational exposure safe limits—for that, exposure information is required. Of course, an EC3 value, whilst not an absolute measure, is quantitative

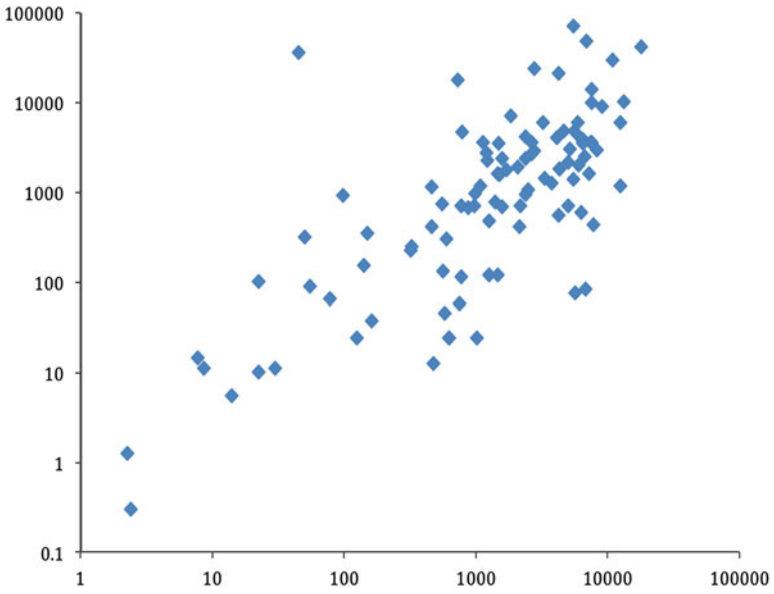

Fig. 4.3 The graph plots sensitization thresholds in the local lymph node assay (LLNA) (in μg/cm^2) on the *x* axis against human experimental test thresholds (also in μg/cm^2) for 102 substances

and has proven to be very reproducible over time and between laboratories (Warbrick et al. 1999; Basketter et al. 2007b).

Owing to the lack of definition of a measure of a no effect level (NOEL) within guinea pig testing strategies, the assessment of skin sensitization risk generally was achieved by the application of comparative toxicology and use of complementary processes such as the human repeated insult patch test (HRIPT). These techniques are detailed elsewhere (Basketter et al. 1996). With the advent of the LLNA EC3 value, which is able to predict the HRIPT induction threshold (Safford et al. 2011), it has been possible to devise an approach to risk assessment similar to that used in many others of repeat dose toxicity. Quantitative risk assessment (QRA) for skin sensitization now uses the LLNA EC3 value (together with any other available information) to predict what is effectively a NOEL in the HRIPT. This value is then adjusted by a number of uncertainty (i.e., safety) factors to determine a maximum acceptable exposure level (Gerberick GF et al. 2001a, b; Felter et al. 2002, 2003; Api et al. 2008). There are several important issues that must be noted. Firstly, exposure is measured in terms of dose per unit area, this being the key measure for skin sensitization (Kimber et al. 2008). Then, the maximum exposure level is calculated for specific product types since the uncertainty factors incorporate elements of the nature of the exposure which may occur (e.g., rinse off vs. leave on) as well as of the vehicle matrix in which exposure occurs. An outline of the QRA process is presented in Fig. 4.4. This process has been evaluated for a range of types of allergen, including fragrances (Gerberick GF et al. 2001a, b; Api et al. 2008), transition metals (Basketter et al. 2003), and preservatives (Basketter et al. 2008; Basketter 2009).

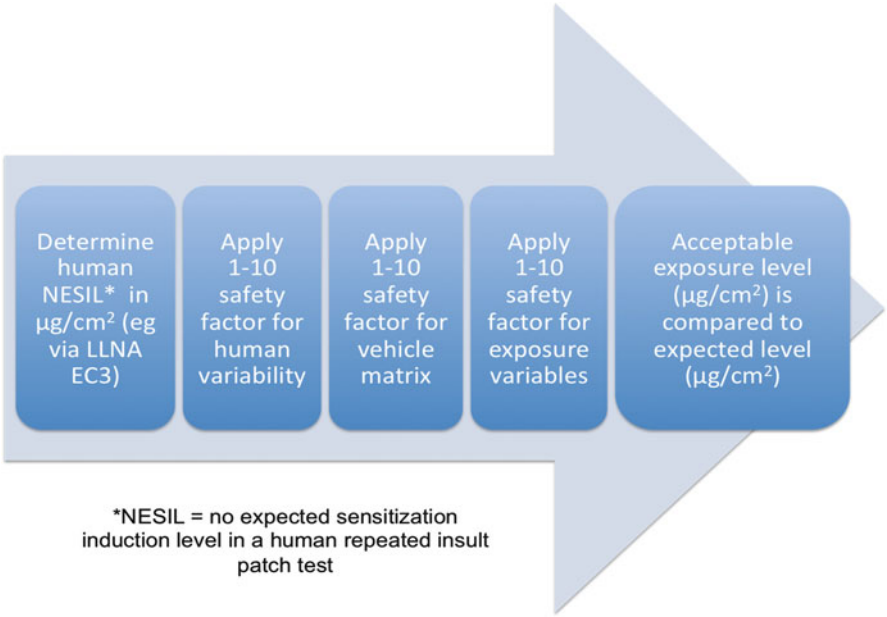

Fig. 4.4 The diagram offers a simplified overview of quantitative risk assessment, demonstrating the principles of applying standard toxicological uncertainty (safety) factors to an experimental human sensitization induction threshold to make it relevant for a specific sensitizing substance being used in a specific exposure situation

It has also been adjusted to take into account exposure at mucosal surfaces (Farage et al. 2003). Furthermore, by combining the daily doses from multiple (product) exposures, it represents a transparent tool for the completion of a more comprehensive strategy for the establishment of safe exposure levels than has heretofore been available. Ultimately of course, as in all areas of toxicology, this risk assessment contains assumptions and requires elements of expert judgment and thus is most appropriately used to guide a final safety decision rather than being regarded as a precision tool.

4.5 In Vitro Alternatives

A question of great current importance is how one can expect to achieve the same quality of skin sensitization safety assessment goal without any use of animals. This seems a more realistic goal than even just a few years ago (Basketter and Maxwell 2007; Kimber et al. 2011), although a recent definitive review suggests that full replacement for risk assessment of all ingredients and in all cosmetic product types may not be feasible before the end of this decade (Adler et al. 2011). It is not appropriate here to recapitulate the details of these publications, so what follows is a

digest. The prospect of predictions on the basis of chemical structure continues to offer limited utility (Patlewicz et al. 2007). Nevertheless, these approaches do offer some very specific benefits, not least that it is not necessary to synthesize the chemical in order to test its sensitizing potential. Most notable has been the definition of mechanistic applicability domains for skin sensitizing chemicals from which a higher degree of confidence in predictions can be achieved (Roberts and Aptula 2008). Such work requires input from a suitably skilled expert in sensitization chemistry. However, the estimation of chemical reactivity by evaluation of peptide binding (Gerberick GF et al. 2004, 2009; Natsch et al. 2007), particularly if combined with other information (Natsch et al. 2009), does provide the confidence that current in vitro efforts are proceeding in a potentially fruitful direction. Indeed, the development of a detailed understanding of reactive chemistry is seen by some groups as the critical step in a non-animal alternative in skin sensitization (Aleksic et al, 2009).

Cell-based assays have also shown some promise, particularly those utilizing dendritic-like cell lines (Ashikaga et al. 2002, 2010; Sakaguchi et al. 2006, Miyazawa et al, 2007; Python et al. 2007). These assays have generally been the subject of some degree of interlaboratory evaluation, but much of the data remain unpublished. All of these cell-based assays (and the other non-animal methods mentioned earlier) suffer from one defining limitation that they provide data only on one aspect of the sensitization induction mechanism. For example, peptide binding assays inform on the potential for chemical reactivity, but do not tell us about skin bioavailability, the capacity to produce danger signals, or the intrinsic antigenicity of the chemically modified self protein structure. It seems probable therefore that data from these various elements of the induction process will have to be combined in order to deliver a non-animal equivalent to the LLNA or guinea pig methods. An outline of the strategic approach to this has been presented already (Basketter and Kimber 2009). A first attempt to translate this theory into practice has recently been published and already shows considerable promise, with a good degree of accuracy (approximately 85%) in terms of both the identification and potency characterization of skin sensitizing chemicals (Natsch et al. 2009). Many of these approaches are now the subject of formal validation activities (Basketter, personal communication).

Figure 4.2 shows a strategy that could be followed for a non-animal approach to skin sensitization evaluation. The simplest way to consider this, at least in terms of simplicity of outcome, is where substantial confidence is gained that the chemical of interest is not a skin sensitizer. In such as case, safety assessment is easy! So, using the numbers 1–4, where each element is "negative" in each component, the more one has consistent in vitro results, the greater the confidence in the absence of skin sensitization. Conversely, if each element is positive, then as the elements overlap and all four inputs converge, then there is a high degree of confidence that the substance is a skin sensitizer. Where data from the four input areas are inconsistent, then a degree of judgment has to be applied, but one relatively obvious decision would be that a negative prediction for bioavailability would not override positive outcomes in all the remaining three areas. In addition, this type of approach might also be adapted to construct a model of the relative potency of newly identified skin sensitizing chemical.

4.6 Concluding Remarks

Dermal allergy to chemicals represents an important cause of skin disease in both consumer and occupational environments. Use of agents such as hair dyes, fragrances, and preservatives carries potential allergic risk. The very nature of some chemicals that give them their functional properties (small molecular weight, protein reactivity) can also bestow allergic potential. Ingenious methods of neutralizing this allergic potential, while preserving their functional properties, are required. Despite our ability (for many decades) to identify sensitizing hazards and a regulatory requirement to do so, the burden of such skin disease remains very much the same. The reality is that it is risk assessment and risk management which impact on health and in this respect it is to be hoped that the drive towards the adoption of in vitro alternatives does not reverse the recent steps forward that have been taken in this respect, notably in QRA. Ultimately, only time will tell, as it is the feedback from dermatology clinics that delivers the final proof of the effectiveness of risk assessment and risk management efforts.

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Chapter 5
Food Allergies

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Abstract Adverse immunologic reactions to food are termed food allergy or food hypersensitivity. For many individuals, having food allergies imposes serious safety and quality of life issues; failure to avoid certain foods can have potentially life-threatening consequences. The prevalence of food allergy appears to be increasing, along with other atopic (allergic) diseases. Allergic reactions to food occur in a variety of target organs, manifesting in the skin, gastrointestinal and respiratory tracts, and cardiovascular system. Severe, systemic responses may result in fatal anaphylaxis. Direct causes of food allergy are unknown, but risk factors identified thus far include genetics, including polymorphisms in genes for immune responses and oxidative stress, intestinal deficiencies, and sensitization to cross-reactive aeroallergens. Evidence also suggests a role for vitamin D, fruit and vegetable consumption, fatty acid intake ratios, and exposure to air pollution.

Key Points

- Food allergies represent a serious public health threat and are increasing in prevalence.
- Along with other atopic diseases, food allergy is more common among industrialized societies and may be a result of modern lifestyles and dietary habits.
- Respiratory sensitization plays an important role in the development of food allergy.
- A role for oxidative stress and inflammation as causative factors is suggested.

Disclaimer The views expressed in this chapter are those of the author and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.

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5.1 Introduction

Adverse immunologic reactions to food are termed food allergy or food hypersensitivity. For many individuals, having food allergies imposes serious safety and quality of life issues; failure to avoid certain foods can have potentially life-threatening consequences. The involvement of the immune system distinguishes food allergy from food intolerance, which may result in similar symptoms but is not immune-mediated, rather resulting from a biochemical inability to process certain foods. Food allergens are typically proteins, but some are chemical haptens (see Sect. 4.2.1). Cross-reactivity can occur when allergens are similarly recognized by the immune system due to shared structures or similar amino acid sequences; this is not limited to foods, but rather can occur with aeroallergens such as pollen. Generally, immune responses to food can be divided into IgE- and non-IgE-mediated reactions, although a given adverse reaction may involve multiple mechanisms. IgE-mediated reactions tend to be immediate and have features clearly recognizable as allergy, such as hives. Non-IgE-mediated reactions often occur in a delayed fashion and tend to be insidious and chronic in nature, often involving damage to the bowel. For example, celiac disease is a non-IgE-mediated immune response to grains, including wheat, and is discussed at length in Chap. 12.

Food allergy, along with eczema, is typically a first sign of closely related atopic diseases which arise in a characteristic sequence commonly called the "atopic march" (Kjellman and Nilsson 1998). There is a tendency for food allergy to arise in infancy and be followed by airway disease later in childhood and adulthood (Wahn and von Mutius 2001). Further, food allergy appears to be associated with more severe asthma; young asthmatics with peanut allergy are more likely to be hospitalized for asthma than asthmatic children without peanut allergy, and use systemic steroids at a greater rate (Simpson et al. 2010). The prevalence of food allergy appears to be increasing, along with other atopic (allergic) diseases including asthma and atopic dermatitis (Grundy et al. 2002; Gupta et al. 2003; Jarvis et al. 2005; Sicherer et al. 2010a; Branum and Lukacs 2009; Liu et al. 2010). A considerable amount of evidence suggests that environmental pollutants are spurring an increase in atopic disease along with other diseases of immune dysregulation. However, pinpointing the exact causes given the broad range of environmental immunotoxicants to which we are exposed is challenging. True prevalence is somewhat difficult to ascertain due to the constraints of food allergy testing (described below), along with reliance on inaccurate self-reporting, and although reports exist of allergic reactions to over 170 foods, prevalence information is only available for relatively common food allergies, namely eggs, milk, soy, wheat, peanuts, tree nuts, fish and shellfish. In 2006, the overall prevalence of food allergy in the United States was estimated to be 3–4% and up to 6% in children (Sicherer and Sampson 2006). More recently, however, a very large study reports the prevalence of food allergy in US children to be 8% (Gupta et al. 2011). For tree nuts and peanuts, well-known for their propensity to elicit severe reactions, the rates for surrogate reported allergy among US children were 0.6 and 0.4%, respectively, in 1997. By 2008, the rates had risen to 2.1 and 1.4% (Sicherer et al. 2010a).

Prevalence rates differ by country, sex, and age group. According to the 2010 Summary of the NIAID-Sponsored Expert Panel Report on food allergy (Boyce et al. 2010), the most common food allergy in the United States is to seafood, affecting 2.8% of adults (0.6% for fish and 2.5% for shellfish). The rate in children is much lower (0.6% for any seafood). With the possible exception of seafood, food allergy in general is more prevalent in children, particularly allergies to cow's milk and egg (Savage et al. 2007; Sicherer and Sampson 2006). However, allergies to milk, egg, and wheat are commonly outgrown, with specific IgE levels for these foods decreasing over time (Bock 1987; Chapman et al. 2006; Eggesbo et al. 2001; Savilahti et al. 2010; Solinas et al. 2010; Wood 2003). Evidence suggests that milk and egg allergies are more persistent than they used to be (Savage et al. 2007; Skripak et al. 2007). Soy allergy may also be somewhat persistent, resolving in 25% of patients by the age of 4 years, 45% by age 6, and 69% by age 10 (Savage et al. 2010). Peanut and tree nut allergies tend to persist into adulthood (Fleischer 2007; Hourihane et al. 1998; Skolnick et al. 2001), and seafood allergy tends to arise in adulthood and persist (Kandyil and Davis 2009). The immunologic mechanisms underlying the natural or therapeutic resolution of food allergy are discussed later in this chapter.

5.2 The Immunology of Food Allergy

5.2.1 Mechanisms of Food Allergy: The Biological Players

Food allergy and food intolerance can result in similar symptoms, and may be stimulated by the same food item, but food allergy is defined as an adverse health effect resulting from a specific *immune response* to that food. Food intolerance is not immune-mediated, but rather results from a biochemical inability to process certain foods. For example, a person may experience abdominal pain and diarrhea after ingesting milk. In some individuals, symptoms are the result of an allergic response to milk components whereas an inability to digest the milk sugar lactose can cause these same symptoms in others, and is known as lactose intolerance. The most common intolerances are to sugars and sugar alcohols (e.g., lactose, fructose, maltitol).

Hypersensitivity reactions to food occur in a variety of target organs, and may be immediate or delayed, predominantly falling under the Type I or IV classification schemes as described by Gell and Coombs (1963), although some may be Type II or III, or occur in combination. In all cases, sensitization is required to recruit and prime the immunologic players that react upon subsequent exposure to the food and cause symptoms during the elicitation phase. Most symptoms classically recognized as allergy result from a Type I or IgE-mediated response, known as immediate or anaphylactic hypersensitivity, which is thought to have evolved as a defense against parasitic and helminth infection. As with other antigens, presentation of food antigens occurs via dendritic cells, macrophages, or epithelial cells. For Type I hypersensitivity responses, antigen presentation occurs in a context that promotes a T helper type 2 (Th2) phenotype, characterized by IL-4, IL-5, and IL-13. This in turn stimulates

eosinophil accumulation and IgE production by B cells; the presence of IgE is a hallmark of allergic sensitization. IgE binds to receptors on basophils and mast cells, thus completing the sensitization phase. Re-exposure to the offending food results in elicitation of an allergic response when the allergen binds to and cross-links the antigen-specific IgE expressed on these cells, causing an immediate release of potent mediators of inflammation (e.g., platelet activating factor) and increased vascular permeability (e.g., histamine). These mediators are responsible for causing overt symptoms, such as hives, swelling or redness in the skin, itching, swelling, or tearing of the eyes, nasal congestion or runny nose, sneezing, itching, swelling of the throat, coughing, wheezing, and labored breathing. The lips or tongue may swell or itch, as is the case with oral allergy syndrome (OAS). Gastrointestinal symptoms, including nausea, vomiting, reflux, pain, or diarrhea, are very common. These types of symptoms have a role in defense against intestinal parasites; intestinal mast cells are involved in expulsion of intestinal helminths, in part by increasing gut permeability (McDermott et al. 2003). Immediate-type hypersensitivity reactions can also affect the cardiovascular system, resulting in rapid heartbeat, decreased blood pressure, dizziness, fainting, or loss of consciousness. A severe, systemic IgE-mediated response may result in anaphylaxis, a potentially fatal reaction.

IgG antibody has also been implicated in Type I allergic responses to food; cross-linking of IgG bound to high affinity receptors on mast cells and basophils causes mediator release in the same fashion as IgE cross-linking (Tkaczyk et al. 2002). IgG antibody also plays a role in Type II and III hypersensitivity reactions. In Type II reactions or cytotoxic hypersensitivity, IgG and complement are involved in the destruction of host cells that have antigen bound to their surface. It is thought that milk-induced thrombocytopenia (loss of blood platelets) is mediated by this type of reaction (Caffrey et al. 1981). Type III reactions, or immune complex hypersensitivity, are characterized by the formation of antigen-antibody-complement aggregates in the tissues, particularly in the joints and kidneys. Generally this reaction, termed an Arthus-type reaction, requires large amounts of antigen available in the bloodstream. There is evidence for this type of reaction in enterocolitis and pneumonia caused by cow's milk allergy, based on intestinal and lung biopsies (Lee et al. 1978; Saavedra-Delgado and Metcalfe 1985). Additionally, there is speculation that arthritis may result from the accumulation of these complexes in the joints and ensuing inflammation, but evidence for this is sparse. One study in patients with rheumatoid arthritis observed symptom improvement in 40% of participants who followed a gluten-free vegan diet, concomitant with a decrease in milk- and gluten-specific IgG (Hafstrom et al. 2001).

Type IV hypersensitivity is mediated by cells as opposed to antibody, and is also known as delayed-type hypersensitivity. As the name implies, these reactions typically occur more slowly (24–72 h after exposure) and are thus somewhat more difficult to diagnose as responses to particular foods. Type IV reactions are characterized by edema and inflammatory cell influx, and often involve the gastrointestinal tract or the skin, causing gastrointestinal enterocolitis and enteropathy, celiac disease, and possibly eczema (roughly 35% of eczema is thought to be due to food allergy, Greenhawt 2010). In these cases, CD4+ T cells release inflammatory Th1

cytokines, including IFN-γ, TNF-α, and TNF-β and chemokines. These mediators recruit and activate macrophages and cytotoxic T cells, which cause tissue damage.

Allergic responses to food can involve any of these four classifications of reactions, but the immune system has mechanisms for limiting such responses to ingested antigens, specifically oral tolerance, which is discussed in the next section.

5.2.2 Oral Tolerance

Our intestinal tracts are exposed to a massive number of foreign antigens, and yet adverse immunologic responses to ingested proteins are relatively rare. This is thought to be due to tolerance, in which the immune system actively ignores proteins encountered in a context perceived to be nonthreatening. As such, food allergy is thought to be a failure to generate oral tolerance or a breakdown in oral tolerance mechanisms. Tolerance may be mediated through the generation of regulatory effector cells or through anergy or deletion of reactive cells. In general, tolerance can occur in any tissue, but antigens in the gut are subject to specialized pathways that lead to oral tolerance, which includes suppression of both cell-mediated and humoral immune responses, particularly IgE and delayed-type hypersensitivity (the Type I and IV reactions) (Christensen et al. 2003; Saklayen et al. 1984). This quality is thought to rely partially on the overall tolerogenic or hyporeactive state of the intestinal immune system, mediated by CD103+ dendritic cells influenced by various factors including CCR7, retinoic acid, TGF-β, and indolamine-2,3-dioxygenase (Vickery et al. 2011). Examination of duodenal biopsies from children with food allergy demonstrated that the primary abnormality in the intestine was not excessive Th2 bias but rather a lack of TGF-β-producing T cells, which maintain tolerance (Perez-Machado et al. 2003).

Another pathway particular to antigens in the gut is transcytosis across the intestinal epithelium, which packages antigens in MHC class II containing exosomes. It is thought that these traffic to the liver via the hepatic portal; blocking the hepatic portal inhibits oral tolerance induction. Antigen processing within the liver leads to the generation of regulatory T cells, many of which express CD103, suggesting that these cells may home to the intestinal mucosa (Hultkrantz et al. 2005; Thomson and Knolle 2010). In mice, oral tolerance can be transferred from a fed animal to a naïve one via liver transplant. In humans, the liver is the least rejected transplant organ, and rejection of other organs is less likely when the donor's liver is transplanted as well, indicating some general tolerogenic function.

Oral tolerance has been found to be defective in neonatal animals (Peng et al. 1989; Strobel and Ferguson 1984), and immaturity of the immune system and intestine may underlie the prevalence of food allergy in children (Sampson 1999). In light of this, however, there does not seem to be good evidence to suggest that avoidance of allergenic foods prevents food allergy. For existing allergy, strict avoidance may allow levels of reactive antibodies and cells to diminish due to lack of stimulation, but frequent exposure to foods may foster the development of oral tolerance.

In animal models, increased exposure to antigens, via larger or more frequent doses, appears to enhance tolerance induction (Bowman and Selgrade 2008; van Wijk et al. 2007). Children who outgrow milk allergy have larger numbers of circulating regulatory T cells and diminished in vitro proliferative responses to stimulation with milk allergen (Karlsson et al. 2004). Resolution of milk allergy is also associated with increased IgG4 epitope binding and decreased IgE epitope binding (Savilahti et al. 2010). Oral tolerance is the goal of oral immunotherapy, in which patients are intentionally fed the offending food. Peanut and cow's milk oral immunotherapy has been shown to increase antigen-specific IgG and IgG4 levels while allowing patients to tolerate increasing doses of allergen (Skripak et al. 2008; Varshney et al. 2011). These antibodies may interfere with IgE-antigen binding and signaling. Stimulation of peripheral blood mononuclear cells (PBMCs) with peanut antigen demonstrated changes in cytokine production, including a decrease in pro-allergic IL-5 and IL-13 and a transient increase in TGF-β, which is associated with tolerance. Additionally, alterations in the CD4+ CD25+ T-cell population suggested the induction of T regulatory cells capable of suppressing allergic responses.

Intestinal health is closely tied to oral tolerance. Integrity of this barrier is important and depends on both physical and immunologic components, including mucus, epithelial junctions, and secretory IgA. Small amounts of antigen do cross the intestinal barrier intact, and generally tolerance mechanisms allow this to occur harmlessly (Kleinman and Walker 1984). However, excess permeability may increase this antigen load and be detrimental. Selective IgA deficiency allows greater entry of food antigens through the intestinal barrier and often results in food allergy (Janzi et al. 2009).

Gut microbes appear to play an integral role in allergy and tolerance (Ly et al. 2011; McLoughlin and Mills 2011). The risk of atopic disease is increased in individuals delivered by cesarean section, possibly due to reduced exposure to maternal flora (Kero et al. 2002; Pistiner et al. 2008; Salam et al. 2006; Xu et al. 2001). The composition of the gastrointestinal flora differs in infants up to 6 months of age depending on the method of delivery (Gronlund et al. 1999). Some evidence suggests that supplementation with probiotics reduces the incidence of eczema (Kalliomaki et al. 2007; Kukkonen et al. 2007), but the role of intestinal flora or antibiotic use in food allergy has not been thoroughly examined (Cochrane et al. 2009).

5.2.3 The Role of the Allergen

Food allergic patients may lack oral tolerance; however, the specificity of allergic reactions indicates that tolerance is lacking only to a small subset of orally encountered antigens. This then begs the question of whether food allergens are somehow excluded from the oral tolerance pathway and therefore unable to dampen the allergic responses produced against them. Animal studies suggest that certain foods, particularly those associated with persistent and severe allergies such as peanut and tree nut, do not induce oral tolerance as readily as foods like egg white, to which

allergies are frequently outgrown (Bowman and Selgrade 2008; Strid et al. 2004). The physicochemical properties of allergens may influence both their ability to sensitize and tolerize. Most food allergens tend to be 10–70 kDa in size, resistant to enzymatic and acid degradation, heat stable and glycosylated. Those food allergens which are labile to acid and enzymes tend to be associated with OAS. Digestion of proteins greatly reduces their immunogenicity, and thus it is thought that the relative stability of allergens makes them available targets for the immune system after trafficking through the digestive tract. Additionally, an allergen must be stable enough to provide two properly spaced epitopes for cross-linking of adjacent IgE molecules on effector cells to elicit allergic symptoms. Alterations in gastric acidity may allow or enhance sensitization or elicitation of allergic responses, as evidenced by the incidence of food allergy arising in patients taking antiulcer drugs (Scholl et al. 2005; Untersmayr et al. 2005) and studies of mice treated with antacids and then exposed to fish or nut allergens (Scholl et al. 2005; Untersmayr et al. 2003). Gastric acidity may also be a key factor underlying the higher incidence of food allergy in early life as infants' stomachs have a relatively high pH (Walker 1986). As previously mentioned, effective oral tolerance induction in mice is achieved through specialized trafficking of antigens across the intestinal tract and to the liver (Thomson et al. 1999; Trop et al. 1999; Yang et al. 1994). Recent evidence suggests that peanut and tree nut allergens survive the stomach but are sensitive to degradation in the intestine, while egg white contains tolerance-inducing proteins that resist both stomach and intestinal enzymes (Bowman and Selgrade 2008). It is possible that the reduced ability of peanuts and tree nuts to induce tolerance in mice and the relative persistence of peanut and tree nut allergies in humans is related to the reduced availability of intact antigen targets for the oral tolerance pathway (Bowman and Selgrade 2008; Strid et al. 2004). Likewise, the ability of egg white proteins to induce oral tolerance in animal models may be related to the frequent resolution of this allergy with age in humans.

Allergens may represent stable targets to which immune responses are mounted when a danger signal such as an adjuvant is present, even if unrelated. Alternatively, allergens may provide these signals themselves. Recent studies have begun to shed light on how proteins or other components in allergenic foods might affect or alter immune signaling or regulation. There is evidence that certain allergens can bind to C-type lectins and activate dendritic cells to produce Th2-related mediators (Shreffler et al. 2006; Wills-Karp 2010). Some allergens also activate complement pathways, which contribute to anaphylaxis (Bergamaschini et al. 1996; van der Linden et al. 1990). For example, extracts of peanut and tree nuts have been observed to activate complement C3a in human and mouse plasma (Khodoun et al. 2009). Activated complement can act as an adjuvant, possibly enhancing sensitization to food allergens (Van den Berg et al. 1991). The processing of foods may increase their allergenicity, particularly when heat induces reactions between the proteins and sugars. Heating egg white protein with sugar enhanced uptake by dendritic cells and activation of antigen-specific T cells to produce IL-4 (Ilchmann et al. 2010). Roasting peanuts renders their allergens less soluble and less digestible, effectively increasing their availability in the gastrointestinal tract for subsequent reactions (Kopper et al. 2005).

A few food allergens and a number of aeroallergens possess protease activity, which is also a feature of parasites (McKerrow et al. 2006). Allergenic proteases have recently been observed to activate production of IL-25 and thymic stromal lymphopoietin (TSLP), which activate Th2 responses (Yu et al. 2010).

5.3 Susceptibility and Risk Factors

5.3.1 Genetics

Genetics play a central role in the probability of developing food allergy. Individuals with first-degree relatives (parents, siblings) having allergic disease are far more likely to suffer from allergy in general (Dold et al. 1992; Sibbald et al. 1980; Taylor and Broom 1981). The production of total IgE is controlled by genetic factors, mapped to several loci within and around the IL-4 gene cluster on chromosome 5q (Cookson et al. 1989; Marsh et al. 1994). Candidate genes include those for IL-4, IL-5, IL-13, all of which promote Th2 responses, and IRF1, whose product may affect IgE production indirectly by influencing expression of IFN-α. In addition to IgE itself, genes for the IgE receptor and other genes involved in regulating Th1/Th2 responses (IFN-γ, STAT6, IL-4R) have been identified as potentially influential in food allergy (Amoli et al. 2002). A promoter polymorphism in the CD14 gene (endotoxin binding protein) is more frequent in individuals with nonatopic asthma and food allergy (Woo et al. 2003). Endotoxin stimulates the release of IL-12 from antigen-presenting cells, which enhances Th1 responses; thus a lack of endotoxin signaling could promote Th2 skewing. Mouse strains defective in endotoxin signaling pathways exhibit strong Th2 bias. IL-12 and TNF-α are also important for delayed-type hypersensitivity reactions. TNF-α is a pro-inflammatory cytokine that contributes to eosinophil recruitment, survival, and activation. An association between a single nucleotide polymorphism in the TNF-α gene (TNF-308) and sensitization to food allergens was observed in a large birth cohort of children in Stockholm (Melen et al. 2008). The polymorphism at position 308 is associated with increased TNF-α production. In eosinophilic esophagitis and atopic dermatitis, genetic variants of TSLP and its receptor are important (Gao et al. 2010; Sherrill et al. 2010). TSLP is an IL-7-like cytokine that contributes to Th2 responses by influencing the nature of T-cell stimulation by dendritic cells (Soumelis et al. 2002).

The ability to recognize and respond to certain allergenic epitopes also depends on genetics. The human leukocyte antigens, or HLA molecules, are critical parts of the cellular machinery involved in presenting antigen to T cells. Specific polymorphisms in both HLA I and II genes are associated with nut and latex/fruit allergies (Blanco et al. 2004; Hand et al. 2004).

Genetics may also influence our ability to cope with environmental stresses which might play a role in food allergy or atopic skewing in general. In particular, oxidative stress has been identified as a key promoter of allergic disease. The glutathione *S*-transferase (GST) supergene family provides protection against damaging

reactive oxygen species (Hayes and Strange 1995; McCunney 2005; Strange et al. 2001). Variants in GSTP1 may confer increased susceptibility to allergic diseases including childhood asthma and allergic rhinitis (Gilliland et al. 2004; Lee et al. 2004). Studies of GST genes and food allergy are rare, but in one study Swedish children with specific polymorphisms in GSTP1 were observed to have an increased risk of sensitization to inhalant and food allergens when exposed to higher levels of traffic pollution (NO_x) during the first year of life (Melen et al. 2008).

5.3.2 Prenatal and Early Life Factors

Because food allergy predominantly affects children, much attention has been given to aspects of early life and development. Although studies offer mixed results (Oddy and Peat 2003), exclusive breastfeeding may prevent food allergy and promote oral tolerance (Gruskay 1982). It also serves to provide the infant with maternal IgA, along with soluble factors to promote maturation of the infant intestinal barrier and immune system. Until fairly recently it was suggested that potentially allergenic foods be avoided, especially for at-risk infants, but current evidence suggests that consumption of allergenic foods promotes tolerance, and delaying the introduction of particular foods can do more harm than good (Katz et al. 2010; Koplin et al. 2010). Peanut allergy has been found to be much lower among Israeli Jews, who typically consume peanuts in infancy, compared to British Jews, who delay peanut consumption until later in life (Du Toit et al. 2008). The role of maternal allergen intake is less clear. Although a number of studies have been conducted, the conclusions vary. A recent study indicates increased risk of sensitization to peanut antigens in atopic infants of mothers who consumed peanut during gestation (Sicherer et al. 2010b). However, restriction of the maternal diet during pregnancy or lactation is not currently recommended. Additionally, although hydrolyzed formula may be used for genetically at-risk infants, soy is not recommended as an alternative (Boyce et al. 2010).

5.3.3 Dermal and Respiratory Sensitization

Several lines of evidence suggest that sensitization to food antigens may occur via skin exposure. The use of peanut oil on children's skin and household peanut consumption are associated with an increased risk of peanut allergy (Fox et al. 2009; Lack et al. 2003). Frequent exposure to latex via the skin or respiratory tract, as is common for healthcare workers, creates risk of latex allergy along with allergy to cross-reactive plant-derived foods (described below) (Kanny et al. 2001). Experimental animals may also be sensitized to food antigens via the skin (Hsieh et al. 2003; Navuluri et al. 2006; Parvataneni et al. 2009; Strid et al. 2005). In these models, animals are shaved before exposure or the skin is otherwise modified, and

Table 5.1 Cross-reactivity between aeroallergens, other foods, or contact sensitizers and foods

Primary allergen	Cross-reactive foods
Birch	Anise, apple, buckwheat, carrot, celery, cherry, coriander, cumin, fennel, hazelnut, honey, kiwi, peach, pear, plum, raw potato, spinach, walnut, wheat
Grass	Cherry, orange, melon, peanut, potato, rye, swiss chard, wheat
Hazel	Hazelnuts (filberts)
House dust mite, cockroach	Shrimp, lobster, mollusks
Latex	Avocado, banana, carrot, chestnut, kiwi, papaya, peach, potato
Mugwort	Apple, carrot, celery, chamomile, kiwi, melon, spices including anise, caraway, coriander, fennel, and parsley
Peanut	Corn, legumes/soy, wheat
Pine	Pine nuts
Ragweed	Banana, chamomile, honey, melon, tomato, sunflower seeds

the impact this has on dermal barrier integrity and potential adjuvancy through irritation cannot be discounted. However, use of peanut oil-containing creams on children generally occurs when a rash is present, so the introduction of an allergen to disrupted or inflamed skin may be a realistic model. Deficiency of filaggrin, a protein which is important in maintaining skin barrier integrity, has not been found to increase the risk of food allergy, but study of this topic has been limited (Marenholz et al. 2009).

Inhalation exposure can be a source of sensitization as well as elicitation of allergic responses to food. Fumes or vapors from cooking, dust from food processing in an occupational or home setting, and exposure to airborne allergens can initiate or exacerbate food allergy (Asero and Antonicelli 2011). In many cases this is due to cross-reactivity, which can occur when allergens from certain food groups share similar 3D structures or amino acid sequences, as occurs with certain nuts, shellfish, fish, grains, and milk from cows, sheep and goats. Antigenic similarity is not necessarily restricted to edible parts of food crops and may extend to aeroallergens such as pollen which is often associated with allergic reactions to foods of the same plant family (see Table 5.1). In addition, aeroallergens such as house dust mite or cockroach antigen may trigger allergies to foods such as shrimp due to the presence of evolutionarily conserved proteins in the arthropod family. Sensitization to cross-reactive aeroallergens is also thought to cause pollen-food allergy syndrome, or OAS, in which allergic symptoms in the mouth and throat occur after ingestion of the cross-reactive food, generally of plant origin. However, symptoms are not necessarily limited to the mouth; progression to systemic effects has been reported in 8.7% of patients, and anaphylaxis occurs in 1.7% of OAS sufferers (Webber and England 2010).

Clinical severity may be tied to the relative stability of the allergenic protein. The lipid transfer proteins (LTPs) resist degradation by both proteases and heat, and their stability is thought to underlie the tendency for LTP-allergic individuals to experience systemic reactions in addition to OAS. Recently it was discovered that an LTP in peanut, Ara h 9, is the predominant protein eliciting allergic responses in

Mediterranean patients, whereas Ara h 2 and Ara h 6 are the peanut proteins most commonly reacted to by peanut allergic in the US individuals (Krause et al. 2009; Lauer et al. 2009). Ara h 9 was found to cross-react with the LTP from peaches, to which allergy is very prevalent in the Mediterranean region, possibly due to genetic factors or local pollen exposures.

5.3.4 Diet and Lifestyle

Food allergy is a disease predominantly affecting industrialized/Western societies, in which the lifestyle and diet differ dramatically from those of our ancestors. In most modern diets, omega-6 polyunsaturated fatty acid intake far exceeds that of omega-3, and there is some evidence that this high ratio of omega-6 to omega-3 fats enhances inflammation and allergy (Dunstan et al. 2003; Hodge et al. 1996; Trak-Fellermeier et al. 2004). Omega-6 fats, which compete for the same metabolic enzymes as omega-3 fats, are readily converted to arachidonic acid, a precursor to PGE_2, which reduces the formation of IFN-γ, affecting the Th1/Th2 balance and IgE production (Black and Sharpe 1997; Roper et al. 1995). A protective effect has been reported for maternal omega-3 fatty acid supplementation in decreasing the risk of food allergy and IgE-associated eczema during the first year of life in infants (Furuhjelm et al. 2009). Infants of supplemented mothers had lower levels of the Th2 cytokine IL-13 in cord blood, and infants of nonallergic supplemented mothers had high levels of the Th1 chemokine CXCL11 and lower levels of the Th2-related chemokine CCL17 (Furuhjelm et al. 2011). In other studies, omega-3 supplementation was associated with some reduced risk of sensitization to food allergens, but this was not statistically significant, and others have found no clear benefit to modification of dietary fatty acid intake in terms of generally preventing atopy (Almqvist et al. 2007; Anandan et al. 2009). A recent systematic review found that omega-3 supplementation during pregnancy, but not lactation, reduced the risk of food allergy (Klemens et al. 2011).

Modern diets also tend to be lacking in fresh produce. A high intake of fruits and vegetables was found to be inversely related to high food-specific IgE levels; most studies point to a protective effect of fruit and vegetable consumption for atopic disease in general, but evidence across studies is not altogether consistent (Rosenlund et al. 2011). It is thought that the antioxidant potential of these foods can limit inflammation and thus curtail allergy. Additionally, fiber or prebiotic intake, supplying intestinal flora with the precursors to anti-inflammatory mediators, has been associated with reduced risk of atopy (Arslanoglu et al. 2008; Gruber et al. 2010).

The modern lifestyle also includes greater amounts of time spent indoors, and vitamin D deficiency is common. Low vitamin D levels have been associated with atopic disease, and a recent study demonstrates an association between low vitamin D levels and IgE levels to peanut and shrimp (but not egg or milk) in children and adolescents, but not adults (Sharief et al. 2011). Notably, vitamin D deficiency was also associated with a higher level of sensitization to aeroallergens that cross-react

with peanut and shrimp. These dietary factors have also been associated with certain autoimmune diseases, and likely influence general immune function.

The hygiene hypothesis contends that reduced exposure to microbes and microbial products is thought to be a contributing factor to the increases in allergic diseases (Strachan 1989). It may be that the modern lifestyle, generally devoid of parasitic and helminth infections, is lacking key components needed to develop immunoregulatory mechanisms. The association of endotoxin binding protein gene promoter polymorphisms with food allergy suggests that microbial products play a role. Although biologically plausible, this is still an area of ongoing research and debate (Bloomfield et al. 2006).

5.3.5 Environmental Insult

The environmental factors contributing to the development of food allergy are not well-characterized, but evidence suggests that exposure to air pollution has significant effects on food allergy as well as other atopic diseases. Sensitization to milk and egg allergens in both girls and boys in Germany was observed to be positively associated with outdoor NO_2 levels, a marker for traffic-related pollution (Kramer et al. 2000). In a laboratory setting, NO_2 increases eosinophil activation in an allergen provocation challenge in human volunteers (Wang et al. 1995). Lead (Pb) is known to have potent immunomodulatory effects, able to suppress production of Th1 cytokines (e.g., IFN-γ) while increasing production of Th2 cytokines (e.g., IL-4). Pb may directly influence dendritic cells to promote Th2 responses, altering CD86/CD80 ratios and diminishing IL-12 output (Gao et al. 2007). Children with higher blood Pb levels have been observed to have higher levels of serum IL-4 and IgE (Annesi-Maesano et al. 2003; Karmaus et al. 2005; Lutz et al. 1999; Sun et al. 2003), and higher blood Pb levels correlate with higher serum IgE levels in environmentally and occupationally exposed adults (Heo et al. 2004; Pizent et al. 2008). However, no studies have been conducted to examine the association of blood Pb levels and sensitization to food allergens. Sensitization to aeroallergens, which confers some risk of cross-reactive food allergy, was found to be positively associated with particulate matter (PM) pollution and residential distance to the nearest main road in a birth cohort study of children in Munich, Germany (Morgenstern et al. 2008). Similarly, an association between skin prick test reactivity to house dust mite and lifetime exposure to outdoor air pollution (NO_2, PM) was observed in a study of children residing in Oslo, Norway (Oftedal et al. 2007) and sensitization to pollen was associated with benzene and PM in French children (Penard-Morand et al. 2010). Skin prick test positivity to indoor allergens was also significantly increased in French children residentially exposed to PM concentrations that exceeded WHO air quality limits (Annesi-Maesano et al. 2007). In experimental studies, PM has been shown to function as an adjuvant in humans and in rodents (Diaz-Sanchez et al. 1996; Takafuji et al. 1987), and increases IgE levels (Diaz-Sanchez et al. 1997, 1999; Fujieda et al. 1998; Steerenberg et al. 2004). Although most of these studies utilize intranasal

exposure, adjuvant activity can be reasonably assumed to apply to other mucosal surfaces as well, and much of the PM we inhale ultimately ends up being swallowed (Eyles et al. 2001). Interestingly, oral exposure to PM has been shown to inhibit oral tolerance in mice (Yoshino et al. 1998).

One of the common features of different forms of air pollution is the ability to exert oxidative stress (Hiura et al. 1999; Kelly 2003). For PM, oxidative potential is closely tied to adjuvant activity (Li et al. 2009; Whitekus et al. 2002). As noted in the section on genetic susceptibility, polymorphisms in GST genes involved in protection against oxidative stress are associated with increased risk of atopic disease in general. In a large birth cohort in Sweden, children with specific polymorphisms in GSTP1 had an increased risk of sensitization to inhalant and food allergens when exposed to higher levels of traffic pollution (NO_x) during the first year of life; for one polymorphism this translated to a nearly 2.5-fold increase (Melen et al. 2008). The interaction between one GSTP1 polymorphism and air pollution was much stronger with respect to sensitization in children carrying a particular TNF-α variant (representing increased TNF-α), indicating a gene–gene–environment interaction.

Oxidative stress may also come from dietary contaminants, including aflatoxin B1, which is detoxified by GSTM enzymes (Eaton et al. 2001). Aflatoxins are produced by some strains of *Aspergillus* fungi. Notably, many allergenic foods are contaminated with aflatoxin B1 or its metabolite (M1) in the case of milk and eggs. It has been speculated that contamination of foods with aflatoxin contributes to their allergenicity. As a potential mechanism, aflatoxin B1 may suppress immunologic factors required for Th1 responses, which could promote allergic skewing (Kocabas and Sekerel 2003). Additionally, aflatoxins are directly toxic to the liver, an organ critical for the generation of oral tolerance.

Contamination of foods with certain pesticides could also potentially contribute to the development of allergy, although evidence for this is limited. Occupational pyrethroid exposure has been associated with higher IgE levels, and it is thought that pyrethroids exert oxidative stress (Shoukry 2011). A cross-sectional study in children observed an association between increased IgE levels (serum and basophil-bound) and higher blood concentrations of dichlorodiphenylethylene (DDE) (Karmaus et al. 2005). Biopesticides, derived from molds or bacteria, may also be of concern. Insecticidal toxins from *Bacillus thuringiensis*, a common soil bacterial species, have been sprayed on US crops since the late 1950s and are now expressed within the tissues of genetically engineered plants. It was originally thought that Bt toxins only bound to the insect midgut and had no activity in mammals. It has now been discovered that particular Bt toxins have cytolytic activity in human cell culture (Tayabali and Seligy 2000), and bind to the rodent intestinal mucosa (Vazquez-Padron et al. 2000). Moreover, one Bt toxin (Cry1Ac) has exhibited intranasal and oral adjuvant activity in mice, whereby administration enhances the immune responses to a co-administered, unrelated antigen (Moreno-Fierros et al. 2000, 2003; Vazquez-Padron et al. 1999; Vazquez et al. 1999). Although it is difficult to compare these experimental exposures with what are probably very low level but chronic dietary exposures, it appears that real world exposures result in detectable levels of Bt toxin in humans. A recent study reports that Cry1Ab was found in the

serum of nonpregnant women and pregnant women as well as their fetuses, suggesting that prenatal exposures are relevant as well (Aris and Leblanc 2011). However, adjuvant activity in mice may not necessarily translate to adjuvant activity in humans. Additionally, the nature of the immune responses elicited in mice (i.e., Th1 vs. Th2) has not been evaluated. It has been shown that IgE antibodies are produced against Bt toxins in greenhouse workers (Doekes et al. 2004), and positive skin prick tests and IgE antibodies to Bt are evident in farm workers (Bernstein et al. 1999), but these studies speak to the immunogenicity and allergenicity of Bt toxins under occupational exposure conditions, not the potential for adjuvanticity via dietary exposure.

Other environmental contaminants also exhibit adjuvant activity, promoting Th2-type responses. Phthalates have been shown to function as Th2 adjuvants in mice (Larsen et al. 2001, 2002), and allergic symptoms in children have been associated with phthalate levels in the home (Bornehag et al. 2004). Bisphenol A (BPA) has been shown to enhance IL-4 and IgE responses in mice, possibly through action at the IL-4 gene promoter (Lee et al. 2003; Tian et al. 2003). Additionally, mice exposed to BPA prenatally or in adulthood exhibited smaller percentages of CD4+ CD25+ cells, suggesting reduced numbers of T regulatory cells; this was particularly evident in prenatally exposed animals (Yan et al. 2008).

5.4 Summary

The prevalence of food allergy appears to be increasing, along with other atopic diseases, including asthma and atopic dermatitis, particularly in industrialized societies. For many individuals and their families, food allergies impose serious safety and quality of life issues, given that failure to avoid certain foods can have potentially life-threatening consequences. Food allergy also worsens disease severity for asthmatics. Although food allergy tends to be a disease of childhood, many allergies persist into adulthood and a rather bleak picture is emerging in which allergies previously thought to be fairly transient, such as those to egg and milk, are increasingly persistent. Treatment options are few; patients are advised to avoid the offending food, but mounting evidence suggests that exposure is the only route to true tolerance. Immunotherapies based on this premise are progressing, but not without pitfalls.

Although the causes of food allergy are unknown, and likely to be a combination of factors, the current evidence suggests a strong role for oxidative stress. Genes involved in protection against oxidative stress have been shown to be important, and types of air pollution and dietary contaminants which exert oxidative stress have been implicated as causal factors, although some of these relationships are tenuous or speculative. Antioxidants have been shown to be protective for a number of different atopic diseases, and the protective effect of dietary intake of antioxidant providing fruits and vegetables is a good example. Inflammation, a known response to oxidative stress, is also implicated, with variants in TNF-α being associated with

allergic sensitization. The role of inflammation is also highlighted by the protective effect of a higher omega-3 to omega-6 fatty acid ratio, which is thought to reduce inflammation and allergy by diminishing pro-allergic PGE_2 levels. Most of these factors have more firmly established connections with respiratory sensitization, but it is clear that respiratory sensitization is closely tied with, and often precedes, food allergy. Cross-reactivity with aeroallergens, both indoor and outdoor, is a major contributor to allergies to both plant and animal-derived foods. Thus the causes of respiratory sensitization can be reasonably expected to contribute to the burden of food allergy. To summarize, the modern lifestyle, with its increasing rates of cesarean sections, early weaning of infants, excessive time spent indoors, diet high in pro-inflammatory foods and low in antioxidants, antibiotic use, and lack of microbial and helminthic stimulation offers a number of options for blame with respect to food allergy along with other atopic diseases, but these etiologies are undoubtedly very complex and unique to individuals and populations.

5.4.1 Recommendations

The environmental influences spurring increases in food allergy prevalence are likely to be the same as those driving other atopic diseases. As such, the ability of environmental contaminants to cause general immune dysregulation by way of inflammation, Th2 skewing, or interference with tolerance mechanisms is of interest. Assessing perturbations in oral tolerance induction is not classically part of immunotoxicity testing, but given the role of oral tolerance in limiting food allergy, this type of evaluation may be warranted for chemicals even in the absence of evidence for other forms of immunotoxicity, particularly when there are indications of liver toxicity. Testing of common food contaminants including pesticidal Bt toxins and mold toxins such as aflatoxin B1, using relevant exposure conditions, could elucidate any potential role for these in stimulating responses against ingested antigens or interfering with oral tolerance. Obtaining a greater understanding of the properties of antigens that make them able to orally tolerize and the application of this information to immunotherapy could potentially be of significant value.

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Part III
Autoimmune and Inflammatory Diseases and Conditions

Chapter 6
Factors Involved in Onset of Type 1 Diabetes

Anne Cooke and Paola Zaccone

Abstract Type 1 diabetes (T1D) is an autoimmune disease where the immune system selectively targets the pancreatic insulin-producing beta cell. This disease is increasing in incidence faster than can be accounted for by genetic change thus highlighting the importance of environmental modifiers. The environmental modifiers of disease onset can vary from agents such as viruses proposed to precipitate diabetes onset to those inhibiting diabetes onset in susceptible individuals. Complications of diabetes such as cardiovascular disease, diabetic retinopathy and nephropathy can develop in affected individuals. Strategies to prevent or cure T1D include the delineation of biomarkers to enable identification of individuals in the process of developing diabetes, the generation of immune tolerance to pancreatic beta cells and exploration of ways of restoring the destroyed beta cell mass. Progress has been made in the development of therapeutic strategies and some of these have been translated to the clinic.

Key Points

- Type 1 diabetes is an autoimmune disease, which is increasing in incidence in the developed world.
- Onset of Type 1 diabetes is influenced by both genetic and environmental factors.
- Infectious agents have been implicated in both the initiation and the inhibition of the onset of Type 1 diabetes.
- Complications including cardiovascular disease, retinopathy, nephropathy and neuropathy may develop in Type 1 diabetic patients.
- Therapeutic strategies involve targeting T cells either specifically or non-specifically.

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6.1 Introduction

Type 1 diabetes (T1D) is an autoimmune disease where the insulin-producing pancreatic beta cells are selectively destroyed by the immune system. This is classically a disease of juvenile onset and is one of the most common chronic diseases of childhood. The incidence of T1D has been increasing dramatically over the last 30 years at a rate faster than can be accounted for by genetic change. The development of T1D is under complex genetic control with environmental factors additionally playing a key role in determining disease onset. T1D is sometimes accompanied by a range of complications such as cardiovascular disease (CVD), nephropathy, retinopathy and neuropathy (Table 6.1).

6.2 Type 1 Diabetes Is an Autoimmune Disease

An autoimmune aetiology for T1D was initially suggested by the seminal observations of Gepts concerning mononuclear cell infiltration around the islets in the diabetic pancreas (Gepts 1965). The development of animal models such as the BB rat and the NOD mouse (Makino et al. 1980; Nakhooda et al. 1977) permitted detailed analysis of disease aetiology and further supported an autoimmune basis for beta cell destruction. Studies of the effects of cyclosporine immunosuppression on diabetes in recent onset patients also suggested an autoimmune aetiology (Stiller et al. 1984). It was, however, the studies of Sutherland and his colleagues that provided concrete evidence that in humans T1D is an autoimmune disease. In these studies, the islets in pancreas grafts from identical twins were rapidly infiltrated and the pancreatic beta cells destroyed in the diabetic twin recipient (Sibley et al. 1985; Sutherland et al. 1989); such destruction was only prevented by concomitant immunosuppression. The observation that T1D can be transferred by human bone marrow cells further confirms the autoimmune aetiology in humans (Lampeter et al. 1993).

Table 6.1 Complications in Type 1 diabetes

Pathology	Complications
Cardiovascular disease	Angina, heart attack, stroke, atherosclerosis and high blood pressure
Nephropathy	Hyperglycaemia and poor blood supply to the nephrons can cause damage to the kidney filtering system, leading to kidney failure
Neuropathy	Hyperglycaemia can cause nerve damage in the periphery with consequential: loss of sensitivity, nausea, vomiting, etc.
Retinopathy	Hyperglycaemia can cause: cataracts, glaucoma and damage the blood vessels of the retina potentially leading to blindness

6.3 Cell Types Involved in the Development of Type 1 Diabetes

Much has been done to clarify the role of different myeloid and lymphoid cells involved in the development of T1D (Table 6.2). The animal models support a role for macrophages, $CD4^+$ T cells and $CD8^+$ T cells playing a role in the demise of the beta cell (El-Sheikh et al. 1999; Hutchings et al. 1990a, b; Miller et al. 1988; Nagata et al. 1994; Phillips et al. 2009; Walker et al. 1988) with dendritic cells (DC) playing key antigen-presenting roles at disease initiation (Turley et al. 2003). It is thought that the key initiating event takes place in the pancreatic draining lymph nodes (Gagnerault et al. 2002). Other cell types are present in the pancreatic draining lymph nodes and in the islet infiltrates including NK cells, NKT cells and B cells, and there is evidence of crosstalk between these populations, which ultimately determines whether diabetes ensues or is regulated (Lehuen et al. 2010). Studies of pancreatic biopsies from humans who had recently diagnosed T1D support the findings of the different cell populations identified in the murine models in the infiltrated pancreases of patients (Itoh et al. 1997). In terms of mechanism(s) of beta cell destruction, several have been proposed, and it is possible that all of them may occur. There are data supporting cytokine-mediated destruction, class I-restricted killing mechanisms as well as involvement of Fas/FasL interactions. Evidence suggests that the beta cell dies by apoptosis and not necrosis (Kurrer et al. 1997), and this would be consistent with these mechanisms of destruction.

Regarding cytokine-mediated destruction of pancreatic beta cells, there is good evidence from in vitro studies highlighting the detrimental effects of cytokines such as IL-1, TNFα and IFNγ and their synergistic effects on beta cell survival (Grunnet et al. 2009; Mandrup-Poulsen et al. 1987, 2010; Nicoletti et al. 1994). As macrophages and T cells elaborate these cytokines, there is likely to be ample production of them in the infiltrated pancreas. In terms of $CD4^+$ T cells involved in the development of T1D, much of the data supports a role for IFNγ-producing Th1 cells rather than IL-4- and IL-5-producing Th2 cells (Boyton et al. 2005; Trembleau et al. 1995; Wang et al. 1997; Yang et al. 2004). However, since the identification of two other $CD4^+$ T cells, Th17 and Th9, identified by the production of their signature cytokines IL-17 and IL-9 respectively (Bettelli et al. 2006; Jager et al. 2009; Veldhoen et al. 2006, 2008), there has been increasing interest in the involvement of these populations in autoimmunity. In part this has arisen through the proposed involvement of these pro-inflammatory cell types in the initiation of other autoimmune diseases and their presence at sites of inflammation. The observation that Th17 cells may manifest plasticity and convert to Th1 cells, not only in vitro but also in vivo, may mean that both $CD4^+$ T-cell subsets play a role in T1D; but the effector $CD4^+$ T cell population may in fact be the Th1 cell type (Bending et al. 2009, 2011; Martin-Orozco et al. 2009).

$CD8^+$ T cells form a predominant cell type in the diabetic human and murine pancreas as well as in recurrence of autoreactivity in pancreas transplants between identical twins (Amrani et al. 2001; Itoh et al. 1993; Santamaria 2003; Santamaria et al. 1995; Sibley et al. 1985). In addition to being potent producers of pro-inflammatory

Table 6.2 Cells involved in Type 1 diabetes

Cell type	Pathogenic role	Protective role
Macrophages	Antigen presentation, inflammatory cytokine secretion (e.g. IL-12, IL-18, NO, etc.)	
Dendritic cells	Self-antigen presentation leading to clonal expansion of effectors/diabetogenic cell	
$CD4^+$ T cells	T helper 1 (Th1) $CD4^+$ T cells infiltrate the pancreas, secrete inflammatory cytokines (e.g. IL-1, IFNγ, TNFα, etc.)	T helper 2 (Th2) $CD4^+$ T cells (secreting IL-4, IL-5)
	The roles of Th17 and Th9 remain to be fully clarified	$CD4^+$ regulatory T cell expansion (IL-10 and/or Foxp3 positive) can counteract the pathogenic role of effector T cells
$CD8^+$ T cells	Cytotoxic $CD8^+$ T cells infiltrate the pancreas, secrete inflammatory cytokines, granzyme and perforin	Generation/expansion of autoantigen-specific $CD8^+$ regulatory T cells
NK and iNK T cells	Although studies in animal models and in man have shown that NK cells are involved in disease progression, the contribution of NK cells in T1D still needs to be fully elucidated	Expansion and activation of iNKT cells (expressing Type 2 cytokines, e.g. IL-4, IL-5)
B cells	Possible role as antigen-presenting cells. Secretion of autoantibodies (e.g. anti-GAD, anti-IA-2 and anti-ZnT8) has not been demonstrated to have a clear pathogenic role	

cytokines, $CD8^+$ T cells mediate cytotoxicity by cytolytic granules containing granzyme and the pore-forming perforin. There is evidence of increased expression of cytotoxicity-related molecules on $CD8^+$ T cells in the pancreatic infiltrates (Graham et al. 2011; Yamada et al. 2010) and deletion of perforin or inhibition of Cathepsin L activity has been shown to inhibit diabetes onset in NOD mice (Kagi et al. 1996; Yamada et al. 2010). Cathepsin L has recently been shown to play a role in granzyme B activation (Yamada et al. 2010). There are numerous studies investigating the role of Fas/FasL interactions in diabetes development. Fas expression is increased in pancreatic beta cells in the diabetic pancreas (Moriwaki et al. 1999; Stassi et al. 1997; Suarez-Pinzon et al. 1999), and activated T cells ($CD4^+$ T cells and $CD8^+$ T cells) through expression of FasL have the potential to kill these endocrine cells. Blockade or genetic loss of FasL prevents diabetes onset in NOD mice (Itoh et al. 1997; Su et al. 2000; Wen et al. 2011). However, it is important to note that non-immunological effects also have been identified in regard to Fas expression on the pancreatic beta cell. Specific targeting of Fas to downregulate its expression in the pancreatic beta cells has been shown to lead to increased beta cell function with increased insulin production and improved glucose tolerance (Choi et al. 2009). Thus, the increased Fas expression on beta cells noted in the diabetic pancreas can result in beta cell deficiency in more than one way.

The role of B cells remains somewhat enigmatic. Autoantibodies that arise during the pre-diabetic period provide useful markers of ongoing beta cell autoreactivity and can serve as diagnostic predictors of disease onset. Those autoantibodies that appear to be the most useful markers are those directed at the antigens insulin, glutamic acid decarboxylase (GAD), IA-2 (a member of the protein tyrosine phosphatase family) and more recently the zinc transporter (ZnT8) (Guo and Achenbach 2008). There is, however, no evidence to support a pathological role for these autoantibodies. Unlike Graves' disease or myasthenia gravis, there is no evidence of maternal transmission of T1D. Indeed, there is one report of T1D development in an individual suffering from X-linked agammaglobulinaemia indicating that autoantibody formation is not necessary for diabetes development (Martin et al. 2001). In the NOD mouse model of T1D, depletion of B cells, either through the use of antibodies pre-diabetically or through genetic means, has been shown in most studies to inhibit insulitis and prevent disease onset (Forsgren et al. 1991; Serreze et al. 1996; Xiu et al. 2008). It is thought that B cells may play a role in diabetes through presentation of islet antigens to autoreactive T cells (Noorchashm et al. 1999). Interestingly, depletion of B cells was associated with an increase in a population of regulatory T cells (Tregs), so-called $CD4^+$ $Foxp3^+$ Tregs or natural Tregs (Marino et al. 2009). Increases in regulatory T-cell activity are often associated with successful therapeutic outcomes in autoimmune disease studies.

6.4 Putative Immune Deficiencies and Type 1 Diabetes

It has been suggested that autoimmune diseases may result from homeostatic expansion in a lymphopenic environment (Stockinger et al. 2004). Early studies in the diabetes-prone BB rat (BBDP) identified a lymphopenia that was associated with diabetes susceptibility (Poussier et al. 1982; Yale et al. 1985). A marked deficiency in regulatory T cells expressing a marker RT6 (now known as ART2) was shown to be a hallmark of BBDP rats. The diabetes-resistant subline of BB rats (BBDR) was found to have this $ART2^+$ regulatory T-cell population, and their depletion was shown to render the BBDP susceptible to diabetes onset (Greiner et al. 1987). Subsequent studies have identified a frame shift mutation in the *Gimap5* gene as being responsible for the peripheral lymphopenia in these rats (Hornum et al. 2002; MacMurray et al. 2002). This mutation in BBDR rats appears to affect the survival of $CD4^+$ $Foxp3^+$ regulatory T cells in the periphery after they leave the thymus resulting in a dysregulated immune response and the development of T1D (Poussier et al. 2005). These studies provide support for selective deficiencies in regulatory populations predisposing to autoimmunity.

There are conflicting data regarding lymphopenia in NOD mice. There are data showing no obvious deficiencies in cell numbers in these mice (Berzins et al. 2003) and in particular no age-related deficiencies in regulatory T cells identified by expression of the transcription factor Foxp3 (Grinberg-Bleyer et al. 2010; Mellanby et al. 2007; Tang et al. 2008). Other studies, however, have suggested deficiencies in

this population of cells particularly in the pancreatic draining lymph nodes (Pop et al. 2005). Depletion of $CD4^+$ $Foxp3^+$ regulatory T cells from NOD mice accelerates onset of diabetes highlighting the importance of these cells in ongoing prevention of autoimmune pathology (Brode et al. 2006; Mellanby et al. 2007). Therapeutic approaches to further amplify this population include both antigen-specific and non-antigen-specific approaches (Cooke et al. 2001; Grinberg-Bleyer et al. 2010).

Another T-cell population identified as being deficient in NOD mice is the Type 1 or iNKT population of cells (Baxter et al. 1997; Godfrey et al. 1997; Gombert et al. 1996). These NKT cells express semi-invariant TCR where the invariant Vα14-Jα18 chain is coupled to Vβ2, 8.2 or 7. In humans, this population of cells expresses an invariant Vα24-Jα18 coupled to Vβ11. NKT cells recognise glycolipids presented by the MHC class I-like molecules, CD1d. These cells rapidly produce cytokines when activated, the nature of which depends very much on the context and the microenvironment in which activation takes place. In this way they have the potential to interact with both innate and adaptive arms of the immune system and can influence the outcome of a response through such cross-talk (Lehuen et al. 2010). The potential contribution of this population to diabetes prevention has been shown by numerous studies designed to increase NKT cells numerically (Hammond et al. 1998; Lehuen et al. 1998) and functionally (Fletcher and Baxter 2009; Hong et al. 2001; Sharif et al. 2001). The mechanism of protection has been shown to involve the generation of anti-inflammatory cytokines combined with effects on other cell populations such as dendritic cells such that immune tolerance prevails and pathogenic T cells do not develop (Beaudoin et al. 2002; Chen et al. 2005; Lehuen et al. 2010).

6.5 Genetics and T1D

The development of T1D is governed by both genetic and environmental factors (Fig. 6.1). Involvement of an environmental effect is exemplified by the observation that the concordance rate for diabetes development in monozygotic twins is not 100% but ranges from 40 to 60% depending on the population under study (Hyttinen et al. 2003). Furthermore, the incidence of T1D has been increasing faster than can be accounted for by genetic change in the developed world, again highlighting the need to consider the effects of environmental change (Dunne and Cooke 2005). Over the last 30 years, the overall annual incidence of T1D has increased by around 3% in Northern Europe. This increase in incidence is particularly notable in children under the age of 5 years (Patterson et al. 2009; Soltesz et al. 2007). The major susceptibility locus for T1D maps to the HLA class II region of humans with relative risk being associated with certain combination of alleles of the *HLA-DRB1*, *DQA1* and *DQB1* genes (Morahan et al. 2011; Pociot et al. 2010). The genotype *DRB1*0301-DQA1*0501-DQB1*0201/DRB1*0401-DQA1*0301-DQB1*0302* for example represents a high-risk genotype. Likewise, the MHC class II region in the NOD mouse and BB rat models was also identified

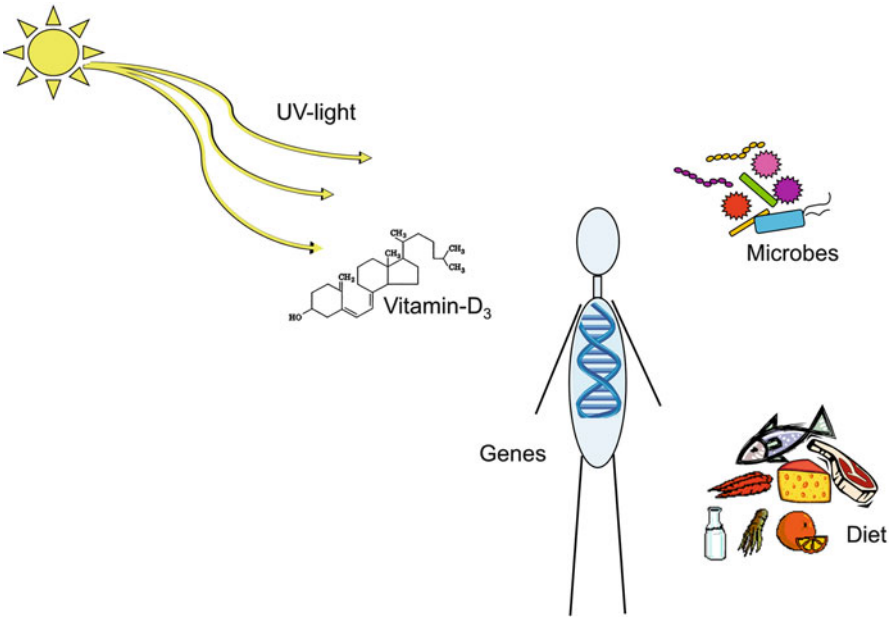

Fig. 6.1 Genetic and environmental components in Type 1 diabetes (T1D). T1D development is influenced by an individual's genetic background and environmental factors such as sunlight, diet and exposure to infections. Vitamin D deficiency combined with low exposure to sunlight (UV) are known contributing factors to the development of T1D. Infectious agents have been demonstrated to have a dual role in controlling T1D, both inducing/precipitating but also preventing T1D (Hygiene Hypothesis). Food intake is an important regulating factor in T1D, contributing for example to the composition of the gut microflora with implications for the host immune system

as playing a major role in conferring susceptibility to this autoimmune disease. The increasing incidence of T1D, which is currently seen in many countries, appears to be due to an increased incidence among individuals bearing a lower-risk HLA haplotype and not due to changes in incidence in those with a high-risk haplotype. The insulin gene locus itself also plays a major role in determining resistance or susceptibility to T1D (Bell et al. 1984). In humans allelic variants with different sizes of variable number tandem repeats (VNTR) flanking the INS locus have been identified, which appear to be associated with differing levels of insulin production in the thymus (Pugliese et al. 1997; Vafiadis et al. 1997). In terms of mechanism, it is proposed that lower level expression of the insulin gene in the thymus might permit the escape of autoreactive insulin-specific T cells into the periphery. Genetic studies have made it clear that T1D is under complex genetic control and that many other loci are involved, albeit with smaller effect than HLA or insulin. Recent genome-wide association studies have shown that there may be as many as 40 other loci playing a role in diabetes susceptibility (Barrett et al. 2009). Likewise, in both the NOD mouse and BB rat models of T1D the development of diabetes is under complex genetic control.

6.6 Environment and T1D

As previously mentioned, the development of T1D is influenced by environment as well as genetic background (Fig. 6.1). Much effort has been expended on trying to identify factors in the environment that might influence onset of T1D. Possible factors include exposure to an infectious agent, diet, toxins, lack of Vitamin D and also lack of exposure to certain infectious agents (Hygiene Hypothesis).

Support for the view that an infectious agent precipitated onset of diabetes arose following the observation that there appeared to be a seasonal onset of diabetes with presentation of disease more common in the spring and the autumn coupled with the anecdotal evidence of an infection prior to onset of T1D. A viral aetiology for diabetes onset seemed conclusively proven by the isolation of a Coxsackie B4 variant from the pancreas of a child that died of T1D and the demonstration of the ability of this virus to mediate hyperglycaemia on transfer to mice (Yoon et al. 1978, 1979). Further support for a viral aetiology was provided by studies demonstrating the presence of a viral superantigen in the pancreas of diabetic individuals (Conrad et al. 1994, 1997). However, subsequent studies failed to replicate these observations of a superantigen (Badenhoop et al. 1999; Jaeckel et al. 1999). Nevertheless, since these observations were made, there has been a great interest in establishing whether viruses and, in particular, human enterovirus might play a role in diabetes development. This is partly because there are several ways in which it might be envisaged that a virus infection could mediate T1D. These would include direct beta cell toxicity by viruses with tropism for beta cells or class I-restricted killing of virus-infected cells by virus-specific $CD8^+$ T cells. Virally induced beta cell lysis could also result in the release of endogenous self-antigens and consequent autoreactive T-cell activation. If viral antigens shared sequences with self-proteins, there is the possibility of molecular mimicry resulting in autoreactive T-cell activation. Sequence homology between a Coxsackie B4 non-capsid antigen P-2C and glutamic acid decarboxylase 65 (GAD65, an islet autoantigen) has been shown and this fuelled the molecular mimicry hypothesis. However, T cells specific for the viral epitope failed to respond to the GAD65 epitope (Varela-Calvino et al. 2004). Studies of enteroviral infections in NOD mice have provided evidence of either induction or prevention of diabetes depending on the dose of virus and the timing of infection (Tracy et al. 2010). Such timing and dose factors might have contributed to the difficulties in formally assigning a viral aetiology to most cases of T1D. Recent association and GWAS studies have added to the debate as polymorphisms in IFIH1 (interferon induced with helicase domain 1) protein were found to be associated with T1D (Liu et al. 2009; Qu et al. 2008; Smyth et al. 2006). This helicase is thought to recognise double-stranded RNA such as arises during RNA virus infection (Kato et al. 2006). Lower IFIH1 activity would be associated with a reduced pro-inflammatory response (Loo et al. 2008), which could lead to a reduced predisposition to the development of T1D following viral infection (Tracy et al. 2010).

Another explanation proposed for the increasing incidence in T1D has been lifestyle changes that have resulted in less exposure to sun and thus less Vitamin D. The activated form of Vitamin D, 1,25(OH)2D3, has been shown to play other physiological

roles in addition to effects on calcium and bone metabolism. There are data suggesting that Vitamin D supplementation in infancy might protect against onset of T1D (Hypponen et al. 2001). Furthermore, studies in NOD mice show that reducing Vitamin D levels exacerbates diabetes while increasing Vitamin D reduces the incidence of diabetes in this mouse model (Mathieu et al. 2004). Vitamin D mediates its effects through the binding of 1,25(OH)2D3 to the vitamin D receptor (VDR), and it might have been anticipated that polymorphisms in this receptor might have shown some associations with T1D. However, there are conflicting data regarding the association of polymorphisms in VDR and T1D with some of the larger studies suggesting no association (Kahles et al. 2009). VDR agonists mimic the effects of Vitamin D and can be used effectively at doses that do not cause hypercalcaemia to prevent onset of diabetes in adult NOD mice (Gregori et al. 2002). In terms of mechanism by which Vitamin D might influence diabetes onset, there are good data showing an effect of Vitamin D on myeloid DC function (Adorini and Penna 2008). Dendritic cells play a pivotal role in the initiation of immune responses, and in terms of diabetes it is thought that DCs in the pancreatic draining lymph nodes play a fundamental role in T1D by presenting islet antigen to autoreactive T cells (Turley et al. 2003). Vitamin D has been shown to consistently skew both human and mouse dendritic cell function towards a more tolerogenic phenotype where they express less co-stimulatory molecules such as CD80 and CD86, less pro-inflammatory cytokine production (IL-1, IL-6, IL-23 and IL-12) and more anti-inflammatory cytokine, IL-10. Therefore, in terms of influence on T1D it is proposed that Vitamin D might skew the immune response from a pro-inflammatory Th1 or Th17 response to a more regulatory response (Adorini and Penna 2008). Vitamin D-induced upregulation of the chemokine CCL22 production by myeloid DCs would furthermore ensure recruitment of regulatory T cells that would inhibit diabetes onset (Montane et al. 2011).

In a study of the influence of household size on the development of asthma, Strachan proposed that less exposure to infections of historical importance might be responsible for the increased incidence of asthma (Strachan 1989). This Hygiene Hypothesis extends also to studies of autoimmunity (Cooke et al. 2004). The immune system co-evolved with infectious agents, and it is interesting to speculate that alleles of some of the genes (or genes in linkage disequilibrium) that predispose towards diabetes onset have been retained in the population as they provide a selective advantage against infection. This is particularly interesting in the context of T1D as insulin therapy, which is required to keep Type 1 diabetic patients alive, was not discovered until the 1920s by Banting and Best (Banting et al. 1922). As T1D is a disease of juvenile onset classically affecting individuals before reproductive age, it has to be supposed that diabetes must somehow not have been developing at high frequency historically and that certain infections inhibited its development (Dunne and Cooke 2005). There has been considerable interest in examining which microbial agents have the potential to modulate autoimmunity and how they achieve this. Studies of the effects of viral, bacterial and parasitic infections on T1D have shown that some, but not all, infectious agents have the potential to modulate diabetes onset (Lehuen et al. 2010). In some cases, prevention of diabetes does not require an actual infection but can be achieved by products of infectious agents (Alyanakian et al. 2006; Zaccone et al. 2003). Studies in NOD mice have highlighted the

importance of timing of exposure to the infectious agent for its ability to inhibit diabetes (Cooke et al. 1999; Stiller et al. 1984; Tracy et al. 2010; Zaccone et al. 2004). This almost certainly reflects the different ways that various infectious agents mediate their inhibitory effects. Detailed analyses of the ways in which infectious agents or their products impact on the development of diabetes have documented effects on DCs, NKT cells and T-cell subset differentiation such that there may be skewing towards Th2 or increased generation of regulatory T cells. Some of these effects are mediated through the interactions of products of infectious agents with Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) (Aumeunier et al. 2010; Burton et al. 2010; Lehuen et al. 2010). Recent studies have focussed on the effects of probiotics and the gut microflora on diabetes development. There is considerable evidence showing that there is an active dialogue between commensal bacteria and the host immune system. Studies of bacterial intestinal colonisation have shown that this plays an important role in the development and maturation of the host gut immune system (Artis 2008; Macpherson and Harris 2004). The availability of molecular techniques to identify the spectrum of organisms resident in the gut coupled with the ability to monitor discrete alterations in the immune system following selective colonisation with distinct bacterial species has enabled a more detailed appraisal of how different commensal bacteria might influence the immune response (Round and Mazmanian 2009). Several studies have shown that segmented filamentous bacteria are able to drive this maturation of immune responses and in particular to induce Th17 responses (Gaboriau-Routhiau et al. 2009; Ivanov et al. 2009). On the other hand, the commensal organisms *Bacteriodes fragilis* and *Clostridium* clusters IV and XIVa have been associated with the induction of Foxp3 expressing $CD4^+$ regulatory T cells (Atarashi et al. 2010; Round and Mazmanian 2010). Imbalance in commensal organisms or dysbiosis could predispose to autoimmune conditions in animals with a susceptible genetic background. For example, segmented filamentous bacteria, through their ability to induce Th17 cells, have been shown to promote arthritis and also autoimmune responses in the CNS (Lee et al. 2011; Wu et al. 2010). In the context of T1D, recent studies have shown that the presence of such segmented filamentous bacteria correlated with a reduced diabetes incidence in NOD mice supporting a Th1-mediated aetiology for this autoimmune disease (Kriegel et al. 2011). This observation complements the finding that alterations in commensal organisms of NOD mice markedly influenced onset of T1D in this mouse model (Wen et al. 2008). Thus it can be seen that marked changes in exposure to antibiotics may have influenced the gut microbiome in such a way as to predispose towards allergies and autoimmune disease. Of course, the microbiome and the host response might also be influenced by enteric viral or bacterial infections which in turn might impact on onset of autoimmune pathology.

6.7 Recommendations

There is considerable interest in preventing onset of T1D as well as developing a cure for this autoimmune condition. Approaches for early diagnosis and therapy are summarised in Table 6.3. Knowledge of the key factors, both environmental and

Table 6.3 Examples of strategies to diagnose/prevent/cure Type 1 diabetes

Diagnosis
Biomarkers (e.g. detection of amino acids, peptides and lipids in the serum)
Autoantibodies (e.g. anti-insulin, anti-GAD, anti-IA-2 and anti-ZnT8)
Genetic (e.g. HLA, insulin and other candidate loci)
Prevention
Induction of antigen-specific tolerance (e.g. to islet antigens such as insulin and GAD)
Induction of non-antigen-specific tolerance (e.g. monoclonal antibodies such as anti-CD3)
Prophylactic treatments (e.g. vitamin D and biomodulators)
Cure
Generation of immune tolerance (e.g. antigen or non-antigen-specific tolerance)
Replacement of islet mass (e.g. islet transplantation, neogenesis, generation of β cells from exogenous stem cells)

genetic, leading to diabetes onset will permit the development of targeted therapies. Therapeutic strategies have not only focussed on targeting T cells involved in beta cell destruction but also on considering whether it is possible to take into consideration some of the environmental factors that may influence diabetes onset such as Vitamin D. Current thought on the microbiome and how that might modulate autoimmune disease as well as harnessing information regarding how the various microorganism-derived biomodulators are able to harness autoreactive T cells and induce immune regulation should be considered as this might broaden the scope for therapeutic intervention.

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Chapter 7
Rheumatoid Arthritis

Jean C. Pfau

Abstract Rheumatoid arthritis (RA) challenges researchers, physicians, and patients with its complexity, its impact on lives, and its refusal to respond predictably to treatment. Because we do not know what causes RA, treatment is, at this time, entirely palliative. Many environmental factors, including smoking, diet, stress, environmental chemicals, and infections, have been implicated in either the etiology or progression of RA, but none of these emerges as a distinct causative agent of RA, in general. As more data becomes available, RA appears to be a cluster of disease entities, which may have unique etiological patterns. Therefore, the best research, current and future, will subdivide RA into disease subsets based on clinical, serological, or environmental parameters. In an iterative process, once RA subsets are defined, common mechanisms may emerge that link them and help explain the complexity, thereby leading to new therapeutic or preventative approaches. This chapter attempts to set the stage for this kind of analysis, using the new paradigm of the gene/environment interaction that unites smoking and major histocompatibility complex haplotype with RA as a critical and revealing example.

Abbreviations

ACPA	Anticitrullinated peptide antibodies
AhR	Aryl hydrocarbon receptor
CCP	Cyclic citrullinated peptide
EBV	Epstein–Barr virus

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HLA	Human leukocyte antigen
HPA	Hypothalamic pituitary adrenal axis
HSP	Heat shock protein
IFN	Interferon
IL	Interleukin
MHC	Major histocompatibility complex
NK	Natural killer
PAD	Peptidylarginine deiminase
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutants
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus
TLR	Toll-like receptor
TNF	Tumor necrosis factor

7.1 Introduction

Anyone who has watched an adult or child deal with the pain, disability, and deformities of rheumatoid arthritis (RA) yearns for better understanding of this disease to elucidate novel treatments that can give more complete and targeted relief. There are many reviews available that describe the clinical symptoms and the current understanding of the pathophysiology of RA (Cooles and Isaacs 2011). It is classified as a systemic autoimmune disease in which there is chronic inflammation of multiple synovial joints which progresses to tissue damage and bone erosion. In addition, it can cause systemic complications that affect peripheral nerves, skeletal muscle, and major organ systems including the lung and kidney. Mortality largely occurs when these latter organ systems are affected. A complete picture of the etiology of RA is elusive, undoubtedly due to the heterogeneity of its clinical presentation, the complexity of the immune dysfunction, and what appears to be a temporal disconnect between the appearance of disease and the environmental factors that may be initiating it. Nevertheless, technologies to delve deeply into the cellular and molecular events, to understand contributions of genetic and epigenetic factors, and to develop novel therapeutic approaches are becoming available. What is needed is an interweaving of disciplines, from molecular biology to epidemiology to animal models of immunotoxicology. Recent workshops and discussions among experts have demonstrated that these conversations are underway, leading to new insights and discoveries (Ball 2010). A key piece of this multifaceted puzzle is the role of environmental exposures, because understanding toxicological factors not only teaches about etiology, but also opens the door to prevention of a costly and crippling disease.

RA was first distinguished from other arthritic syndromes by Garrod in 1859 (Short 1974). The value of separating RA from diseases such as gouty arthritis,

spondylitis, and osteoarthritis was evident as diagnostic criteria and therapeutics subsequently evolved along different lines. New understandings about possible subsets within the RA diagnosis will likely lead to similarly critical tailoring of diagnostic parameters and therapeutic approaches. Current criteria continue to rely on symptomatic patterns (Aletaha et al. 2010), but new data regarding molecular pathogenesis and genetic contributions to RA demand a new approach that requires further subdividing RA into subsets in order to elucidate specific triggers in various gene/environment settings. A major objective of this review is to explore not only the putative environmental triggers for RA, but also to explore these more complex gene/environment interactions that likely lie at the heart of "causation." It may be that, through an iterative process in which RA is first subdivided based on differences, then compared for commonalities, a more unifying hypothesis of RA etiology can be deduced and explored.

7.2 Prevalence: Genetics vs. Environment

Despite new biological therapies that take advantage of a growing understanding of the disease at the molecular level, the burden of the disease personally, socially, and economically is high (Boonen and Severens 2011). RA cripples in the prime of life, with early symptoms being in the hands and feet, often rendering the person unable to work at their normal job, or even to carry out daily activities. Because there is no cure, some kind of medication or treatment for their symptoms becomes a way of life. The prevalence of RA is around 1% worldwide, with a higher prevalence in females (Gabriel and Michaud 2009). There has been an interesting downward trend in incidence of RA since the 1950s, decreasing from 61.2/100,000 individuals during 1955–1964 to 32.7/100,000 during 1985–1994 (Gabriel and Michaud 2009). However, that trend appears to be modulating or in fact reversing in women, rising to 53.1/100,000 between 1995 and 2007 (Myasoedova et al. 2010). The fact that this reversal appears to be occurring only in women may provide clues about environmental changes that may be responsible. Female-specific issues being explored include changes in oral contraceptives, increased smoking among women relative to men, vitamin D deficiency, and a variety of other dietary and socio-economic factors (Myasoedova et al. 2010).

Twin concordance data from 1986 showed a 12.3% concordance for monozygotic same-sex twins and 3.5% for dizygotic same-sex twins (Aho et al. 1986). These numbers were reported to be consistent with studies in families (Aho et al. 1986). A Danish historical cohort study of 37,338 twins from a twin registry showed even lower concordance among twins, with the monozygotic twin concordance rate being essentially zero (Svendsen et al. 2002). These data clearly support a role for the environment in the etiology of RA, and minimize the role of genetics. Nevertheless, it has been suggested that twin concordance data may underestimate the contribution of genetics to RA (MacGregor et al. 2000).

The variability in clinical features, subjectivity of some of the diagnostic criteria, case/control definitions, variability in disease latency, and the overall challenge of clinical diagnosis could all contribute to discrepancies in twin concordance studies (Silman 1997). MacGregor et al. explored the heritability of RA using variance components analysis among twins, looking at sex, age, age at onset, and various clinical characteristics (MacGregor et al. 2000). Using this technique, the rate of "heritability" was found to be over 50%, leading to the conclusion that genetic factors play a much larger role in RA than previously believed when based solely on twin concordance data. In addition, it has become clear from animal model studies that environmental factors implicated in the development of autoimmunity actually cause autoimmune disease only in the presence of a genetically permissive background (Kim and Moudgil 2009). Therefore, the most parsimonious assumption is that the etiology of RA is based on gene/environment interactions.

7.3 Immune Dysfunction in RA

Before exploring the various environmental factors implicated in RA, it is important to briefly review the immune dysfunction seen in the disease, while keeping in mind that this is a generalization regarding a very complex and heterogeneous disease process. In an affected joint, one would expect to find macrophages, synoviocytes (synovial fibroblasts), natural killer (NK) cells, and mast cells. The innate immune system, particularly macrophages, is correlated with the production of key inflammatory cytokines such as tumor necrosis factor-α (TNF-α), IL-1, and IL-6, and the progress of inflammation and joint destruction in RA. Despite the importance of macrophages, synoviocytes are also key players, producing a similar array of inflammatory cytokines and proteolytic enzymes. Even platelets (platelet microparticles) are found in the synovial fluid, where they interact with lymphocytes and synovial fibroblasts (Boilard et al. 2010; van Eijk et al. 2010), and trigger production of IL-8 and IL-6. Interestingly, platelet depletion in a mouse model of inflammatory arthritis was protective (Boilard et al. 2010). Therefore, the innate immune system alone presents multiple potential therapeutic targets, and also many mechanisms whereby environmental exposures could trigger or exacerbate the disease.

While much of RA appears to be an autoinflammatory disease in which the key feature is severe inflammation mediated by the innate immune system, T and B cells are also clearly involved. This point is illustrated by differences between osteoarthritis and RA, and the fact that basic measures of inflammation itself and expression of the NALP3 inflammasome do not differ between RA and osteoarthritis (Kolly et al. 2010), suggesting that the difference may lie at the level of the adaptive immune response. The T cells in RA are predominantly CD4+ T helper cells with a Th1 phenotype, although there are some Th17 components that have led to the description of an "unstable" Th17/1 phenotype that may be particularly

pathogenic (Nistala et al. 2010). T cell activation occurs early in the development of RA, and key cytokines include interferon gamma (IFN-γ) and IL-17. The T cells within the synovium tend to be oligoclonal, which suggests that their activation is antigen-driven, but identification of the specific antigens has been extremely difficult. Activated T cells also interact with B cells leading to increased levels of immunoglobulins.

Interestingly, within the synovium, T and B cells are sometimes arranged in germinal center-like clusters (Kim et al. 1999). The function of these "ectopic lymphatic tissues" is not entirely clear. However, the presence of these synovial germinal centers has been detected early in the course of RA, and this was correlated with the degree of joint inflammation (van de Sande et al. 2011). In order to further explore their potential role in RA, Humby et al. used a variety of techniques to study these ectopic lymphoid structures, and found that they contain activated follicular dendritic cells and proliferating B cells that appear to be undergoing class-switching, as well as antibody-producing plasma cells (Humby et al. 2009).

Autoreactive B cells have a central role in the development of RA, producing autoantibodies that might be involved in tissue damage in RA, such as rheumatoid factor (antibodies to host Ig Fc) and antibodies to citrullinated peptides. One of the puzzles of autoimmunity is how peripheral tolerance to self-antigens is broken, leading to antibodies that bind to the host's own proteins and elicit tissue damage through complement activation or Fc-receptor-mediated cytotoxicity. It has long been hypothesized that posttranslational modifications such as phosphorylation, glycosylation, or methylation could alter the three-dimensional structure or specific epitopes of a protein, making that protein appear "foreign" to the immune system. Alternatively, proteins that are tolerized in a posttranslationally modified state could become antigenic if they lost those modifications. Therefore, posttranslational modifications that occur in excess, at the wrong "time," or in the wrong tissue compartment could set up an autoimmune situation. Events such as oxidative stress and apoptosis have been shown to alter enzymatic and nonenzymatic posttranslational modifications, and autoantibodies to modified proteins are found in several organ-specific and systemic autoimmune diseases (reviewed in Uysal et al. 2010). In RA, the key posttranslational modification appears to be citrullination, which is the deimination of the amino acid arginine to citrulline (Schellekens et al. 1998). Because this puts a neutral amino acid where the positively charged arginine used to be, this changes the conformation of the protein, revealing altered epitopes to the immune system. Although citrullination occurs normally, as a part of inflammatory responses (Vossenaar et al. 2004), RA so far appears to be the only autoimmune disease that manifests with autoantibodies to citrullinated proteins (Uysal et al. 2010), and testing for anticitrullinated peptide antibodies (ACPA) has become the most sensitive and specific diagnostic marker for RA. Initially, a test was developed that used an artificial autoantigen (cyclic citrullinated peptide, or CCP) to detect a wide variety of ACPA, and this revolutionized early detection of RA. Currently, based largely on the specificities of autoantibodies found in RA patients, much more specific tests have been developed (Pruijn et al. 2010). Autoantibodies to several citrullinated proteins are seen

in RA, including antibodies to filaggrin, vimentin, alpha-enolase, and fibrinogen. Interestingly, polymorphisms in the genes for peptidylarginine deiminases (PAD) were shown to be associated with the risk of RA in a study in Japan, suggesting that alterations in the enzymes responsible for citrullination may contribute to the development of not only the autoantibodies, but also the disease itself (Suzuki et al. 2007). Further evidence that ACPA could contribute to disease comes from the collagen-induced arthritis mouse model, in which immunization with citrullinated collagen I led to a more severe form of the disease than noncitrullinated collagen, and passive transfer of antibodies to citrullinated collagen could accelerate disease in this model (Lundberg et al. 2008).

If left at that, this novel and important risk factor and diagnostic marker would continue to help with the diagnosis of RA, but many unanswered questions would remain, including the presence of a significant subset of RA patients who are not positive for ACPA. Fortunately, in the subsequent sections of the chapter, a discussion of environmental risk factors will build a case for a critical gene/environment interaction that may resolve many of these puzzles.

7.4 The Basis of Etiological Hypotheses

The most current data available suggests that rather than specific causes, RA's etiological processes may be defined in categories. Using this model, RA may stem from immunotoxicological events that affect exposure to shared/mimicked epitopes, cytokine patterns, complement activation, iron/redox reactions, posttranslational modification (especially citrullination) of self-antigens, epigenetic regulation, and the developing immune system. Within this model, it may be assumed that the inciting event occurs within the context of a genetic background in which a permissive condition exists, due to the status of susceptibility genes such as human leukocyte antigen (HLA) genes, *PTPN22*, *IRF5*, and *STAT4* (Delgado-Vega et al. 2010), and the genes for PAD (Suzuki et al. 2007) and glutathione *S*-transferase (GST) locus (Mikuls et al. 2011).

7.4.1 Epigenetic Regulation of Gene Expression

Epigenetics has emerged as an incredibly important mechanism by which genes are expressed, providing insights into the ways in which whole concerts of gene activity are beautifully directed and coordinated. As human genome mapping has taught us that the actual number of genes is not nearly as vast as originally thought, the focus turns to how that finite number of genes leads to such complex outcomes as organ development, immune regulation, tissue healing, and disease manifestation. Epigenetic regulation includes factors that affect gene availability, such as DNA coiling and uncoiling through modification of histones, and base modifications

in promoter regions that welcome or turn away transcription factors, such as DNA methylation, acetylation, or phosphorylation. It also can include factors that affect gene replication and repair, which can then ultimately affect rates of mutation in specific regions of the genome (reviewed in Ballestar 2011). In RA, DNA demethylation leads to dysregulated expression of matrix metalloproteinases (MMP) and IL-6 in synovial tissues (Nile et al. 2008). In fact, synovial fibroblasts from RA patients, which are highly activated, show reduced levels of DNA methytransferase 1 (DNMT-1) and distinct demethylated promoter sites (Karouzakis et al. 2009). Using a hypomethylation drug, 5-azacytidine, normal synovial fibroblasts were induced to the activated phenotype seen in RA. Environmental conditions that can affect DNA methylation include inflammatory cytokines, oxidative stressors, certain pharmaceuticals, and nutrition. This occurs through the regulation of key epigenetic modifying enzymes such as DNA methyltransferases and acetylases. Interestingly, it has been hypothesized that one of the mechanisms by which methotrexate works is through reversal of DNA demethylation in RA synoviocytes (Kim et al. 1996). To date, most of the epigenetic research in RA has focused on the synoviocytes, but other cells such as macrophages and B cells can also be regulated in this way. Therefore, an important area of research needed is to learn which environmental factors may be driving RA's DNA demethylation and other epigenetic regulatory mechanisms.

7.4.2 Infections

One of the earliest etiological hypotheses for RA was that a microbial infection would be discovered that either exposed the immune system to an antigenic epitope that closely resembled a self-antigen, thus leading to autoimmunity, or that induced a cytokine environment that led to lost immune tolerance to self-proteins. Although there is still no "smoking gun" for such an organism, the research in this area has revealed tremendously valuable clues, and certainly infection may play at least a contributing role in the pathogenesis of RA.

There is a long list of microorganisms, especially viruses and bacteria, for which infection has been associated with autoimmune responses in either humans or animal models. This variety of exposures suggests either that there is something in common between all of those microbes, such as a shared epitope, or something similar about the immune responses they evoke. Several viruses have been explored as possible etiologic agents of RA, including Epstein–Barr virus (EBV), parvovirus B19, cytomegalovirus, and human herpesvirus-6. Similarly, a diverse array of bacteria have also been interrogated for their role in RA, including *Mycoplasma*, anaerobic oral infections, *Proteus* strains, and *Mycobacteria*, either for the presence of the infections in patients with RA or for the role of potentially cross-reacting antibodies to their specific antigens.

The strongest data associating RA with infections come from animal models, particularly in genetically susceptible strains of rodents, in which injection with

microbial antigens can induce a pathology similar to human RA. The microbial antigens tend to be conserved proteins such as heat shock proteins (HSPs), or heavily glycosylated microbial products such as *Streptococcus* cell wall peptidoglycan-polysaccharide (Wilder et al. 1989). An interesting model is the SKG mouse, which spontaneously develops an RA-like disease that includes autoantibodies to type II collagen as well as antibodies that react with *Mycobacterium tuberculosis* HSP-70 (Sakaguchi et al. 2006). The genetic susceptibility is rooted in a mutation in the gene for ZAP-70 which is involved in T cell signaling. Although there is no clear evidence of an active infection (especially of *M. tuberculosis*), these mice do not develop the disease if they are housed in a strictly pathogen-free (SPF) environment, implicating a microbial trigger of some kind (Yoshitomi et al. 2005).

An intriguing debate right now focuses on the reported therapeutic effects of various antibiotics in the treatment of RA (Ogrendik 2009; Voils et al. 2005). The ability of antibacterial agents to emiliorate RA symptoms has been intrepreted as evidence that the bacteria are playing an etiological role (Ebringer and Rashid 2006; Ogrendik 2009). However, while these data certainly call out for further exploration of the stains of bacteria, such as those involved in oral and urinary tract infections, causation has yet to be demonstrated.

There are a couple of possible mechanisms whereby microbial antigens could lead to immune dysfunction and autoreactivity. The "molecular mimicry" model suggests that there is cross-reactivity between a microbial antigen and a host antigen that is so similar to the microbial target that an autoimmune pathology is evoked. The best characterized example, of course, is the cross-reactivity between *Streptococcus pyogenes* M-protein and human glycoproteins, leading to the development of rheumatic fever. In support of this model, mycobacterial antigens injected into disease-permissive rats led to tissue destruction due to cross-reactivity with joint capsule proteoglycans (van Eden et al. 1987). The resulting autoantibody targets include HSPs, and RA patients do indeed manifest immune reactivity to certain mycobacterial HSPs (Danieli et al. 1992). As in the rat studies above, there does not need to be a Mycobacterial infection, due to the highly conserved nature of HSPs, not only between bacterial strains, but also mammals (Kim et al. 2006). Epitope spreading, the diversification of immune specificity away from the original dominant epitope, is a mechanism which may explain why it has been extremely difficult to identify any specific etiological infection.

In 2009, Kivity et al., proposed a set of criteria that would need to be met in order to support the "molecular mimicry" mechanism, including (1) that the pathogen is associated with the onset of the autoimmune disease; (2) that the pathogen causes an immune response that cross reacts with host antigens; (3) that the host antigen can induce the disease in an animal model; and (4) that T cells or autoantibodies from the cross-reactive response can induce the disease in animal models (Kivity et al. 2009). However, no specific infections have clearly met these criteria for RA, challenging the mimicry hypothesis as a primary etiological pathway. The reason for this may be that the autoimmunity induced by infection is not specific to the pathogen, but is relatively nonspecific: a bystander or adjuvant effect.

7.4.3 *Bystander or Adjuvant Effects*

Therefore, another mechanism proposed for a connection between infection and RA is that of a bystander or adjuvant effect (Israeli et al. 2009). This would imply a nonspecific immunotoxicity in which innate immune cells such as antigen-presenting cells or NK cells would be affected, leading to altered immune responses. It is well known that viral or bacterial components can serve as adjuvants. In these cases, the adjuvant is not the target antigen, but rather enhances or accelerates the antigen-specific response. Adjuvants are used to improve the response to vaccines, and the source microorganism for the adjuvant can help tailor the response appropriately. For example, virosome adjuvants contain viral components that can enhance an antiviral response (Moser et al. 2011). Deviation of the inflammatory environment toward Th17/Th1 by an adjuvant effect could nudge the immune response toward autoimmunity. In support of that notion, activated Th17/Th1 cells have been found in the joints of children with RA-like arthritis (Nistala et al. 2010). Other adjuvant effects that could predispose to autoimmunity include increased antigen presentation, especially by dendritic cells, and activation of the NALP-3 inflammasome. Again, despite seemingly incriminating evidence, adjuvants may induce autoimmune or autoinflammatory responses without actually causing autoimmune disease. Of course, if adjuvants can cause immune dysfunction leading to RA, then the possibility exists that adjuvant-containing vaccines could theoretically do the same thing. While more work needs to be done to determine whether this might be particularly the case in certain genetically susceptible individuals or in children, a population-based study in Sweden found no increased risk of RA following any of several adult vaccinations, including flu, tetanus, diphtheria, hepatitis A, B, C, polio, pneumococcus (Bengtsson et al. 2011). Another study of adult vaccinations for influenza (H1N1) virus included 41 RA patients and 21 systemic lupus erythematosus (SLE) patients in addition to 25 healthy controls (Ori et al. 2011). There were no changes in symptom profiles or activity scores in any patients, suggesting that this vaccine also does not appear to exacerbate RA in adults.

It has been suggested that certain infections lead to activation of toll-like receptor (TLR) responses that subsequently tip the scales to an autoimmune response (Monaco et al. 2011). TLR respond to specific microbial antigens, usually molecules with conserved and repetitive sequences (Pathogen-Associated Molecular Patterns, or PAMPs) and induce signaling that help tailor the response to the type of microbe. Some adjuvants may work by this mechanism. Multiple TLRs are highly upregulated on several cell types in the synovial tissues of RA patients as compared to healthy subjects or patients with osteoarthritis (Roelofs et al. 2005; Tamaki et al. 2011), and innate immune cells show heightened responses to TLR ligands (Roelofs et al. 2005). Because increased expression of TLRs occurs during infections, these data provide evidence that at least an initial infection may trigger this on-going inflammatory responsiveness. However, in addition to the microbial ligands for TLR, there also are several endogenous ligands, aka "alarmins," that can trigger TLRs and their signaling cascades. In fact, and this is so important for our understanding

of RA, citrullinated proteins have been shown to trigger signaling through TLR4 (Sokolove et al. 2011). Thus repeated trauma, tissue damage, or other cellular stress and apoptosis could release danger signals that serve as TLR ligands, activating any TLR-expressing cells, including macrophages and synovial fibroblasts (Monaco et al. 2011; Santegoets et al. 2011).

Another complication to the evidence linking RA with infections is that many patients have been treated with drugs that affect the immune system. Thus, if various infections are associated with RA, and in fact with progressing RA, it could be that the patients are simply susceptible to those infections due to immunosuppression. An interesting example of this is that, while there are several lines of evidence that EBV infection is associated with RA (Costenbader and Karlson 2006), it is possible that RA and/or its treatment could simply cause re-activation of dormant EBV. Therefore, there appears to be a "chicken or the egg" issue that will not be easily resolved. Lastly, many pathogens, including rubella, human T cell leukemia virus-1 (HTLV-1), and parvovirus B19, induce a polyarthritic syndrome during the progression of infection (Masuko-Hongo et al. 2003), adding to the diagnostic complexity of rheumatoid vs. infectious arthritis. It will be extremely difficult to determine causation unless a clear pathogenic link is found.

7.4.4 Oxidative Stress: Iron Metabolism and Diet

Telling a classroom of undergraduate students that aging is essentially the process of rusting always manages to get a few chuckles scattered about the lecture hall. Nevertheless, it is a fairly effective way to get them to think about the process of aging, and the role of oxidative stress. The "rusting" analogy may be more applicable in some forms of inflammatory arthritis in which iron metabolism plays an important role. The connection between oxidative stress and inflammation is well established, and the picture emerges as a viscious circle in which oxidative stress increases inflammation, and inflammation leads to oxidative stress (Chiurchiu and Maccarrone 2011; Maicas et al. 2011). In trying to understand the environmental contributions to RA, we are faced with another "chicken and egg" connundrum. One piece of the puzzle may lie in some studies of iron overload or sideropexia, which occurs particularly in people with hereditary hemochromatosis (HH). HH is an autosomal recessive disorder, with mutations in the HFE gene, that presents with excess tissue iron (sideropexia), liver disease, fatigue, and arthropathy. The arthropathy has been misdiagnosed as RA (Lonardo et al. 2001), and an influx of innate immune cells such as macrophages into the synovium causes HH arthropathy to mimic RA, in some cases, more than osteoarthritis (Heiland et al. 2010). It has been suggested that HH could progress to RA (Wernicke et al. 2006), and the implication is that oxidative stress, in this case due to iron-induced redox imbalance, is the trigger that leads down the RA path. In addition to HH, high levels of pro-hepcidin, a hormone involved in tissue uptake of iron, has been found in RA patients and correlated with disease activity (Kim et al. 2010). Current evidence suggests that

oxidative stress can indeed contribute to the bone destruction of RA (Wruck et al. 2011), leading to the hypothesis that potentially anything that sets up a chronic inflammatory state in joints could trigger RA, especially in a genetically permissive setting (Mikuls et al. 2011). This has led to exploration of therapeutic approaches that may reduce oxidative stress, including various nutritional factors such as antioxidant vitamins and polyphenols.

7.4.5 Dietary Factors

The obvious next step in the discussion of possible environmental triggers for RA is to look at the effects of dietary risk factors that would affect systemic oxidative stress (reviewed in Choi 2005). Iron-containing heme of red meat, even when consumed, has been hypothesized to catalyze widespread oxidative reactions, resulting in tissue damage (Tappel 2007). High levels of ferritin iron-binding protein were found in the synovial membranes of RA patients (Blake et al. 1984). A study by Pattison et al. suggested that consuming high levels of red meat is an independent risk factor for inflammatory polyarthritis, including RA (Pattison et al. 2004). The authors admitted two caveats: (1) that the criteria used in the study did not definitively select RA cases and other types of arthritis may have been included, and (2) that although red meat appeared to be a risk factor, it may not be causal. Subsequently, another prospective study analyzed intake of protein and iron in 546 cases of RA (Benito-Garcia et al. 2007). This study found no association between RA and either protein or iron intake, no risk from high intake of any form of meat (red meat, poultry, or fish) or iron itself.

Another component from meat that can affect inflammation is fat or oil. Studies in animal models of various autoimmune diseases, fish oils containing omega-3 fatty acids such as eicosapenaenoic acid (EPA) and docosahexaenoic acid (DHA) were protective, but very little has been done specifically looking at RA models. A recent study using the collagen-induced arthritis model in susceptible DBA/1 mice showed that krill oil and fish oil (both high in EPA and DHA) protected the mice from arthritis inflammation (Ierna et al. 2010). The mechanistic hypothesis behind fish oil benefits has been that these fatty acids tip the balance away from proinflammatory lipids and toward antiinflammatory lipids. Red meat, on the other hand, contains the fatty acid arachidonic acid, which is the metabolic precursor to the eicosanoids, including prostaglandins and leukotrienes, which are highly inflammatory. By analyzing dietary lipid intake in several countries and examining the prevalence or RA in those countries with high meat-fat intake, Grant et al. concluded that meat and offal may be significant risk factors for RA (Grant 2000). However, because there has been no reported association between RA and milk/milk products, which have a similar lipid composition as meat, the specific etiological role of meat fats remains in question. The major issue here may be to distinguish between (a) etiology and (b) symptom severity or disease progression. There is a booming commercial market for fish oil supplements, based on reported benefits in several conditions, including RA

and heart disease. Several double-blind randomized trials explored the potential for omega-3 fatty acid supplementation to improve symptoms in RA patients, and the results of these tended to agree in that at least a moderate improvement was seen (reviewed in James et al. 2003). It also may be that a combination of supplementing with omega-3 fatty acid works best when combined with elimination of arachidonic acid (omega-6 fatty acid) (Adam et al. 2003). Most recently, James has proposed that part of the reason a clear-cut role of this kind of supplementation has not been established is because most of these studies have been done on established RA rather than on evolving, early RA, and that a different study design is needed in order to determine whether early supplementation in high-risk patients would show significant improvements in disease progression (James et al. 2003).

Because fruits and vegetables often contain a combination of purported antioxidants and antiinflammatory agents including vitamins and lipids, systematic study of the role of diet in etiology or symptomology of RA is extremely difficult. A recent meta-analysis of the literature reviewed the efficacy of dietary supplements in both osteoarthritis and RA (Rosenbaum et al. 2010). The results did not convince the authors that available studies support the use of dietary supplementation as an effective treatment, although they recommended further research to determine whether such dietary approaches might be able to reduce dosages of nonsteroidal antiinflammatory drugs that patients need to take to control symptoms. Costenbader et al. recently performed a large cohort study of women diagnosed with RA or SLE (Costenbader et al. 2010). Using a novel method to evaluate dietary intake of antioxidants, called a "ferric-reducing ability of plasma" score, the authors found no relationship between antioxidant intake and either RA or SLE.

Keeping in mind that each of these studies used different approaches to identify their cases, evaluate iron, lipid, or antioxidant capacity, and to analyze the data, and that none of these studies explored these putative risk factors specifically in a permissive genetic context, it is clear that no definitive conclusions regarding diet in the etiology of RA can be drawn at this point. A systematic review of dietary interventions in RA found that overall, the evidence is uncertain and limited by study size, reporting problems, and drop-out rates (Smedslund et al. 2010). Nevertheless, the hypotheses are well-founded, and dietary components certainly merit exploration as possibly having an impact in the severity or symptoms of RA, even if not in the actual incidence of the disease.

Multiple sclerosis is a disease that has long been linked to latitude, with hypotheses related to the amount of sun exposure, vitamin D deficiency, and diet (Pierrot-Deseilligny and Souberbielle 2011). Recent studies show that women living in northern latitudes in the U.S. also have a higher risk of RA (Costenbader et al. 2008a, b; Vieira et al. 2010). People living in colder northern climates receive minimal sunlight several months of the year, and since sunlight is important for production of cholecalciferol, the precursor to the biologically important vitamin D3, such individuals are at risk of vitamin D deficiency if it is not provided in the diet. Zold et al. reviewed the evidence in support of an immunomodulatory role of vitamin D, suggesting, in fact, that vitamin D deficiency may impact immune tolerance, increasing the risk for autoimmunity (Zold et al. 2011). The mechanism is deduced from the fact that vitamin D3 receptors are expressed on immune cells and immunomodulatory

properties have been demonstrated for dendritic cells, Th17 cells, Th1 cells, and B cells (Wen and Baker 2011). People with early/evolving RA have been shown to have low levels of serum vitamin D, although this correlation did not hold for established RA (Heidari et al. 2011). In addition, a study by Costenbader et al. showed no effect of vitamin D on the risk for RA (Costenbader et al. 2008a, b). Due to these discrepancies, a cautious interpretation and more studies are warranted (Welsh et al. 2011).

7.4.6 Stress, Hormones, and Oral Contraception

Environmental factors can include emotional and physical stressors, often measured as dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Hormones produced in this endocrine loop include a variety of steroids that can have a large impact on immune function. There are receptors for adrenocorticotropic hormone (ACTH) and corticosteroids on immune cells, and activation of the HPA axis is generally associated with immune suppression through the action of corticosteroids. However, in RA there appears to be a muted stress response leading to low levels of cortical hormones (Dekkers et al. 2001). This may explain why the stress response in RA leads to enhanced inflammation (Straub and Kalden 2009). Boscarino et al., have shown an association between posttraumatic stress disorder (PTSD) and adult-onset RA in a twin-control study (Boscarino et al. 2010). Some of the possible mechanisms for this association have been reviewed, focusing on hormonal effects, but not ruling the possible contributions of other behavioral, environmental, or genetic factors (Boscarino et al. 2010).

Other HPA axis hormones include the gonadal hormones, or sex steroids. The best studied of these are the combination estrogen/progesterone products marketed as oral contraceptives. Although a few studies have suggested that the use of oral contraceptives have a protective effect on RA (Brennan et al. 1997; Doran et al. 2004; Hazes and van Zeben 1991), other studies showed no effect (Pikwer et al. 2009). Thus, hormonal regulation appears to be another example of a reasonable hypothesis, with intriguing, though enigmatic, supportive data, and further studies in the context of genetic susceptibility must be planned for the future.

7.4.7 Inhalation Toxicology

An association between inhalation of crystalline silica and RA has been repeatedly established since first described by Caplan in 1953 (Caplan 1953; Makol et al. 2011; Stolt et al. 2005). Exposure to asbestos has also been associated with an increased risk of RA in Libby MT, a population exposed to high levels of amphibole asbestos (Noonan et al. 2006). Possible mechanisms by which silica (or asbestos) might cause this immunotoxicological effect has been reviewed by Brown et al. (2005). The short version is that silica and asbestos are capable of providing a double-hit environment.

First, these dusts can serve as "adjuvants," increasing the production of oxygen radicals, many proinflammatory cytokines, recruiting immune cells to the lung, increasing antigen presentation and changing the balance of T cell and B cell subsets (Blake et al. 2007; reviewed in Brown et al. 2005). These events challenge the mechanisms of peripheral tolerance, predisposing the exposed individual to autoimmune responses. Second, these silicate materials are not easily cleared from the lung by macrophages, and over time a significant amount of cell death occurs as the phagocytes continuously attempt to get rid of this toxic material. Accumulation of dead cell debris, in an environment with compromised immune tolerance, may lead to the production of autoantibodies against that self-material (Blake et al. 2008; Pfau et al. 2004). Although associated with RA, or at least a rheumatoid-like syndrome (Caplan's syndrome), neither silica nor asbestos appear to induce ACPA independently (Pfau et al. 2005; Stolt et al. 2010). It is possible that a combined exposure to these dusts along with smoking is required for ACPA expression (Stolt et al. 2010). Another potential risk factor that will need to be watched carefully is the widespread use and production of nanomaterials that are manufactured as "nanowires" or "nanobelts." These elongated carbon, silica, or titanium-based materials have been shown to induce effects similar to asbestos and silica (Donaldson et al. 2011; Hamilton et al. 2009).

In addition to silica and asbestos, other inhalation exposures have been linked to RA, including proximity to high levels of traffic pollution (Hart et al. 2009) and cigarette smoking (Serra-Bonett and Rodriguez 2011). Smoking has become the most recognized and validated environmental risk factor for RA, yet ironically the discovery was made somewhat serendipidously by a group studying the role of oral contraceptives on RA (Vessey et al. 1987). The relationship appears to have a dose effect, as measured by pack-years (numbers of cigarette packs per day over the number of years the person smoked) (Costenbader et al. 2006). In addition, cigarette smoke condensate triggers early development and severity of arthritis in the collagen-induced arthritis mouse model (Chujo et al. 2010; Okamoto et al. 2011). Interestingly, people who have smoked and also experience silica exposure have a sevenfold increase in the risk of having RA, which is much more than either exposure alone (Stolt et al. 2010). There are numerous chemicals and particulates in cigarette smoke which can have a variety of effects. One of these is dioxin, a polychlorinated biphenyl (PCB) that exerts its biological activity by binding to the aryl hydrocarbon receptor (AhR). Activation of AhR in cigarette smokers with RA has been demonstrated (Kobayashi et al. 2008), and such activation can lead to up-regulation of proinflammatory cytokines, including IL-1, IL-6, and IL-8. Nicotine is a cholinergic agent that aggravated arthritis in a rat model of RA and increased Th1/Th17 cytokines, both in vitro and in vivo (Yu et al. 2011).

7.5 Anti-Citrullinated Peptide Autoantibodies: A Putative Link

Major histocompatibility complex (MHC) Class II variations, especially in *HLA-DRB1*, are known genetic risk factors for RA, but new evidence suggests that this association is restricted to ACPA-positive RA (reviewed in Klareskog et al. 2011).

ACPA-positive RA has a different disease course than ACPA-negative RA (de Vries-Bouwstra et al. 2008), so this distinction in genetic susceptibility is a critical piece of knowledge related not only to treatment approach, but also by providing a mechanistic clue. MHC Class II molecules are involved in presentation of foreign antigens to the immune system and are critical for maintaining tolerance to self-proteins. Normally, immune cells expressing MHC Class II molecules that can hold and present self-antigen are deleted, thereby tolerizing the host to immune responses against its own proteins. However, this is not a perfect system, and slightly modified self-peptides can be presented which increases the risk of autoimmunity. Variations in MHC Class II molecules lead to subtle changes in the antigen-binding groove and increase the ability to present certain epitopes. Therefore, it is not surprising that a combination of posttranslational protein modification, along with a particular MHC Class II allele that accommodates that modified peptide can lead to an immune response that, through epitope spreading, begins to attack even the normal, unmodified peptides possibly even at remote sites of the body.

A paradigm shift has occurred in our understanding of the etiology of RA with the recent discovery that smoking, a key environmental exposure associated with RA, primarily increases the risk of ACPA-positive RA, and not for ACPA-negative RA (Klareskog et al. 2006). Therefore, logically, being a smoker in the context of the *HLA-DRB1* genetic background is a high-risk recipe for ACPA-positive RA. And indeed this has been shown to be the case (Klareskog et al. 2006; Morgan et al. 2009; Pedersen et al. 2007). The idea thus emerged of a "shared epitope," a peptide-binding groove expressed by *HLA-DRB1* alleles that permit the binding and presentation of citrullinated autoantigenic peptides, as the gene/environment interaction responsible for ACPA-positive RA. Thus, RA is subdivided into at least two disease entities based on ACPA positivity and the shared epitope of *HLA-DRB1*, permitting intense exploration of each entity to understand the pathogenesis of RA.

How then do environmental risk factors for RA lead to citrullinated peptides that become the autoantigens? And are the resulting autoantibodies (ACPA) pathogenic?

Citrullination, as described early in the chapter, is not a rare event. There are several forms of PADs expressed in various tissues of humans, and they are active during infection, inflammation, and apoptosis (Vossenaar et al. 2004). Thus, many of the environmental agents associated with RA could potentially activate PADs and lead to citrullination. In fact, two isoforms of PAD are found in the synovial membranes of RA patients (Foulquier et al. 2007). Because ACPA can be found years before clinical disease develops (Kokkonen et al. 2011), early exposures could initiate a series of events that culminate in ACPA and subsequent disease. Early exposures could be second-hand smoke, infections, or even stress (Colebatch and Edwards 2010). Smoking increases the expression of PAD in the lung (Makrygiannakis et al. 2008). Infections are also associated with ACPA. In 2010, Lima performed a literature-based review of evidence linking infections with anti-CCP autoantibodies (Lima and Santiago 2010). A number of publications were identified that had found antiCCP (ACPA) antibodies associated with various infections, with the highest frequency of association being found with tuberculosis. *Porphyromonas gingivalis* is a member of the human oral normal flora, and has been

associated with peridontitis (Ogrendik 2009; Lundberg et al. 2010). Because antibodies to *P. gingivalis* have been found in RA patients, this bacterium has been implicated in disease pathogenesis (Hitchon et al. 2010). Interestingly, *P. gingivalis* is the only known bacterium to express a form of PAD, and the bacterial form appears to have a citrullination pattern that is unique from that seen with human PAD (Wegner et al. 2010). Although oral infections with this particular microorganism are associated with RA and may drive the disease through bacterial PAD activity, ACPA associated with most infections are clearly due to citrullination by human PAD enzymes.

The answer to the second question appears to be, yes, ACPA can be pathogenic, although certainly much more research is needed. Injection of ACPA antibodies into mice induced arthritis or exacerbated arthritis, and tolerization to specific ACPA led to reduction in symptoms (Kuhn et al. 2006; Lundberg et al. 2008; Uysal et al. 2009). These studies, however, only explore synovial symptoms, in an animal model. It will be very exciting to follow the research to see whether this model can also help explain some of the severe systemic manifestations of RA that affect mortality as well as morbidity of RA. The fact that high levels of ACPA are associated with severe forms of RA that include pulmonary involvement (Aubart et al. 2011) illustrates the importance of this research focus.

7.6 Future Directions of ACPA and RA

This model of gene/environment interaction in RA brings up many areas for study. There is much to be learned about citrullination and its regulation, including questions about the tissue specificity of PADs, the genetic contributions to PAD expression and activation, and the role of various exposures to activate this and other posttranslational enzymes. The shared epitope and cigarette smoking have now been shown to occur even in ACPA-negative RA (Bang et al. 2010; Mikuls et al. 2010) implicating other factors from smoking such as nicotine, particulate matter, or dioxin-like compounds (PCBs). Thus, the subdividing of RA by molecular categories based on these discoveries is already teaching us about discrete environmental contributions to the etiology of RA, and will undoubtedly lead to tremendous advancement in our understanding of RA in the near future.

7.7 Summary

This review of the environmental contributions to RA etiology and immune dysfunction yields three major themes. First, the environmental factors that have been implicated in RA fall into categories that make sense in the current understanding of autoimmune pathologies, including oxidative stressors, adjuvant effects, hormonal impacts on immune and inflammatory responses, apoptosis, and posttranslational

modification. However, no specific etiological pathway has been delineated all the way from initial exposure, through the immune dysfunction, and to the ultimate pathology. Nevertheless, those common pathways help formulate the mechanistic hypotheses that can be tested in animal models, and then translated back to therapeutic and preventive approaches. The second theme is that of gene/environment interactions for which the interaction between smoking and the shared epitope of HLA-DRB1 provides an excellent example that has opened up whole new realms of research possibilities. This is the closest research has come to achieving the goal of characterizing a full etiological pathway for RA. Third, new discoveries are allowing—and in fact mandating—the subdivision of RA into subsets of diseases based on the previous two themes. These new characterizations should bring far better, more targeted, diagnostic tools to physicians and hopefully better outcomes for their patients. Just as important, these discoveries dramatically augment our understanding of the environmental triggers for RA, leading to education that can actually begin to prevent this chronic and crippling disease.

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Chapter 8
Environment, Immune Dysfunction, and Systemic Lupus Erythematosus

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Abstract In recent years, there is accumulating evidence for the role of various external environmental factors in the pathogenesis of systemic lupus erythematosus (SLE). The main environmental factors that were found to be associated with SLE include chemicals, such as silica, mercury, phthalate, trichloroethylene, and pesticides; cigarette smoking, infectious agents, drugs, vaccines, and ultraviolet radiation. In this chapter we review these associations and the possible mechanisms underlying the immune dysregulation they trigger.

Key Points

- Systemic lupus erythematosus (SLE) development is determined by the interplay between the host genetic factors and external stimuli such as environmental and hormonal factors.

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- Environmental factors that were found to be associated with SLE development include exposure to chemicals, drugs, infections, UV light, smoking, and vaccines.
- The main chemicals that were shown to have a role in SLE development are silica, solvents (phthalate), mercury, and agricultural pesticides.
- Epstein–Barr virus (EBV) infection was shown to be linked to SLE development, among others. However, some infections were shown to have a protective role, such as malaria and other parasites.
- There is an increasing evidence for the relationship between vaccines and the development of autoimmunity, including SLE.

8.1 Introduction

Autoimmune diseases (AD) affect between 5 and 10% of the Western population (Shoenfeld et al. 2008b; Lleo et al. 2009; Shapira et al. 2009a, b) and are responsible for an extensive socioeconomic burden (Shapira et al. 2010). One of the most notorious of diseases is the systemic lupus erythematosus (SLE). This systemic autoimmune disease may literally involve any organ or system and is characterized by a chronic relapsing course, increased mortality rate, and female predominance (Borchers et al. 2009).

The etiopathogenesis of AD, in general, and SLE, in particular, is commonly explained by the "Mosaic of autoimmunity" (Shoenfeld and Isenberg 1989). The latter refers to a combination of factors associated with induction or triggering of autoimmunity, including genetic, immune mediated, hormonal and environmental factors. Different combinations of those factors may affect the clinical presentation of a certain disease within the wide spectrum of autoimmunity. In other words, in order to develop a specific AD, one has to be genetically susceptible and at the same time be exposed to one or more external stimuli, which ultimately manifest as a loss of self-tolerance (Shoenfeld et al. 2008a; de Carvalho et al. 2009). SLE is a complex genetic disease with various genetic alterations, as well as many environmental influences associated with its presence (Molokhia and McKeigue 2006; Dieude 2009).

Currently, an avalanche of environmental factors has been gradually revealed to play a role in the pathogenesis of SLE. In this chapter we will review those factors and try to decipher the subtle interaction between the host and the environment leading to immune dysfunction and eventually SLE.

8.2 Exposure to Chemicals and Risk of SLE

In the modern world, the exposure to chemicals has been significantly increased due to global industrialization. In recent years there is an increased concern about a possible link between chemical exposure and autoimmunity induction, including SLE. Recently, the association between exposure to chemicals in the form of vaccines'

adjuvants was defined, and termed Autoimmune or autoinflammatory Syndrome induced by Adjuvants (ASIA), discussed in more detail below (Shoenfeld and Agmon-Levin 2011). Several studies have examined the effect of exposure to chemicals on the development of SLE. The main chemicals that were linked to SLE are silica dusts, mercury, and solvents such as phthalate and agricultural pesticides.

8.2.1 Silica Dusts

Silica is a mineral-inhaled dust such as quartz and crystalline silica. In lupus-prone mice, silica was shown to accelerate the development of SLE including increased production of autoantibodies and accelerated kidney disease (Brown et al. 2003). In humans, exposure to silica generally occurs by inhalation of particles, which is common to certain occupations, such as construction, mining, powder manufacturing, farming, and dental technicians (Finckh et al. 2006; Cooper et al. 2010). The association between silica exposure and human SLE development was shown extensively in the literature summarized below.

Increased prevalence of SLE was found in workers in a factory producing scouring powder that contained a high amount of silica; out of 50 workers, 3 developed SLE and 5 developed SLE overlapping with scleroderma (Sanchez-Roman et al. 1993). Another example of increased prevalence of SLE after exposure to silica was in a German cohort of 28,000 uranium miners that were exposed to inhaled silica, in whom 28 definite and 15 possible cases of SLE were diagnosed, showing more than tenfold increased prevalence of SLE than expected (Conrad et al. 1996). In southeastern USA, a population-based case-control study has shown that occupational exposure to crystalline silica, mostly in farming or trades, has been significantly associated with risk of SLE development, with a dose-dependent relationship between levels of exposure and risk of disease (Parks et al. 2002). Urban women exposed to silica for longer than 1 year had 4.3 times increased risk for SLE development (95% CI, 1.7–11.2). It was also shown that the longer the exposure persisted the greater the risk (Finckh et al. 2006). Another study documented that increased number of exposures to silica increases the risk for SLE (Cooper et al. 2010). More epidemiological evidence of the risk of SLE after silica exposure exists and summarized in a review by Parks and Cooper (2006).

The exact mechanism by which silica predisposes to the development of SLE is unknown. Generally, silica has an adjuvant effect causing an increased production of proinflammatory cytokines (Parks and Cooper 2006). Silica was shown to stimulate T cells in vitro (Davis et al. 2001) and to decrease the number of regulatory T cells in lupus-prone mice (Brown et al. 2004). It was also shown that cement workers exposed to silica dusts have significantly lower percentages of T regulatory cells. Moreover, the proinflammatory cytokines levels (Interleukin [IL]-1β, IL-2, IL-4, IL-10, and Interferon γ [IFNγ]) were significantly elevated compared to the control group (Carlsten et al. 2007). Silica was also shown to induce cell apoptosis in New Zealand mixed mice and, therefore, theoretically could increase the exposure of cellular epitopes and exacerbate the development of SLE in these mice (Pfau et al. 2004).

8.2.2 Mercury

Mercury is a common heavy metal throughout the world in water-soluble forms and vapors that can be ingested or inhaled. It is used for scientific research and in amalgam material for dental restoration, among other applications. The major occupational exposure to mercury occurs in dental workers. Only one study in humans showed that occupational exposure to mercury is a risk factor for development of SLE, mainly among dental workers (Cooper et al. 2004). In rats, mercury caused an immunological syndrome including production of specific autoantibodies against nuclear components and phospholipids, increased immunoglobulin levels, and a transient glomerulonephritis with immunoglobulin deposits (Vas and Monestier 2008). In lupus-prone mice, including NZB/W and MRL/lpr, exposure to mercury caused an elevation in immunoglobulins levels, antinuclear and antichromatin antibodies (Abs), immune complex deposition in the kidney (Pollard et al. 1999), and also activation of TH2 response (Kroemer et al. 1996; Hudson et al. 2003).

8.2.3 Solvents

8.2.3.1 Phthalate

Phthalate is a commonly used chemical seen in polyvinyl chloride (PVC) polymers that are being used in children's toys and medical devices. It can also be used as a solvent for cosmetic products including nail polish and fragrances. Another type of phthalate is used for producing synthetic fibers. In October 2000, the Centers for Disease Control and Prevention and the National Toxicology Program in the USA published data showing increased exposure to phthalate, especially in women at ages of 20–40 years (Potera 2005). Lipstick, which contains phthalate, was first suggested as a risk factor for SLE by Burry (1969) but until today, only one study examined this issue. In this case-control study, increased frequency and duration of lipstick use were significantly associated with the development of SLE (Wang et al. 2008). It is presumed that the mode of exposure to lipstick compounds is by ingestion that occurs during eating or after rubbing the lips together after the lipstick is applied (Kurien and Scofield 2008).

The mechanism of SLE induction by phthalate is not clear. Chemically, phthalate has several isomers (*ortho*-, *meta*-, *para*-) that were all shown to induce the production of anti-DNA Ab's and SLE-like syndromes in animals. The use of ortho-phthalate induced the production of anti-DNA Ab's both in BALB/c and NZB/W F1 mice. The latter brand had aggravation of nephritis and a high mortality rate due to activation of autoreactive B cells. It was shown that BALB/c mice did not develop nephritis, because they have developed idiotype-specific CD8+ suppressor T cells against the autoreactive B cells (Lim and Ghosh 2003, 2004, 2005).

8.2.3.2 Trichloroethylene

Trichloroethylene (TCE) is commonly used as an industrial solvent for degreasing metals. Several studies in $MRL^{+/+}$ mice have shown that TCE exposure accelerated autoimmune response and promoted inflammation, including production of autoantibodies, immunoglobulins, and IFNγ (Khan et al. 1995; Griffin et al. 2000; Cai et al. 2008). In contrast to mice, epidemiological studies in humans did not find an association between exposure to TCE and development of SLE (Cooper et al. 2004; Finckh et al. 2006).

8.2.4 Agricultural Pesticides

Pesticides are a group of chemical or biological substances aimed at pest prevention or extermination. One of the subgroups of pesticides is insecticides that are used against insects and consists of organochlorine compounds (e.g., DDT), organophosphates, and more.

One study showed that occupational exposure to pesticides was significantly associated with high risk for SLE development (Cooper et al. 2004). Nogales, Arizona, in the United States (US) is a city in which the prevalence of SLE is two to seven times higher than in the normal US population. Toxicological analysis of blood and urine samples from residents of the city showed high levels of 1,1-dichloro-2,2-bis-(*p*-chlorophenyl) ethylene-(DDE), a metabolite of 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) and organophosphate metabolites (Balluz et al. 2001). However, the association between exposures to this substances and SLE was found to be insignificant. In a different study on postmenopausal women, personal use of insecticides was shown to be a risk factor for the development of SLE or rheumatoid arthritis (RA). This was shown to be dose-dependent with correlation to increased frequency and duration of use (Parks et al. 2010).

The mechanisms of pesticides' modulation of the immune system were studied in mice. Treatment of ovariectomized NZBxNZW F1 mice with organochlorine pesticides known for their estrogenic effects (chlordecone, methoxychlor, and *o*,*p*′-dichlorodiphenyltrichloroethane [*o*,*p*′-DDT]) resulted in earlier appearance of renal disease (Sobel et al. 2005). Chlordecone treatment also caused an early appearance of elevated antidouble-stranded DNA (ds-DNA) antibodies. It was hypothesized that the effect of those pesticides is due to their estrogenic properties; however, it was shown that uterine hypertrophy (a marker of estrogenicity) did not correlate with pesticides exposure (Sobel et al. 2005). In another study, it was shown that chlordecone and 17 beta estradiol (E2, a sex hormone) share several common mechanisms of immune modulation, such as activation of splenic B cells and germinal center reactions and reduction of apoptosis of B cells. However, in contrast to E2, chlordecone does not change the composition of splenic B-cell subsets, implying additional mechanisms other than estrogenic effects (Wang et al. 2007). Also, BALB/c mice that were treated with chlordecone did not develop autoantibodies or

renal disease, showing that chlordecone itself is not sufficient for SLE development and that genetic background is essential for the development of autoimmunity (Sobel et al. 2006). Other pesticides that were shown to have an effect on SLE pathogenesis include malathion, which was shown to exacerbate SLE progression in MRL/lpr mice (Rodgers 1997), and hexachlorobenzene that was shown to increase the level of anti-ds-DNA Ab's in rats (Schielen et al. 1993).

8.3 Cigarette Smoking and SLE

Cigarette smoking is an addictive habit with an established role in the development of cancer, cardiovascular, and pulmonary diseases. Smoking has immunomodulatory effects and is linked with autoimmunity and autoimmune diseases (George et al. 1997; Skurnik and Shoenfeld 1998; Shoenfeld et al. 2008c; Arnson et al. 2010) such as RA, Graves' disease, multiple sclerosis, Crohn's, primary biliary cirrhosis, SLE, and others (Shovman and Shoenfeld 2000; de Carvalho et al. 2009; Arnson et al. 2010).

A meta-analysis published by Costenbader et al. (2004) demonstrated a significantly elevated risk for developing SLE among current smokers with an odds ratio of 1.50 (95% CI, 1.09–2.08). However, past-smoking was not found to be a risk factor for SLE in several studies (Ghaussy et al. 2003), and was further supported while estimating smoking with SLE incidence, as well as with specific manifestations of the disease, such as dermatologic features, serositis, neuropsychiatric symptoms, and end-stage renal failure (Harel-Meir et al. 2007). On the other hand, current smoking increases the risk of cutaneous lupus erythematosus (CLE) (Boeckler et al. 2009), and is associated with decreased response to antimalarial therapy (Rahman et al. 1998).

Cigarette smoking is a major risk factor for atherosclerosis and cardiovascular diseases (CVD). Both diseases are relatively prevalent among SLE patients and are a leading cause of morbidity and mortality in this disease. Up to 36% of SLE-associated deaths are considered to be secondary to cardiovascular morbidity (Scalzi et al. 2009). The high prevalence of atherosclerosis among SLE patients cannot be attributed solely to "traditional" risk factors (i.e., Framingham study risk factors), but relies also on certain immune-mediated ones (de Leeuw et al. 2009; Sherer et al. 2009). Among the above are systemic chronic inflammation, endothelial cell activation, and the use of immunosuppressive drugs (de Leeuw et al. 2009). Ahmad et al. (2007) have studied the prevalence of carotid plaque in an ethnically homogenous cohort of female SLE patients compared to healthy controls. They found that SLE patients have a higher prevalence and different distribution of carotid plaque than controls. Race may also play a role in the predisposition to atherosclerosis in SLE patients. This was shown by Scalzi et al. (2009) who documented that Afro-American SLE patients with a smoking history had a significantly higher carotid medial intimal medial thickness (mIMT) compared to those who did not smoke. This difference was not observed in Caucasian patients.

Several biological mechanisms have been postulated regarding the causal relationship between smoking and SLE. An immense amount of free radicals is contained in cigarette smoke which causes a cascade of production and activation of endogenous free radicals. These free radicals can interact with DNA and cause mutations and activation of genes that may contribute to the development of the disease (Shoenfeld et al. 2008c; Arnson et al. 2010). Moreover, free radicals induce tissue hypoxia and cellular necrosis that may lead to the exposure of intracellular antigens (de Carvalho et al. 2009; Arnson et al. 2010), which in turn, may serve as autoantigens and result in the production of autoantibodies such as anti-ds-DNA antibodies (de Carvalho et al. 2009). The latter are characteristic of SLE and were shown to be more prevalent among current smokers (Freemer et al. 2006). With regard to free radicals, several studies have shown that the use of antioxidants, such as vitamin E and C, reduced anti-ds-DNA titers and disease activity as reviewed by Zifman et al. (2008).

Smoking increases inflammation as reflected by the elevation of serum inflammatory markers such as fibrinogen, C-reactive protein (CRP), soluble-intercellular adhesion molecule type 1, and E-selectin (Shoenfeld et al. 2008c; de Carvalho et al. 2009). The presumed mechanisms for this includes the induction and production of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), IL-1, IL-6, IL-8, and Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Arnson et al. 2010).

Other immune alterations that are attributed to smoking entail the overexpression of Fas, an apoptotic factor, on B and T lymphocytes (de Carvalho et al. 2009) and the stimulation of autoreactive B and peripheral T-lymphocytes by cigarette smoke product, polyphenol-rich glycoprotein (Arnson et al. 2010).

Hence, the association of cigarette smoking to SLE is multifaceted and is linked to SLE development, course, and outcome. It also has an additive effect to the development of atherosclerosis in SLE patients, which is a leading cause of morbidity and mortality in those patients. These data support, as suggested in numerous studies, avoidance or cessation of cigarette smoking, in general, and among SLE patients, in particular (Ghaussy et al. 2003; Freemer et al. 2006; Doria et al. 2008; Arnson et al. 2010).

8.4 Infections and SLE

Infections are the environmental factors most strongly linked with autoimmunity, as they were proven to be both inducers and triggers of autoimmune diseases (Shoenfeld et al. 2008a, c; Kivity et al. 2009). The biological mechanisms by which infectious agents induce autoimmunity are diverse and include *molecular mimicry*, which is a cross-reactivity between the infectious agent's epitope and a selfantigen, as well as *epitope spreading*, a switch from one dominant epitope into several subdominant (cryptic/neo) epitopes by molecular mimicry to the dominant epitope, protein processing of it, and antigen presenting of the neo-epitopes, resulting in an immune response to the neo-epitopes (Kivity et al. 2009).

Bystander activation is another mechanism by which the infectious agents and their products cause increased cytokine production and as a result an inflammatory microenvironment is produced. This increases the number of autoreactive T cells causing a greater risk for an overt disease to develop. *Viral persistence and polyclonal activation* is a result of prolonged viral infection leading to ongoing immune response, thereby inducing a nonspecific proliferation of B cells that may lead to the production of autoantibodies and immune complexes. Viral and bacterial *Super antigens* may also be related to autoimmunity induction. Viral super antigens have the ability to bind different major histocompatibility complex (MHC) class 2 molecules and the variable domain of the beta chain of a T cell receptor. Thus, they may bind and activate a broad spectrum of T cells regardless of their specificity including autoreactive ones (Shoenfeld et al. 2008c; de Carvalho et al. 2009; Kivity et al. 2009).

In the context of SLE, perhaps the Epstein–Barr virus (EBV) is the most notorious agent with the strongest evidence of association with this AD (Shoenfeld et al. 2008c). EBV is a common infection and a member of the Herpes-virus family. Infection with EBV usually causes "infectious mononucleosis" but is also associated with Burkitt's lymphoma and nasopharyngeal carcinoma (Barzilai et al. 2007a; de Carvalho et al. 2009). The connection between EBV and SLE was first described in 1971 when EBV antibodies were observed in SLE patients' sera in higher prevalence than in control population (Evans 1971). Further studies supported this observation demonstrating EBV early antigen IgG and EBV viral capsid antigen IgG to be significantly more prevalent in SLE patients (Barzilai et al. 2007b; Berkun et al. 2009; Esen et al. 2012). EBV infection is not just more prevalent among SLE patients but may determine the clinical manifestations of the disease. Exposure to EBV may predict milder SLE disease accompanied by high titers of anti-Ro antibodies, skin and joint manifestations (Zandman-Goddard et al. 2009).

The role of EBV in pathogenesis of SLE was suggested by several hypotheses. The most profound is the molecular mimicry (Poole et al. 2006; Barzilai et al. 2007a; de Carvalho et al. 2009) in which EBV produces the viral protein EBV nuclear antigen 1 (EBNA-1). The exposure of susceptible individuals to EBNA-1 causes a humoral response that manifests in the production of long-standing cross-reactive antibodies against SLE-specific antigens like Sm, Ro, and ds-DNA. Of note, in the normal population, the humoral response towards EBNA-1 is a limited response without production of those long-standing cross-reactive antibodies (Poole et al. 2006; Barzilai et al. 2007a; Shoenfeld et al. 2008c). Strengthening this hypothesis, an animal study showed that immunization of rabbits and mice with EBNA-1 fragments has led to the development of lupuslike autoantibodies in both animal models (Poole et al. 2008).

On the one hand, it seems that EBV infection may precede SLE and contributes to its development and clinical presentation. On the other hand, some studies suggest an increased susceptibility to EBV infection among SLE patients. Anti-EBV antibodies are more prevalent in SLE patients (99%) compared with healthy subjects (90%). Additionally, an aberrant specific immune response to EBV was observed in SLE patients. The normal response to EBV infection is proliferation of B cells that eventually become latent memory B cells. In SLE, due to the immune

dysregulation, those memory B cells may become resistant to apoptosis and their number is increased. In addition, those B cells act as antigen-presenting cells and further stimulate the proliferation of T cells and cytokine production (Kang et al. 2004; Zandman-Goddard et al. 2009).

Other infectious agents such as *CMV* and *Parvovirus B19* have also been suspected as a trigger to the development of SLE (de Carvalho et al. 2009; Gualtierotti et al. 2010). CMV IgM and IgG levels were significantly higher in SLE patients than controls (Barzilai et al. 2007b; Berkun et al. 2009; Esen et al. 2012). Moreover, *Rubella* and *Mumps* infection in the first year of life was shown to have a significant association with appearance of antinuclear antibodies (ANA) in adulthood (Edwards et al. 2006). Recently, high titers of Rubella IgM antibodies were found to be marginally associated with psychosis and depression in SLE patients (Zandman-Goddard et al. 2008).

Last but not least, the "hygiene hypothesis" suggests a protective role for some infections in the development of autoimmune and allergic conditions (Kivity et al. 2009). This hypothesis may explain the inverse or protective association between some endemic infectious agents and SLE such as malaria, in which its parasite *Plasmodium berghei* was shown to prevent lupus in mice, and certain other parasites. This was further supported by the low prevalence of SLE in West African population (endemic area) as compared to Africans living in Europe and North America (Butcher 2008; Borchers et al. 2009; Kivity et al. 2009; Shapira et al. 2010). In accordance with these observations, a recent finding shows a protective role of Hepatitis B virus (HBV) infection in SLE patients from Columbia (Ram et al. 2008).

To summarize, a bi-directional relationship of infections and SLE have been extensively documented. While some infectious agents are clearly related to the induction or triggering of SLE, others may play a protective role.

8.5 Vaccines and SLE

Vaccines have succeeded in eradicating several infectious diseases and are considered to be one of the greatest medical developments. However, similarly to most medical intervention, vaccines are not free of adverse events, including triggering of autoimmune phenomena, and SLE (Shoenfeld et al. 2008c; Agmon-Levin et al. 2009a).

Vaccines may be related to various adverse events by *temporal and causal* associations. Neither of which are easy to prove. As for temporal association, the time frame between immunization and the emergence of postvaccination autoimmunity may vary between days to weeks and even years (Israeli et al. 2011). Regarding postvaccination autoimmunity, causal associations are rare and the criteria to determine such a cause and effect association are not fully established. Nevertheless, several such links have been accepted by the medical community (Agmon-Levin et al. 2009a).

The mechanisms by which vaccines may induce autoimmunity are diverse. Similarly to infectious agents, vaccine can induce autoimmunity via mechanisms

such as molecular mimicry, bystander activation, epitope spreading, and polyclonal activation (Shoenfeld and Aron-Maor 2000; Molina and Shoenfeld 2005; Agmon-Levin et al. 2009a). Moreover, in recent years we have become more aware of the adjuvant role in autoimmunity induction (Shoenfeld and Agmon-Levin 2011). Adjuvants were previously considered to be inert substances and were added to most vaccines in order to enhance the antigen-specific immune response. Alas, an amassed amount of data supports the concept of adjuvants-induced autoimmune phenomena (Israeli et al. 2009). This has led, in the last year, to the definition of a new syndrome termed "ASIA"—Autoimmune or autoinflammatory Syndrome induced by Adjuvants (Shoenfeld and Agmon-Levin 2011). The most common adjuvant in human and animal vaccines is aluminum. This adjuvant was linked to the immune-mediated disease Macrophagic Myofasciitis (MMF) (Israeli et al. 2011) as well as the presence of alum–immune complexes following vaccination (Shoenfeld and Agmon-Levin 2011). Other adjuvants such as pristane (tetramethylpentadecane—TMPD) and squalene have also been correlated to autoimmune phenomena (Israeli et al. 2009; Shoenfeld and Agmon-Levin 2011). In animal models, injection of pristane, a hydrocarbon oil, to the peritoneum of BALB/c mice induced a lupus-like disease characterized by autoantibodies against nuclear components and clinical manifestations of SLE (Reeves et al. 2009). In another study, following intraperitoneal immunization of Atlantic salmon with oil-adjuvanted vaccines, autoantibodies, granulomatous inflammation of the liver and immune-complex glomerulonephritis were apparent (Koppang et al. 2008). Squalene may induce arthritis in rats and the production of SLE-autoantibodies in mice (Shoenfeld and Agmon-Levin 2011).

Several studies reported on the plausible *association between vaccinations and the induction or exacerbation of SLE*. In animal models, immunization of healthy young dogs elucidated the production of nine autoantibodies including lupus-associated antibodies (Hogenesch et al. 1999). In humans, a temporal association between hepatitis B vaccine and SLE has been suggested (Older et al. 1999; Aron-Maor and Shoenfeld 2001; Cooper et al. 2002; Geier and Geier 2005; Agmon-Levin et al. 2009b). In a case-control epidemiological study based on the vaccine adverse events reporting system (VAERS), an increased risk of SLE emergence following hepatitis B vaccine was shown (OR = 9.1, $p < 0.0001$, 95% CI = 2.3–76) (Geier and Geier 2005). In another study of 265 newly diagnosed SLE patients, an increased risk of disease was documented following immunization against hepatitis B, although this did not reach statistical significance (OR = 1.4, 95% CI 0.9–2.1) (Cooper et al. 2002). Several case series and case reports also suggested a temporal association between hepatitis B vaccine and the emergence of SLE. Older et al. (1999) described five cases of SLE within 2–3 weeks after the second dose of hepatitis B vaccination in healthy soldiers. Two additional cases also documented the appearance of SLE manifestation within weeks following hepatitis-B immunization (Tudela et al. 1992; Guiserix 1996). Recently, Agmon-Levin et al. (2009b) described ten patients who developed SLE following hepatitis B vaccination. The clinical manifestations of this group of SLE patients were somewhat similar to patients diagnosed with drug-induced SLE. Special manifestations that were observed in this group include a relatively high rate of liver involvement (20%) as well as neurological (80%) and pulmonary (70%) manifestations compared

to a relatively low rate of renal and hematologic features. Description of association between SLE and other vaccines, such as typhoid, influenza, meningococcal, and others is rare and was reported in some case reports summarized by Older et al. (1999).

Taking it all together, it seems that postimmunization autoimmunity was observed both in animal models and humans. As for SLE disease, although the data is limited and such cases are estimated to be a very rare, a plausible link between hepatitis B vaccine and the emergence of SLE cannot be excluded.

8.6 Drugs and SLE

Drug-induced autoimmunity is an idiosyncratic reaction resulting in the production of autoantibodies and/or cell-mediated immune response against selfepitopes. Of note, although autoantibodies usually precede and even predict the risk of disease, it is not equivocal to an overt AD. In other words, most patients that develop autoantibodies in response to a drug will not develop a full-blown AD (Chang and Gershwin 2009).

Drug-induced lupus erythematosus (DILE) accounts for up to 10% of newly diagnosed SLE cases, and its incidence in the USA is 15,000–20,000 per year (Vedove et al. 2009). DILE is defined by a lupus-like syndrome following drug administration that resolves both clinically and serologically after discontinuation of the culprit drug (Katz and Zandman-Goddard 2010). Classical DILE shares many similarities with idiopathic SLE, even though clinical and serological differences do exist (Sarzi-Puttini et al. 2005). Moreover, ACR (American College of Rheumatology) criteria for SLE cannot always be applied to DILE, for which there are currently no standard diagnostic criteria (Vedove et al. 2009).

Nevertheless, at present more than 80 drugs have been associated with DILE and can be classified according to their relative risk. For instance, procainamide confers the highest risk for DILE followed by hydralazine. A moderate risk was related to quinidine, whereas most other drugs are considered to be of a lower risk (Sarzi-Puttini et al. 2005; Chang and Gershwin 2009; Katz and Zandman-Goddard 2010) (Table 8.1).

DILE can be classified to a systemic form (DISLE) and a cutaneous form (DICLE). The latter may be further divided into subacute cutaneous and chronic cutaneous variants (Antonov et al. 2004).

8.6.1 Drug-Induced Systemic Lupus Erythematosus

This systemic form is the most common variant of DILE and is frequently apparent following the use of hydralazine, procainamide, isoniazid, and minocyclin (Marzano et al. 2009). DISLE resembles idiopathic SLE and usually presents as a mild disease. Several unique features characterize DISLE (Antonov et al. 2004), such as equal distribution of gender and the occurrence at a relatively older age (Sarzi-Puttini et al. 2005). Clinically, it manifests with arthralgia (90%), myalgia (50%), serositis, skin manifestations, and

Table 8.1 A list of drugs associated with SLE

Drugs related with SLE	Drugs possibly associated with SLE
Hydralazine	*Anticonvulsants*: Carbamazepine, etosuximide, phenytoin, diphenyl-hydantoin, pirimidine, trimethadione, valproate, and zonisamide
Procainamide	*Antithyroid drugs*: Propylthiouracil, methimazole, and thiamazole
Isoniazid	*Anti infectious agents*: Terbinafine, penicillin, streptomycin, tetracycline, griseofilvin, ciprofloxacin, rifampin, alpha-interferon, and *para*-aminosalicilic acid
Methyldopa	*Beta-blockers*: Practolol, acebutolol, labetalol, propranolol, pindolol, atenolol, metoprolol, and timolol
Chlorpromazine	*Other antihypertensive drugs*: Captopril, calcium channel blockers, clonidine, and hydrochlorothiazide
Quinidine	*Anticancer agents*: Fluorouracil agents (fluorouracil, tegafur/UFT), hydroxyurea, interferon, and gemfibrosil
Minocycline	*Antirheumatic*: Sulfasalazine, gold salts, and interleukin-2
Penicillamine	*Statins*: Lovastatin, simvastatin, and fluvastatin
Etanercept	*Others*: Phenylbutazone, reserpine, lithium, clobazam, clozapine, tocainide, lisinopril, zafirlukast, estrogens, and oral contraceptives
Infliximab	

fever. Skin manifestations of DISLE are usually mild and include photosensitivity, erythema nodosum, and purpura, while malar rash, alopecia, oral ulcers, and discoid lesions are relatively rare in comparison with SLE (Vedove et al. 2009). In contrast to idiopathic SLE, renal and central nervous system are rarely involved (Vedove et al. 2009; Katz and Zandman-Goddard 2010). The laboratory findings in DISLE include elevated erythrocyte sedimentation rate (ESR), mild cytopenia, normal complement levels (Vedove et al. 2009; Katz and Zandman-Goddard 2010), and a unique profile of antibodies. Antihistone antibodies are positive in up to 95% of DISLE patients and are considered to be a serological marker of this condition. These antibodies can be detected also in idiopathic SLE and other rheumatic diseases, and thus are not defined as pathognomonic to DISLE (Marzano et al. 2009; Vedove et al. 2009; Katz and Zandman-Goddard 2010). Other autoantibodies commonly found in DISLE are ANA that appear in a homogenous pattern by immuno-fluorescence techniques and do not fix complement, antisingle-stranded DNA (anti-ssDNA) and antichromatin (antinucleosome) antibodies (Katz and Zandman-Goddard 2010). In contrast, anti-ds-DNA and antiextractable nuclear antigens (ENA) are rare in DISLE (Vedove et al. 2009; Katz and Zandman-Goddard 2010). The detection of antismith (anti-Sm) antibodies is very rare and their presence is regarded as an evidence of idiopathic SLE (Katz and Zandman-Goddard 2010).

8.6.2 Drug Induced Subacute Cutaneous Lupus Erythematosus (Drug-Induced SCLE)

This form of DILE is very similar to idiopathic SCLE in clinical manifestations and laboratory findings (Vedove et al. 2009) and is more common among females (Katz and Zandman-Goddard 2010). Drug-induced SCLE can appear following the usage of cardiovascular drugs including: calcium channel blockers, angiotensin-converting enzyme

inhibitors, thiazide diuretics as well as other drugs such as terbinafine and TNF-α antagonists (Marzano et al. 2009; Vedove et al. 2009). Clinical manifestations of this form of DILE include cutaneous lesions in sun-exposed areas of the skin, which are usually symmetric, nonscarring annular polycyclic or papulosquamous lesions (Marzano et al. 2009; Vedove et al. 2009). The autoantibodies profile of drug-induced SCLE consists of the appearance of ANA, anti-Ro/SSA, anti-La/SSB, and antihistone antibodies (Marzano et al. 2009; Vedove et al. 2009; Katz and Zandman-Goddard 2010).

8.6.3 Drug-Induced Chronic Cutaneous Lupus Erythematosus (Drug-Induced CCLE)

In contrast to drug-induced SCLE, drug-induced CCLE is a very rare disease that manifests with discoid lesions. It is associated with administration of fluorouracil agents, NSAIDS, and TNF-α antagonists (Marzano et al. 2009; Vedove et al. 2009).

8.6.4 Mechanisms of DILE

Several mechanisms by which DILE develops have been proposed, although none of them has been proven (Vedove et al. 2009). These mechanisms can be classified into patient-related and drug-related ones. Patients that are considered to be of high risk to develop DILE include those with genetic predisposition, namely carrier of HLA-DR4, DR2, or DR3 and patients with only one allele of C4 complement factor (Vedove et al. 2009). In addition, being a "slow acetylator" is another risk factor for developing DILE as acetylation is the process by which most drugs are metabolized in the liver (i.e., hydralazine, procainamide, isoniazid, and sulfonamides). Slow acetylation of drugs leads to a decreased elimination of the drugs and increased exposure to reactive metabolites (Antonov et al. 2004). Several drug factors may contribute to induction of DILE. Drugs that increase the production of reactive oxygen species and can affect mitochondrial DNA and cause hypomethylation enhance the risk of DILE, i.e., procainamide and hydralazine (Katz and Zandman-Goddard 2010). Drugs that act as haptens and bind to carrier proteins may become immunogenic and induce an immune response against the drug as well as other proteins (Chang and Gershwin 2009; Katz and Zandman-Goddard 2010). Certain drugs may further induce a nonspecific immune dysfunction through lymphocyte activation and break of immune tolerance (Katz and Zandman-Goddard 2010).

8.6.5 Biological Drugs and SLE-Like Disease

In the last decade DILE was linked to a new group of biological drugs such as anti-TNF and interferon (IFN) α. SLE that is induced by biological drugs is regarded as

a unique entity of DILE, as this variant may be irreversible and is associated with different immune-mediated mechanisms (Antonov et al. 2004; Vedove et al. 2009; Katz and Zandman-Goddard 2010). In addition, it is believed that at least in some patients, biological drugs unmask latent idiopathic SLE rather than inducing DILE (Katz and Zandman-Goddard 2010).

Interestingly, up to 50% of patients treated with infliximab, adalimumab, or etanercept will become seropositive for ANA during therapy, though only a very small minority, estimated to be 0.1–0.2%, will develop true SLE (Katz and Zandman-Goddard 2010). Anti-TNF-α-induced SLE manifests similarly to idiopathic SLE with renal and dermatologic involvement, hypocomplementemia, high titers of anti-ds-DNA antibodies and low titers of antihistone antibodies (Katz and Zandman-Goddard 2010). The disease was reported to occur 2 months and up to more than 30 months following the initiation of treatment with TNF-blockers. Different mechanisms were suggested to play a role in this condition, of which down regulation of mechanisms that controls B cell hyperactivity and the induction of T cells apoptosis are the most acceptable ones (Antonov et al. 2004).

IFNs are mainly used for the treatment of multiple sclerosis, cancer, and chronic viral hepatitis. IFNα is the most notorious exogenous IFN to induce lupus. This is not surprising since IFNα contributes to SLE pathogenesis by disrupting peripheral tolerance. IFNα and type 1 IFN-inducible genes (termed also "IFN signature") were found to be increased in the sera of idiopathic SLE patients and has a concordance with disease activity (Obermoser and Pascual 2010). Similarly to TNF-induced lupus, IFNα-induced lupus resembles idiopathic SLE with renal involvement, hypocomplementemia, and presence of anti-ds-DNA abs (Antonov et al. 2004).

8.7 Ultra Violet Radiation and SLE

Photosensitivity is one of the ACR criteria for SLE (Rahman and Isenberg 2008) and its prevalence among SLE patients is approximately 30–50% (Reefman et al. 2006). Ultra violet (UV) radiation is an electromagnetic radiation known for its ability to ionize molecules, leading to chemical reactions in the skin and resulting in skin cancer and exacerbation of immune-mediated diseases. UV light was shown to induce or exacerbate skin lesions of SLE (Kuhn et al. 2007, 2010; Lehmann and Homey 2009; Maverakis et al. 2010). Moreover, UV exposure increases the risk of SLE development, as seen in a case-control study showing that outdoor work in the previous year increases the risk of SLE by twofold (Cooper et al. 2010). Apart from inducing cutaneous manifestations, UV exposure can also induce or exacerbate the systemic manifestations of SLE (Vila et al. 1999).

UV radiation is absorbed in DNA molecules within keratinocytes leading to apoptosis, which is a cancer-protective mechanism (Lehmann and Homey 2009; Maverakis et al. 2010; Kuhn et al. 2010). Normally, keratinocytes undergoing apoptosis release a small amount of nuclear material in the first 24 h in order to selfprocess it and then be cleared by phagocytosis. Subsequently, phagocytes that clear apoptotic cells

release antiinflammatory cytokines such as transforming growth factor beta (TGFβ) (Reefman et al. 2006). It has been suggested that the susceptibility to apoptosis in SLE is increased, while the clearance of apoptotic cells is somewhat defected (Lehmann and Homey 2009). It was shown that in SLE patients, autoantibodies recognize the autoantigens that have been exposed on the apoptotic cells surface, and clearance of these antigens is mediated by Fc-gamma receptor (FcγR) leading to the release of proinflammatory cytokines (Reefman et al. 2006), which in turn activate plasmacytoid dendritic cells (pDCs) and induce the release of type 1 IFN (Lehmann and Homey 2009). Moreover, UV is also responsible for re-distribution of intracellular antigens such as SS-A/Ro and SS-B/La to the apoptotic cell surface and, therefore, to their exposure to the immune system. Interestingly, antiRo Abs are a photosensitivity marker found in SLE patients; their binding to Ro antigens on the apoptotic cell surface enhances cytotoxicity of the cell and release of nonprocessed nuclear material, enhancing the autoimmunity process (Maverakis et al. 2010).

Intriguingly, although the data presented herein support the recommendation given to SLE patients to avoid sun exposure, this may lead to an increased deficiency of vitamin D. The latter has been linked to SLE severity and increased thrombosis (Shapira et al. 2009a, b; Agmon-Levin et al. 2010; Amital et al. 2010; Ben-Zvi et al. 2010; Hajas et al. 2010; Souberbielle et al. 2010; Toubi and Shoenfeld 2010; Arnson et al. 2011; Cutolo et al. 2011; Kivity et al. 2011; Lerner et al. 2011; Oren et al. 2011).

To summarize, UV radiation can induce or exacerbate skin lesions in SLE patients and systemic manifestations and also increases the risk for SLE development due to a specific combination of UV properties with local immune responses and specific immune dysfunctions. On the other hand, avoidance of UV light is associated with vitamin-D deficiency, which is also linked with SLE prevalence and severity. Thus, it may be suggested that for SLE patients the best intervention is a combination of decreased exposure to UV radiation with increase supplementation of vitamin D.

8.8 Summary

The role of environmental factors in pathogenesis and clinical manifestations of autoimmune diseases, particularly SLE, has been established. SLE development is determined by the interplay between the host genetic factors and external stimuli such as environmental and hormonal factors. In this chapter we have gathered the current evidence relating various factors with SLE disease. Of these, exposure to chemicals and the role of cigarette smoking, as well as exposure to infectious agents, drugs, vaccines, and UV radiation are delineated. This wide profile of environmental factors may contribute to SLE development, acceleration, and specific manifestations. Thus, suggesting that the multifaceted SLE depends on the patient's genetic profile and his/her array of environmental exposures. In other words, the specific combination of factors determines the individual "mosaic of SLE" and its clinical expressions. The definition of factors that increase the risk of SLE may enable physicians and patients to utilize different preventive methods.

8.9 Recommendations

Primary prevention: Screening for patients at risk of being diagnosed with SLE.

One should avoid excessive sun exposure, cigarette smoking, chemical exposure, and several drugs.

Secondary prevention: It is also important for SLE patients to avoid the above factors, Moreover, administration of oral vitamin D is very important for them as well. In addition, the use of vaccines should be done when the patient is in remission.

Future studies should be done in order to identify better the interplay between environment and genetics in SLE. The ability to determine which genetic factors lead to one's susceptibility to a specific environmental factor and eventually to SLE will be a great progress in understanding the pathogenesis of SLE.

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Chapter 9
Multiple Sclerosis, Alzheimer's Disease, and Inflammation: A Hypothetical View

Margaret S. Bynoe and Christophe Viret

Abstract Neurodegenerative diseases share many common characteristics. In this chapter we will present current understanding of the genetic, immunologic, and environmental basis of multiple sclerosis (MS) and Alzheimer's disease (AD). The etiology of AD and MS is not known, but one factor that is common to the pathogenesis of these diseases is inflammation. We will discuss the pathogenesis of the different types of mechanisms that environmental and genetic factors can impose on these diseases with special emphasis on inflammation or inflammatory responses.

Key Points

- Mechanisms underlying MS pathogenesis
- Autoimmunity
- Genetic susceptibility
- Infection hypothesis in MS
- Disruption of central nervous system barriers
- Dysregulated inflammation at mucosal sites can impact MS
- Neonatal inflammation
- Purinergic signaling in MS pathogenesis—a damage model
- Therapy
- The immune system in AD pathogenesis

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- BBB functioning in AD
- Genetic susceptibility to AD
- Factors of risk for AD
- Infectious agents associated with AD
- Immuno-intervention

Abbreviations

AD	Alzheimer's disease
ApoE	Apolipoprotein E
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
BBB	Blood–brain barrier
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CR	Complement receptor
CSF	Cerebrospinal fluid
DCs	Dendritic cells
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein–Barr virus
GA	Glatiramer acetate
HHV6	Human herpes virus 6
HLA	Human leukocyte antigen
HSV	Herpes simplex virus
IBD	Inflammatory bowel diseases
ICAM-1	Intercellular adhesion molecule 1
IFN-β	Interferon beta
IFN-γ	Interferon gamma
IL	Interleukin
IPEX	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked
LRP	Lipoprotein receptor-related protein
MAC	Membrane attack complex
MAG	Myelin-associated glycoprotein
MBP	Myelin basic protein
M-CSF	Macrophage colony-stimulating factor
MHC	Major histocompatibility complex
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
NTFs	Neurofibrillary tangles
PLP	Proteolipid protein

RAGE	Receptor for advanced glycation end products
TGF-β	Transforming growth factor beta
Th	T helper
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
VCAM1	Vascular cell adhesion molecule 1
VLA4	Very late antigen 4

9.1 Introduction

9.1.1 Multiple Sclerosis

Multiple sclerosis (MS) is a multifactorial neuroinflammatory disease of which genetic and environmental factors contribute to its pathogenesis (Krone and Grange 2011; Libbey and Fujinami 2008; Weiner 2009). The first clinical description of MS dates back to the fourteenth century and was first described by both Jean Cruveilhier and Robert Carswell in 1835 (Medaer 1979). The pathogenic mechanisms of MS result in demyelination and axonal injury and loss resulting in marked disability (Libbey and Fujinami 2008). The disease affects women more frequently than men (2:1) and onset typically begins in young adulthood (El-Etr et al. 2010; Lombardi et al. 2010). It affects about 2.5 million people worldwide (Libbey and Fujinami 2008). MS has several phenotypes (Weiner 2009). It may follow a relapsing/remitting course with bouts of relapses and remissions or a primary progressive course that may quickly progress to disability and brain atrophy (Stuve and Oksenberg 1993). Over time, it can take on a gradual progressive course (secondary progressive) as a result of consistent axonal loss (Stuve and Oksenberg 1993).

9.1.2 Alzheimer's Disease

Alzheimer's disease (AD) is one of the most common causes of dementia that is associated with progressive memory loss, cognitive deficits, visual impairment, communication loss, and defective judgment (Mancuso et al. 2011). It is a multifactorial disease that is associated with environmental and genetic factors. AD affects 5.4 million Americans and its prevalence is increasing (Mancuso et al. 2011). Onset of AD symptoms begins around age 60 or older. However, an AD phenotype called early-onset familial AD, which is caused by inherited-dominant mutations in the amyloid precursor protein (APP) and the presenilin-1 and 2 genes, occurs early in life (Ho and Shen 2011; Mancuso et al. 2011). The disease is characterized by

neurofibrillary tangles (NFTs) caused by hyperphosphorylation of tau cytoskeletal protein and formation of amyloid plaques composed primarily of accumulated beta-amyloid deposition. This results in brain atrophy, synaptic loss, inflammation, and neuronal death (Ho and Shen 2011). Amyloid plaques are characterized by the presence of activated microglial, reactive astrocytes, and inflammatory immune cells (Khandelwal et al. 2011). The inflammation induced is believed to result in blood–brain barrier (BBB) leakiness and progressive central nervous system (CNS) deterioration (Khandelwal et al. 2011).

9.2 Multiple Sclerosis

9.2.1 Pathogenesis of MS

9.2.1.1 Mechanisms Underlying MS Pathogenesis

Immunopathogenesis. MS is a multifaceted neurological disease. It is still being debated whether MS is caused by an autoimmune attack on CNS tissue or whether autoimmunity is secondary to CNS injury (Lehnardt 2010; Schmitz and Chew 2008). Regardless of the order of its cause, the immune system plays a critical role in the development and progression of MS. MS is characterized by infiltration of immune cells into the CNS (Biegler et al. 2011; Merson et al. 2010). These include T cells, B cells, and macrophages (Fernandez et al. 2010). The pathogenic T cell phenotypes in MS are T helper (Th)1, Th17, and CD8 T cells (Caminero et al. 2011; Jadidi-Niaragh and Mirshafiey 2011b). Th2 cells, however, have a protective effect in MS (Jadidi-Niaragh and Mirshafiey 2011b). In the CNS, T cells are presented with myelin components by antigen-presenting cells (macrophages, dendritic cells [DCs], and B cells) and microglia cells (Kaushik et al. 2011). Microglia cells, which are also referred to as brain macrophages, express toll-like receptors (TLRs, which are pattern recognition receptors) and recognize and respond to invading pathogens and their products (Kaushik et al. 2011). They are the first line of defense against pathogens and have the ability to destroy infected and/or damaged cells (Kaushik et al. 2011).

9.2.1.2 Mechanisms That Can Potentially Trigger MS

- In response to a pathogen in the CNS, activated microglia cells produce proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, nitric oxide (NO), and induced the recruitment of adaptive immune cells and astrocytes to the site of injury (Lehnardt 2010; Schmitz and Chew 2008). This has detrimental consequences to the host. It can result in axonal damage, neurotoxicity, and the release of myelin antigens (Lehnardt 2010).

The inflammatory response mounted by adaptive immune cells that are presented with myelin components can perpetuate MS (Lehnardt 2010; Schmitz and Chew 2008).

- Generation of an immune response to CNS antigens such as myelin can be induced in the absence of a pathogen (t Hart et al. 2009). For example, during CNS damage, activated, proinflammatory immune cells are recruited to site of damage. These immune cells can produce factors that induce activation of microglial cells. Activated microglial cells increase their expression of major histocompatibility complex (MHC) Class II molecules, produce proinflammatory mediators and present CNS antigens to immune cells including potentially autoreactive immune cells (Zepp et al. 2011). Microglia cells can also induce tissue injury through local production of cytotoxic molecules (Steinman 2004).

9.2.2 Autoimmunity

A key argument in the causality of MS is a lack of tolerance (Goverman 2011). Tolerance or self-education occurs during immune system development and during maturation (Goverman 2011). While no specific genetic defect besides human leukocyte antigen (HLA) or inflammatory genes has been found to explain a lack of central or peripheral tolerance, it is hypothesized that there are defective tolerogenic mechanisms in MS (Goverman 2011).

Normal individuals (without MS) and normal mice carry small numbers of autoreactive T and or B cells specific for self-antigens such as myelin (Rosenling et al. 2011). These autoreactive cells are kept in check by several layers of peripheral tolerance mechanisms, including clonal deletion, clonal anergy, and suppression by regulatory T cells (Prud'homme and Vanier 1993). An individual may be predisposed to develop MS under conditions where inflammation or brain trauma causes damage to the BBB and allows those small number of myelin-reactive cells to gain entry to the brain (Holman et al. 2010). Once in the CNS, these autoreactive cells will encounter abundant CNS antigens to which they will respond and multiply, thereby setting up a vicious cycle of inflammation and destruction. This, in principle, is why we can induce experimental autoimmune encephalomyelitis (EAE), the animal model for MS in various normal mouse strains (Rosenling et al. 2011).

9.2.3 Animal Models of MS

The autoimmune contribution to the pathogenesis of MS is best exemplified by studies in the animal model, EAE (Rosenling et al. 2011). In EAE, subcutaneous immunization with myelin antigens such as myelin oligodendrocyte glycoprotein (MOG),

myelin basic protein (MBP), or proteolipid protein (PLP) emulsified in complete Freund's adjuvant causes the activation and expansion of very small numbers of myelin-specific autoreactive T cells (Miller et al. 2007; Rosenling et al. 2011). This is followed by administration of *Bordetella pertussis* toxin. It is not yet clear what specific effect *pertussis* toxin has on the immune response or on BBB modulation. However, it is believed to alter BBB permeability thus allowing activated cells into the brain (Miller et al. 2007). These autoreactive T cells are usually CD4 Th cells that produce either interferon (IFN)-γ (Th1) or IL-17 (Th17) proinflammatory cytokines and CD8 T cells (Peron et al. 2010). In the CNS, these autoreactive T cells mount an attack against myelin and cause activation of microglial cells that can further exacerbate the damage in the CNS (Fletcher et al. 2010). Although EAE has some important limitations in presenting some of the neurological features of MS, it has served well as a model to elucidate some immunomodulatory mechanisms that might impact MS pathogenesis. One of many important outcomes from studies in EAE is elucidation of the role of T regulatory cells in controlling inflammation and autoreactivity both in the periphery and in the CNS (Bynoe et al. 2007) and establishment of the plasticity between T regulatory and Th17 cells (Jadidi-Niaragh and Mirshafiey 2011a; Lee et al. 2009). Also, the EAE model has led to some of the treatment modalities currently used to treat MS symptoms.

9.2.4 T Regulatory Cells (Treg) Role in MS

Treg cells are a subset of suppressor CD4 Th cells that express CD25 (CD4+ CD25+) and may or may not express the Fork head box transcription factor 3 (FoxP3) (Lee et al. 2009). FoxP3 controls development and function of Treg cells. The mechanisms by which FoxP3 modulates Treg cell function are not yet clearly defined. Treg cells produce the suppressive cytokines IL-10 and transforming growth factor beta (TGF-β), and can suppress the activity of Th17 cells in vitro and in vivo (Jadidi-Niaragh and Mirshafiey 2011a). Thymus-derived Treg cells are referred to as natural Treg cells to distinguish them from inducible Treg (iTreg) cells that are induced to express FoxP3 in naïve T cells in the periphery (Jadidi-Niaragh and Mirshafiey 2011a; Lee et al. 2009). Another subset of Treg cells are type 1 regulatory T cells or Tr1 cells that develop in the periphery from naïve T cells and are induced by tolerogenic DCs to produce IL-10 by which they exert their suppressive function (Kushwah and Hu 2011). Treg cells can also be induced in vitro upon treatment with TGF-β and IL-6 and/or IL-23 (Lee et al. 2009). Studies suggest that Th17 and Treg cells exhibit developmental plasticity whereby Treg cells can be induced to differentiate into IL-17-producing T cells. IL-6 is regarded as influential for Th17 and Treg cell development (Lee et al. 2009). IL-6 can induce Th17 development in the presence of TGF-β but can also inhibit TGF-β and thereby inhibit Treg cell development (Kimura and Kishimoto 2010). The impact of Treg cell's suppressive function in maintenance of peripheral tolerance is best demonstrated in patients with mutations in their FoxP3 gene that develop a life-threatening autoimmune disease called

immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome (Passerini et al. 2011). IPEX patients develop diabetes mellitus, thyroiditis, eczema, and elevated serum IgE levels (Passerini et al. 2011). Similar to IPEX patients, mice with mutations in the FoxP3 gene, called scurfy mice, develop spontaneous multiorgan inflammation and lack FoxP3+ Treg cells (Sharma and Ju 2010). Interestingly, IPEX patients do not develop MS.

9.2.5 Immune Regulatory Factors Whose Alteration Can Contribute to MS Pathogenesis

- *Treg cells in MS*: MS patients have altered Treg cell numbers and/or function (Dalla Libera et al. 2011). Th17 cells are pathogenic in EAE and MS (Jadidi-Niaragh and Mirshafiey 2011b). Disregulation of the Treg/Th17 axis can promote autoimmunity due to developmental plasticity between Treg/Th17 cells (Kimura and Kishimoto 2010). This plasticity can potentially be impacted upon by environmental factors such as infection that may shift the cytokine profile, such as overproduction of IL-6, thereby favoring Th17 development. This can trigger an autoimmune reaction in a background predisposed to MS development.
- *Neuropilin-1* (*Nrp1*): An emerging player in the regulation of peripheral immune responses is Nrp1. Nrp1 is a neuronal cell surface molecule that was originally identified for its role in neuronal development. Recently, Nrp1 has been identified to have important roles in immune regulation, specifically in immune suppression and control of autoreactivity (Glinka et al. 2011; Solomon et al. 2011). It is not yet known if Nrp1 plays a role in MS.

9.2.6 Genetic Susceptibility

MS, like most autoimmune diseases, is linked to several susceptible gene polymorphisms (Bahreini et al. 2010; Goodin 2010). Monozygotic twin studies show about 25% concordance rate to developing MS vs. about 5% for dizygotic twins (Willer et al. 2003). A worldwide population study showed that genetic polymorphisms in the HLA gene is strongly associated with susceptibility to MS (Nischwitz et al. 2011; Shahbazi et al. 2011; Wang et al. 2011). In addition to HLA, a genome wide study identified non-HLA genes that have shown linkage to MS (Louboutin et al. 2011). These include apolipoprotein E (ApoE), IL-1β, TNF-α/β, and CCR5 all of which are involved in inflammation or immune cell mobilization (Louboutin et al. 2011). The identification of linkage of non-HLA genes to MS helps to explain, in part, why HLA-DRB1 accounts for less than 50% of MS genetics and of those patients that have HLA susceptibility alleles, less than 5% are susceptible to MS (Tuller et al. 2011). The HLA allele has gained great interest because of its association

with several autoimmune diseases (ankylosing spondylitis and systemic lupus erythematosus) including MS (Bahreini et al. 2010). MHC class II, HLA-DR, and HLA-DQ alleles are of interest because of their association with CD4 Th cells, their involvement with B- and T-cell-mediated immune responses and the potential for autoimmune development (Kallaur et al. 2011).

The recognition of self-epitopes by CD4+ T cells in the context of susceptibility MHC class II alleles can induce aberrant T cell immune responses. This can lead to loss of tolerance to self-tissues and autoimmune development in several ways:

1. Such disease-associated MHC class II molecules may differ from other MHC molecules in their peptide-binding cleft, which can alter antigen presentation and recognition by CD4 T cells. This can also affect the T cell affinity for the peptide and can lead to T cell activation and immune response against self.
2. Disease-associated MHC class II alleles can cause autoimmunity due to aberrant presentation of normal "cryptic" epitopes that can lead to enhanced T cell recognition and response against such epitopes (Deng et al. 2007).

Convincing circumstantial evidence strongly indicates a role for genetics in MS development (Kallaur et al. 2011). However, genetic predisposition does not fully explain why only a small percentage of people with HLA polymorphisms actually develop MS (Bahreini et al. 2010). Strong circumstantial evidence suggests that environmental factors or triggers such as infection by pathogens or inflammatory stimuli play a role in MS pathogenesis (Ascherio and Munger 2007). Regardless of the factors involved in MS pathogenesis, the immune response is impacted, and this in turn contributes to the triggering or exacerbation of MS. In this chapter, we will focus on infection and inflammation, combined with host genetics, in the pathogenesis of MS.

9.2.7 Role of Environmental Factors in MS Pathogenesis

9.2.7.1 Infection Hypothesis in MS

It is hypothesized that an infectious agent is responsible for triggering MS (see Table 9.1) (Owens et al. 2011). Viruses are the most frequent amongst pathogens associated with MS (Owens et al. 2011). These include adenovirus, human herpes virus 6 (HHV6), Epstein–Barr virus (EBV), herpes simplex virus (HSV), coxsackie virus, and rhino viruses (Brahic 2010). Despite these overwhelming associations, no study has actually linked a specific virus to the cause of MS. The prevailing theory purports that infection by a virus can trigger an autoimmune reaction by distinct mechanisms: Molecular mimicry (Harkiolaki et al. 2009) or epitope spreading.

1. The molecular mimicry hypothesis requires that an immune response directed against an infectious agent cross-reacts with the host tissue (Bach 2005). Although a specific molecular mimic has not been "caught in the act" or definitely identified, extensive blast studies show high percentage sequence homology between known viruses such as EBV, coxsackie virus, HSV, and HHV6 with host tissues, such as MOG, MAG (myelin associated glycoprotein), and claudin 11 (Carter 2012).

Table 9.1 Environmental risk factors proposed to contribute to MS pathogenesis

Risk factors	Proposed mechanism
Viruses	Trigger inflammatory immune responses, CNS damage, demyelination
Smoking	Nitric oxide-mediated demyelination, axonal loss[a]
UV light/vitamin D deficiency	Vitamin D has immunomodulatory functions
Sex hormones	Alter immune response, differences in male and female immune response
Contribution by other factors on MS pathogenesis	
Infection by other pathogens	*Chlamydia pneumonia*
Other inflammatory diseases	Inflammatory bowel diseases
Stress	Impact on immune response, dysregulation of the hypothalamic-pituitary-adrenal axis

[a]Handel et al. (2011)

2. In epitope spreading, an immune response directed at a specific epitope or region of an antigen from a pathogen can be spread to other or more diverse determinants including self-determinants, thereby triggering an autoimmune response against self (Giraudon and Bernard 2009). EBV and HHV6 are among the most common pathogens associated with MS (Giraudon and Bernard 2009). A large number of MS patients are seropositive for EBV (Kakalacheva and Comabella 2010).

9.2.7.2 Pathogens Associated with MS

EBV: It is reported that MS risk increases soon after infection with EBV, the causative agent of infectious mononucleosis (Ascherio and Munger 2010; Lindsey and Hatfield 2010). Approximately 99% of pediatric MS cases are seropositive for EBV compared to 72% for age-matched control patients (Waubant et al. 2011). More than 95% of all MS patients are seropositive for antibodies against EBV (Lindsey and Hatfield 2010). Epidemiological studies show strong association amongst individuals with a history of infectious mononucleosis and MS onset or relapses are synchronized with infections by EBV, and also by HSV, coxasackie virus, influenza virus, and rhino viruses (Levin et al. 2005). High levels of EBV antibodies detected during adolescence or young adulthood during EBV infection are believed to increase the risk of MS development in such individuals (Levin et al. 2005; Waubant et al. 2011).

HHV6: HHV6 is a large double-stranded DNA virus found ubiquitously in humans (Yao et al. 2010). Neuropathological studies support the association of HHV6 infection with MS pathogenesis (Voumvourakis et al. 2010). HHV6 has the highest prevalence of the herpes viruses in MS patients with 57% of MS patients' vs. 43% in control groups that are seropositive or are positive for viral antigens in their brains (Voumvourakis et al. 2010). HHV6 infects a wide variety of cell types including neurons (Yao et al. 2010). The virus is acquired early in life and is regarded as a commensal virus (Voumvourakis et al. 2010). This has raised the question as to whether HHV6 can cause neurological disease. Its frequent appearance in cerebrospinal fluid

(CSF) and brains of MS patients combined with its ability like other Herpes viruses to cause acute and chronic inflammation and neurological disabilities including encephalitis and myelitis and neuronal injury makes it a prime candidate in MS pathogenesis (Giraudon and Bernard 2009). Seropositivity for HHV6 occurs usually before or at the time of puberty into adulthood, which coincides with epidemiological data showing time of exposure matching MS onset (Yao et al. 2010).

- Synergy of genetic and environmental factors in MS pathogenesis
  It is conceivable that polymorphisms in HLA promote aberrant presentation of antigens from an infectious agent such that the processed peptide determinants are structurally similar or have close sequence homology to self-determinants. In this case, the "newly presented" antigen when presented to CD4 T cells could elicit an immune response that cross-reacts with self-tissues. Under these conditions, the immune response that is generated can persist even after the infectious agent is cleared. Another scenario is the induction of an immune response directed against a pathogen such as EBV or HHV6 in a host with genetic polymorphisms in non-HLA genes such as TNF-α and IL-1β, two potent proinflammatory cytokines known to cause significant tissue damage. Under such conditions, the stage is set for the perpetuation of an inflammatory response after the pathogen is cleared and even more so for those pathogens such as HHV6 that remain for the life of the host and can be reactivated. TNF-α is strongly associated with MS. High levels of TNF-α is found in CSF of MS patients and correlates with disease severity and progression (Caminero et al. 2011; Montgomery and Bowers 2011). It is also associated with demyelination and is found in brain lesions of MS patients in postmortem studies. Antibodies against TNF-α ameliorate EAE.
- Disruption of CNS barriers
  The BBB is comprised of a tight-knit layer of endothelial cells that line the CNS vasculature (Kim et al. 2006). These brain endothelial cells limit access of cells and molecules into the CNS. Specialized receptors and pumps expressed on BBB endothelial cells selectively permit or exclude wanted and unwanted substances from the brain. Brain endothelial cells are equipped with tight and adherens junction molecules that seal the spaces between neighboring endothelial cells and prevent paracellular diffusion into the CNS (Carvey et al. 2009). Accessory cells such as astrocytes and pericytes provide structural and biochemical support to endothelial cells to maintain proper endothelial permeability (Carvey et al. 2009; Kim et al. 2006). Together, the endothelial cells and supporting cells are referred to as the neurovascular unit. Alterations in BBB permeability have long been implicated in the pathogenesis of MS (Kim et al. 2006). Inflammation by pathogens and their products and activated immune cells cause damage to the BBB. Inflammatory mediators such as TNF-α, lipopolysaccharide (LPS), and the anticoagulant, thrombin, increase CNS barrier permeability making it accessible to immune cells and other molecules that alter CNS homeostasis (Carvey et al. 2009). It is hypothesized that persistent viral infection in the CNS or systemically and the chronic inflammation they

cause can increase BBB permeability (Carvey et al. 2009). It is not known exactly whether increased BBB permeability precedes MS or whether it occurs after MS. While there is precedent for both situations to occur, increased BBB permeability allows the infiltration of inflammatory cells into the brain and the resultant pathology (Alvarez et al. 2010). It is proposed that the movement of inflammatory cells across the BBB can disrupt the BBB making it accessible to more cells to infiltrate and unwanted substances that can exacerbate MS (Alvarez et al. 2010; Gaitan et al. 2011).

- Dysregulated inflammation at mucosal sites can impact MS
  Inflammatory bowel diseases (IBDs) are chronic inflammation of the bowel that includes the well-characterized Crohn's disease and ulcerative colitis (Bellizzi et al. 2010). Like MS, IBDs are driven by genetic and environmental factors that result in dysregulated immune responses and chronic inflammation causing pathology (Matricon et al. 2010). Recent studies show that alteration in commensal gut microflora reduce EAE severity (Ochoa-Reparaz et al. 2009). Several studies reported associations between patients with IBD and MS (Ochoa-Reparaz et al. 2009; Pokorny et al. 2007). This link between IBD and MS may be due to changes in systemic as well as the mucosal immune response impacted by a shift in commensal microflora composition and induction of chronic global inflammation (Charach et al. 2008). In this context, chronic global inflammation can alter CNS barrier permeability to the entry of inflammatory immune cells. Or, a host that carries an HLA polymorphism can aberrantly process and present commensal bacterial antigens to cells that will respond to and cause damage, thereby triggering MS. Further, diseases such as IBD can alter the host's immune suppressive mechanisms, thereby inducing mucosal inflammation that impact distant sites in the host.
- Neonatal inflammation
  Inflammation, especially in early life can have a detrimental consequence on the developing brain (Stolp and Dziegielewska 2009). It is proposed that a number of neurological diseases may be due to early exposure to ongoing inflammation that cause the BBB to be malformed (Stolp and Dziegielewska 2009). In this scenario, a pathogen such as an herpes virus that is neurotropic can gain access to the CNS of the fetus or neonate and cause damage to neurons. This can set the stage for a cascade of events that lead to MS later in life, both in hosts with and without genetic predispositions. The hygiene hypothesis states that early exposure to germs or infectious agents can induce lifetime protection to those agents (Power et al. 2010). The contrast may be also feasible whereby early exposure to chronic inflammatory stimuli can cause BBB damage and set a stage for neuroinflammation and neurodegeneration. This plastic phenomenon of the CNS can be impacted by the immune system. A recent study demonstrated that pericytes, which make up part of the neurovascular unit, are necessary for formation of BBB during development (Armulik et al. 2010). Acute and chronic inflammation early in life can affect pericyte function and, hence, normal BBB formation. This can have pathological consequences with resultant neurodegeneration in later life.

- Purinergic signaling in MS pathogenesis—a damage model
  Inflammation or inflammatory responses are an important part of host response to pathogens or to damaged or dying cells. It is necessary for clearance of pathogens, and it signals that there is damage to host tissue that needs to be repaired. However, inflammation is costly (Doherty et al. 2011). If uncontrolled, it can cause irreparable damage to the host and even death. Some consequences of uncontrolled inflammation are autoimmunity and septic shock (Doherty et al. 2011). Inflammation is, therefore, a highly regulated process involving many mechanisms. One important mechanism involved in the regulation of inflammation is the purinergic system. The major players in the purinergic signaling pathway are adenosine triphosphate (ATP) and adenosine (Hasko et al. 2008). Adenosine mediates its functions through P1 receptors, which comprise four G-protein coupled receptors: A1, A2A, A2B, and A3. These receptors bind adenosine with different affinities and signal through modulation of adenyl cyclase (Hasko et al. 2008). The A1 and A3 receptors mediate some cellular function by decreasing cyclic adenosine monophosphate (cAMP), whereas A2A and A2B receptors increase cAMP (Hasko et al. 2008). ATP mediates its functions via activation of P2 receptors of which there are two classes, P2X and P2Y. P2X receptor subtypes are ionotrophic, while P2Y are metabotrophic (Ferrero 2011). Both P1 and P2 receptors are expressed on almost all cell types. These include immune cells (T cells, B cells, DCs, macrophages, and neutrophils), and CNS resident cells (neurons, microglia, astrocytes, endothelial cells, and oligodendrocytes) (Burnstock et al. 2011; Ferrero 2011). Therefore, purine nucleotides play a critical role in the modulation of immune cell function as well as CNS cell function. The role of ATP and adenosine in the CNS is both protective and pathological. Studies show that, depending on the extracellular levels and their duration in the CNS after damage, ATP and adenosine can be neuroprotective or damaging (Matute and Cavaliere 2011).
- Purinergic signaling in the peripheral immune response
  Adenosine, its receptors and ectoenzymes (CD39 and CD73), are critical players in the regulation of immune responses (Bynoe and Viret 2008; Hasko et al. 2008). During inflammation and the initiation of primary immune responses, ATP is released into the extracellular environment following cell damage (Hasko et al. 2008). ATP, a damage-associated molecule and a potent activator of the immune response, increases the production of proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and IL-12. ATP also enhances the potency of the oxidative stress response of activated macrophages (Ferrero 2011; Hasko et al. 2008). The immune system has developed mechanisms to resolve these inflammatory signals. One such mechanism is activated by the extracellular adenosine generated through the activities of CD39 and CD73 (Ferrero 2011; Hasko et al. 2008). CD39 catabolizes extracellular ATP to adenosine monophosphate (AMP) and CD73 (a 5′-ectonucleotidase) converts extracellular AMP to extracellular adenosine (Matute and Cavaliere 2011). Adenosine inhibits production of TNF-α, IL-1β, IL-6, IL-12, and IL-23 by monocytes and DCs, and promotes the production of IL-10 (Bynoe and Viret 2008; Hasko et al. 2008). Therefore, CD73-generated adenosine acts as a negative feedback

signal to prevent uncontrolled inflammation that would otherwise cause collateral damage to healthy tissues.

- Purinergic signaling in response to CNS damage
  Inflammation is the host's natural response to damage such as during stroke, trauma, or during chronic inflammatory diseases like MS and AD (Fuxe et al. 2010). After CNS injury, persistent release of ATP from damaged neurons, activated microglia cells, and astrocytes occurs at the damage site (Hamilton et al. 2009; Mills et al. 2011). This can induce detrimental and/or beneficial effects via activation of ionotropic P2X receptors and metabotropic purinergic receptors. Massive release of ATP is toxic to neurons and causes activation of glial cells (Fang et al. 2009; Fumagalli et al. 2011). This results in the degradation of ATP by CD39 and CD73 ectonucleotidases that triggers the activation of the P1 adenosine receptors and the release of high levels of adenosine (Mills et al. 2011). Extracellular adenosine within the CNS acts as a signal for cellular damage and can be both protective as well as have deleterious consequences in the CNS. These signaling molecules induce the recruitment of immune and resident CNS cells to damage sites in the CNS (Matute and Cavaliere 2011). These immune cells, if presented with myelin constituents by activated microglial cells or macrophages, will cause more damage to axons thereby perpetuating axonal destruction and death.

9.2.8 Mechanisms of ATP and Adenosine Signaling in CNS Pathology and MS Pathogenesis

9.2.8.1 ATP

High extracellular levels or prolonged or sustained release of ATP can have deleterious effects on the CNS microenvironment in the following ways:

- Cause microglia cell activation and enhance their production of inflammatory factors such as NO and IL-1β (this usually results in neuronal damage and can initiate or exacerbate neurodegeneration. Such conditions can trigger MS).
- Is directly toxic to neurons and can cause neuronal death in acute or chronic CNS injury.
- Induce oligodendrocyte exocitotoxicity via P2X7 receptor.
- Sustained activation of P2X7 receptors causes lesions in the CNS that have features similar to MS plaques.
- Mice lacking P2X7 receptor are protected from EAE, the disease model for MS.

9.2.8.2 Adenosine

Adenosine receptors are expressed on CNS resident cells including astrocytes, microglial cells, endothelial cells, and neurons (Burnstock et al. 2011). Adenosine regulates neuronal excitability via its receptors (Fuxe et al. 2010; Matute and Cavaliere

2011). Its levels in the extracellular environment are related to ATP catabolism and the degree of synaptic activity. Adenosine can also be released in the extracellular environment under high stress via bidirectional and nonconcentrative nucleoside transporters that function to maintain an extracellular and intracellular gradient (Fumagalli et al. 2011; Matute and Cavaliere 2011). Adenosine plays a role in oligodendrocyte biogenesis and in myelin formation. While adenosine plays a protective role in the CNS, its functions can also lead to CNS damage in the following ways.

- Release of extracellular adenosine from the breakdown of ATP signals the recruitment of cells to the site of injury. These include inflammatory immune cells. The recruitment of immune cells to damaged CNS sites in the case of MS or damage to myelin can result in an inflammatory response by immune cells. This can worsen MS pathology.
- Ablation of adenosine ectonucleotidase CD73, that converts AMP to adenosine, blocks immune cells entry into the CNS and inhibits EAE (Mills et al. 2008). And mice deficient in the A1 receptor develop more severe EAE than their wild-type littermates (Tsutsui et al. 2004).
- Studies show that inhibition of A2A adenosine receptor or blockade of all adenosine receptors inhibit EAE and block migration of immune cells into the CNS (Mills et al. 2008).

9.2.9 Other Factors That May Contribute to MS Pathogenesis

While the identity of the scope of environmental factors that contribute to MS pathogenesis is not unequivocally known, several candidates are strongly associated with MS. Some environmental factors associated with MS pathogenesis include UV light, cigarette smoking, and sex hormones (Wingerchuk 2011). MS has strong association with geographic location and disease prevalence. This relationship is associated with exposure to ultraviolet light or vitamin D (Correale et al. 2011). MS is more prevalent in women than men by a 2:1 ratio (Caruso et al. 2010). This suggests that sex hormones may play a role in the frequency of MS development. It is proposed that sex hormones act on or influence the immune response in MS. Epidemiological studies show increases in MS prevalence among individuals that smoked at some point in their life compared to nonsmokers (Handel et al. 2011). The mechanism of cigarette smoking on MS pathogenesis is not known.

- Therapy
  Most therapies for MS fall into the categories of antiinflammatory, immunosuppressive, or immunomodulatory agents (see Table 9.2) (Mendes and Sa 2011). These therapies are aimed at dampening the immune response, limiting leukocyte infiltration, and the local inflammatory response (Mendes and Sa 2011). T cells represent one of the major culprits in MS pathogenesis (Dhib-Jalbut and Marks 2010). Theoretically, therapies aimed at depleting autoreactive T cells while leaving nonautoreactive T cells intact would be an ideal approach (Steward-Tharp et al. 2010). A plethora of therapies directed at modulating the immune

Table 9.2 Disease modifying therapies for MS. GA and IFN-β are considered first-line therapies. Natalizumab and mitoxamtrone are second-line therapies

Immunomodulatory therapy	Type	Proposed mechanism of action	Most adverse side effects	Effects
IFN-β (IFN-β 1a and IFN-β 1b)	A 166 amino acid glycoprotein secreted by fibroblasts for defense against viral infections	Reduces T cell activation, downregulates MHC II, decreases expression of co-stimulatory and adhesion molecules (ICAM-1, VCAM-1, and VLA4)	Flu-like symptoms, injection site reaction, depression, allergic reaction	Reduces clinical relapse rate
Glatiramer acetate (GA)	A four amino acid synthetic polypeptide	Binds directly to MHC class II, interferes with myelin-specific T cells, inhibits IFN-γ, immune deviation from Th1 to Th2	Injection site reaction, chest palpitations, anxiety	Reduces clinical relapse rate
Natalizumab	A human monoclonal antibody: anti-VLA4 expressed on leukocytes	Blocks lymphocyte a dhesion to VCAM1 on BBB endothelial cells	Concerns about development of progressive multifocal leukoencephalopathy, anaphylaxis, hepatotoxicity, hypersensitivity	Reduce no. of MS lesions
Mitoxantrone	A synthetic cytotoxic chemotherapeutic agent	General immune suppression	Anaphylaxis, neutropenia, hemorrhage, cardiotoxicity	Decrease relapse rates, improves disability score

response have shown promise in MS clinical trials but failed as long-term MS therapies.

A monoclonal antibody used to block the leukocyte integrin, very late antigen 4 (VLA4), such as Natalizumab is aimed at blocking leukocyte migration across CNS barriers (Comabella and Vandenbroeck 2011; Mendes and Sa 2011). However, this drug has limited efficacy in reducing MS symptoms and presents severe side effects thereby restricting its use (Comabella and Vandenbroeck 2011; Yeh 2011). Treatment with the cytokine interferon beta (IFN-β) is regarded as one the mainstays of MS therapy and is used for treatment of relapsing-remitting MS (Mendes and Sa 2011; Yeh 2011). It is produced by innate immune cells, is involved in defense against viral infections, and has a range of antiinflammatory properties (Noyes et al. 2011; Steward-Tharp et al. 2010). It downregulates MHC Class II expression on antigen-presenting cells, interferes with antigen processing and presentation, and hampers the ability of T cells to gain entry into the brain (Mendes and Sa 2011).

Another therapy with beneficial effects in alleviating relapsing-remitting MS symptoms is glatiramer acetate (GA), a synthetic polypeptide composed of four amino acids enriched in MBP (Csepany 2011; Mendes and Sa 2011). Its protective mechanisms include induction of bystander suppression of autoreactive T cell response and immune deviation—a shift in T-cell responses to an antiinflammatory Th2 phenotype (Mendes and Sa 2011).

Ongoing studies that may well prove effective in altering the immune response in MS without affecting the global immune response is the use of epicutaneous or transcutaneous immunization with myelin products that has been first shown to be efficacious in inhibiting EAE (Bynoe et al. 2003). A recent small clinical study in Poland, showed that transcutaneous/epicutaneous immunization of MS patients with a cocktail of myelin constituents protected 80% of MS patients from MS without side effects (Jurynczyk et al. 2010).

Other therapies that in the future may prove beneficial in treating MS is the modulation of adenosine receptors that has shown to be effective in blocking immune cell entry into the CNS and inhibit EAE (Mills et al. 2008). Interestingly caffeine, which is an antagonist for adenosine receptors, also inhibits EAE (Mills et al. 2008). These studies, although preliminary, may potentially prove to be effective MS therapies.

9.3 Alzheimer's Disease

AD is a neurodegenerative pathology that affects neuronal functioning and survival. It is a major cause of progressive dementia, characterized by alteration of attention, memory, language, and learning. The symptoms generally start after 65 years of age in the sporadic (or late onset) form of the disease that is the prominent form. In about 1% of cases, the disease corresponds to a familial form transmitted on a dominant mode with clinical symptoms starting as early as 30 years of age (early onset AD).

The main regions affected by neuronal loss are the temporal, parietal, and frontal neocortex as well as the hippocampus. Two major neuropathological features are linked to neuronal degeneration and dementia in AD. The first feature is an extracellular deposition of various types of amyloid plaques (or senile plaques). Under amyloidogenic conditions, intracellular processing of the membrane protein APP by the β-site APP-cleaving enzyme (BACE β secretase) generates APP-sβ and CTF β products. CTF β is then targeted by γ-secretases called presenilins that produce β amyloid (Aβ) peptides. Two fibrillogenic isoforms of Aβ peptides ($A\beta_{1-40}$ and $A\beta_{1-42}$) are produced that assemble into highly neurotoxic oligomeric aggregates within multivesicular bodies as well as into mature amyloid fibrils (especially $A\beta_{1-42}$). Larger extracellular deposits, either nonfibrillar or fibrillar, assemble upon sustained Aβ peptides secretion and constitute plaques. Plaques accumulate in the brain parenchyma and can be observed in vessel walls as well. Although the physiological function of APP is not understood, its controlled level may be important during development and normal brain functioning. The second feature corresponds to the intracellular inclusion of filaments (NFTs) essentially composed of abnormally hyperphosphorylated forms of tau, a microtubule-associated protein. NFTs cause both morphological and functional alterations in neuronal cells and appear instrumental for AD development. The degree of NFTs development appears to correlate well with the magnitude of dementia. It is possible that the local accumulation of Aβ peptides facilitates the assembly of abnormal tau into insoluble bodies and the emergence of NFTs. However, despite simultaneous occurrence, there is no definitive evidence that NFTs and plaque formations are truly dependent on each other.

9.3.1 The Immune System in AD Pathogenesis

The dysregulated processing of APP and the accompanying progressive accumulation of neurotoxic proteinaceous materials are thought to be instrumental for synaptic alteration, abnormal activity, neuronal death, and AD development (a model referred to as the "amyloid cascade hypothesis," Hardy and Selkoe 2002). In the recent years, the immune system, in general, and neuroinflammation, in particular, has been considered a likely important player in AD development/progression.

9.3.1.1 Neuroinflammation in AD

The high level of proinflammatory factors such as interleukin-1 (IL-1), IL-6, or tumor necrosis factor alpha (TNFα) within the CNS as well as the marked microglial cell activation/accumulation seen in AD brain are reminiscent of other neurodegenerative disorders that are also characterized by neuron death and point to a possibly important role for inflammation in AD development (Gonzalez-Scarano and Baltuch 1999; McGeer et al. 1993). For instance, IL-1 is abundant in cortical areas in AD and could play a catalyzing role in Aβ accumulation and plaque

formation because it can promote APP synthesis (Shaftel et al. 2008). Microglial cells surrounding plaques and NFTs are likely to sustain production of inflammatory factors during AD pathogenesis because Aβ peptides upregulate gene expression and protein production of proinflammatory cytokines or chemokines (IL-1β, IL-8, IL-10, IL-12, TNF-α, MIP-1β, MIP-1α, and MCP-1) and NO in primary microglial cells (Heneka and O'Banion 2007; Schwab and McGeer 2008). Upon phagocytosis, Aβ can activate the inflammasome in microglial cells resulting in the cleavage of pro-IL-1β to active IL-1β that is released within the CNS. In turn, IL-1β promotes the recruitment of microglial cells to Aβ plaques (Halle et al. 2008).

The disposal of plaque material by phagocytic microglia appears greatly enhanced by the presence of immunoglobulin (Ig) G deposition (Kellner et al. 2009). Microglial cells are the major immune effector cell type within the CNS; they are dynamic macrophages that normally ensure the constant immunosurveillance of the brain parenchyma (Nimmerjahn et al. 2005). They respond to neuroinfection or CNS lesions by removing dead cells, by producing multiple proinflammatory factors, by increasing the expression level of MHC I/II molecules and of various receptors including receptor for advanced glycation end products (RAGE) and TLRs. Such activation can be balanced by antiinflammatory factors such as IL-10 and transforming growth factor 1 beta (TGF-1β). In situations of tissue injury, microglial cells quickly project their mobile processes to the exact site of insult (Davalos et al. 2005; Haynes et al. 2006). The detection of extracellular nucleotides from altered cells by purinergic receptors participates in this rapid response that precedes the accumulation of microglial cells at sites of degeneration for the disposal of cellular debris (Kim and Dustin 2006) or at sites of amyloid deposit for phagocytosis of fibrillar material (Ard et al. 1996; Frautschy et al. 1998).

Highly conserved innate immune receptors participate in cell-mediated Aβ elimination in the brain. The engagement of TLR2, TLR4, and TLR9 facilitate the internalization and elimination of Aβ by microglial cells (Chen et al. 2006; Richard et al. 2008; Tahara et al. 2006). In line with this, the sustained engagement of TLR9 was associated with a substantial reduction of Aβ deposit in the brain and ameliorated cognitive functions in a mouse model of AD (Scholtzova et al. 2009). In addition, CD14 reacts with fibrillar $A\beta_{1-42}$ and promotes its phagocytosis (Liu et al. 2005). Microglial cells from AD brain indeed show elevated CD14 expression and polymorphism of the CD14 gene appears associated with increased AD risk (Combarros et al. 2005). In fact, macrophages from AD patients downregulate TLR3, TLR4, TLR5, TLR7, TLR9, and TLR10 expression upon Aβ stimulation (Fiala et al. 2007). Alteration of the equilibrium between Aβ production and removal, for instance, due to inefficient phagocytosis by macrophages/microglial cells, could be a key event in AD pathogenesis. In macrophages from AD patients, the trafficking of Aβ through endosomes and lysosomes appears in fact defective. The altered capacity of macrophages to recognize and clear Aβ could rely on a reduced TLR2 expression level. In line with this notion, the decline of cognitive functions is exacerbated under conditions of TLR2 deficiency (Richard et al. 2008). Hence, expression of innate immune receptors by macrophages and microglial cells appears as a mechanism to control Aβ accumulation within the CNS. Possibly, a defective engagement of such

receptors could cause a reduced capacity of microglial cells to eliminate extracellular neurotoxic material and contribute to the pathogenesis of AD and of other neurodegenerative disorders as well. The targeting of such receptors could be the basis of new therapeutic strategies for AD.

Even in nonpathological situations, peripheral infections can cause rapid microglial cell activation through the detection of pathogen-associated molecular patterns (PAMPs) present in the blood via the highly vascularized circumventricular organs (Rivest 2009) or through induced lipid mediators (Ek et al. 2001). The activation of microglial cells residing in the brain parenchyma is more progressive (Nadeau and Rivest 2000; Nguyen et al. 2002), and it also takes place upon brain injury and in condition of chronic pathology. Besides activation of resident microglia at sites of lesions, microglial cell precursors are recruited from the blood and activated (Soulet and Rivest 2008). Bone-marow-derived microglial cells associated with Aβ deposits limit the progression of the pathology by eliminating Aβ from the brain (Simard et al. 2006). In mice, neutralization of the TGFβ-dependent signaling pathway in peripheral macrophages ameliorated AD-like pathology. Within the brain parenchyma, the Aβ load was reduced and both cerebral vessels and Aβ deposits were surrounded by elevated numbers of Aβ-containing peripheral macrophages (Town et al. 2008). Patients with limited cognitive deficit or presymptomatic AD show low levels of macrophage colony-stimulating factor (M-CSF). Along with reduced levels of other hematopoietic cytokines, this status indeed predicts the rapid evolution of the disease towards dementia (Ray et al. 2007). In vitro, M-CSF promotes lysosome acidification and the processing of internalized Aβ in mouse microglial cells. In a spontaneous AD mouse model, the administration of M-CSF prevented the occurrence of learning/memory deficits or attenuated them in animals already affected. This correlated with an increased number of microglial cells and a markedly reduced Aβ level in the brain. Hence, bone-marrow-derived microglial cell precursors can effectively eliminate plaques. Facilitating the recruitment of such cells to the brain by modulating TGFβ signaling or by provision of M-CSF promotes Aβ elimination and ameliorates cognitive functions in diseased mice (Boissonneault et al. 2009; Simard et al. 2006; Town et al. 2008).

Altogether, these results suggest that AD treatment might benefit from the targeting of innate immune cells. However, a large number of studies have identified numerous detrimental effects of microglial cells and inflammatory molecules in AD. Proinflammatory cytokines and prostaglandins are produced in the brains of mice with AD-like disease and in AD patients. Inhibition of cytokine signaling (e.g., IL-1β) attenuate disease progression in animal models. Administration of exogenous cytokines and cyclooxygenase 2 overexpression in the brain increases plaque formation and accelerates cognitive impairment. In addition, antiinflammatory drugs are beneficial in animal models of AD (reviewed in Wyss-Coray 2006). Moreover, initial clinical trials involving the treatment of susceptible patients with non-steroidal anti-inflammatory drugs (NSAIDs) before the development of disease have indicated that inhibiting the immune response decreases the probability of developing the disease. Epidemiological studies have revealed that long-term treatments with NSAIDs may protect against and lower the risk of AD development.

The protective effect of antiinflammatory treatments could rely on the direct attenuation of microglial cell activation and/or on the reduction of Aβ production (Mackenzie and Munoz 1998; Weggen et al. 2001). However, more recently, the treatment of AD patients with antiinflammatory drugs was without effect on the decline of cognitive functions and was even deleterious in some cases (Martin et al. 2008). Thus, neuroinflammation can be both beneficial and deleterious in AD.

9.3.1.2 Complement System Activation in AD Pathogenesis

The complement system is a natural system of defense against pathogenic microorganisms composed of a large set of soluble and cell-bound proteins. The classical, lectin, and alternative pathways of complement activation induce factors that mediate inflammatory reaction (anaphylatoxins), chemotaxis of and clearance by, phagocytic cells (opsonins) as well as cytotoxicity (membrane attack complex [MAC]). Neurons, microglial cells, astrocytes, and oligodendrocytes all express components of the complement system. Many neurons also express complement receptors (CRs) such as receptors for anaphylatoxins (e.g., C5aR or C3aR).

Several lines of evidence indicate that the complement system is activated in the AD brain suggesting that it may play a role in the pathogenesis of AD or influence its evolution (reviewed in Alexander et al. 2008; Veerhuis et al. 2011). Various complement components including C1q, C1r, C1s, and C2 to C9 are known to be upregulated in AD brain and are expressed in pyramidal neurons. The alternative pathway of complement activation is activated in the frontal cortex of AD patients. C1q and cleavage product of C4 and C3 are associated with plaques and NFTs and MAC with dystrophic neuritis (Loeffler et al. 2008; McGeer and McGeer 2002; Veerhuis et al. 1996). Microglial cells from AD patients express components C1–C4, and around amyloid plaques activated microglial cells express CR3 and CR4 (Akiyama and McGeer 1990; Kobayashi et al. 1998) suggesting that these phagocytes are available for disposal of complement-coated material. The C1q transcript level is highly increased in brain areas that accumulate NFTs and plaques and correlates with the number of C1q-positive plaques in brain sections from AD. C1q is upregulated in areas with fibrillar Aβ in AD mouse models as well. Although alternative pathway products occasionally colocalize with plaques in both AD patients and mouse models, the components prominently associated with Aβ deposits and extracellular tangles in AD brain are activation products of the classical pathway (Fonseca et al. 2011; Strohmeyer et al. 2000). Finally, as we will see below, the association of AD with polymorphisms in clusterin/ApoJ (a complement inhibitor well expressed in AD brain) and CD35/CR1 (a regulator of phagocytosis) genes also suggests a role for complement in AD (Fonseca et al. 2011; Lambert et al. 2009; Strohmeyer et al. 2000).

Consistent with these observations is the fact that Aβ deposits directly interact with various complement factors. C1q is known to interact with $A\beta_{1-42}$ (Jiang et al. 1994; Kishore et al. 2003; Tacnet-Delorme et al. 2001). The C1q–Aβ interaction, which is influenced by the level of Aβ fibrillation, triggers phagocytes recruitment and activation (Guan et al. 1994; Webster et al. 1997). Nonfibrillar $A\beta_{1-42}$ induces

C1q-independent activation of C4 (Bergamaschini et al. 2001), and Fibrillar Aβ can activate the alternative pathway (Bradt et al. 1998; Itagaki et al. 1994).

Complement activation can exert positive effect during pathogenesis. AD mouse models revealed an exacerbated Aβ accumulation and neuronal degeneration upon C3 deficiency or C3 inhibition, suggesting a protective effect of C3 possibly through its opsonic effect for Aβ deposits (Maier et al. 2008; Wyss-Coray et al. 2002). Such a protective role is consistent with the fact that the induction of C1q and C3 is associated with the control of Aβ accumulation in other mouse models (Chakrabarty et al. 2010). The complement regulators, factor H and C4-binding protein, which are abundant in Aβ deposits coated with C4b and C3b, are likely to inhibit further activation and facilitate CR3/CR4-dependent Aβ internalization by microglia cells (Sjoberg et al. 2009; Strohmeyer et al. 2002). Thus, complement activation may protect against Aβ-induced neurotoxicity and may reduce Aβ accumulation and/or promote the clearance of Aβ and apoptotic neurons. In addition, animal studies have suggested that the anaphylatoxin C5a may protect from excitotoxicity via activation of the neuroprotective mitogen-activated protein (MAP) kinase. C5-deficient mice are indeed prone to hippocampal excitotoxic lesions (Loeffler 2004). Finally, the neuroprotective effects of complement activation might also involve oligodendrocyte regeneration and induction of nerve growth factor.

However, besides neuroprotective effects, complement activation might also show deleterious effects. In mouse models of AD, C1q deficiency led to a certain level of neuroprotection suggesting a relative detrimental role in disease evolution (Fonseca et al. 2004; Zhou et al. 2008). In line with this notion, disease development was accelerated when studied on a mouse background with high serum hemolytic activity (Fonseca et al.). In addition, the pharmacological inhibition of C5aR attenuates neuropathology and improves cognitive functions in mouse models (Fonseca et al. 2009). Functional C5aR is expressed within the cortex and various cells of the hippocampus (Farkas et al. 2003; Klos et al. 2009; Sayah et al. 2003). C5a-C5aR signaling also synergizes with signaling of TLRs among which TLR2 and TLR4 that can mediate detrimental effect of Aβ (Jana et al. 2008; Jin et al. 2008; Udan et al. 2008). C5a–C5aR interaction appears thus to promote the inflammatory response of glial cells and enhances neuropathology. Deregulated activity of complement inhibitors could also contribute to AD pathogenesis. In the hippocampus and frontal cortex of AD brain, unlike the terminal MAC factor C9 which is induced, CD59, which controls MAC, is reduced (Yang et al. 2000) possibly due to Aβ which negatively regulates CD59 transcript. Finally, via C1q, which increases reactive oxygen species level in neurons, the complement system also exacerbates the oxidative burst in the brain possibly aggravating neuron survival in AD (Luo et al. 2003).

9.3.1.3 T Lymphocytes in AD

Experiments from animal models have shown that activated T cells can enter the CNS. Such T cells are not retained in situ unless their antigen receptor (TCR) is able to react to antigens presented on MHC molecules and to cause accumulation and

differentiation. Tissue injury, neuroinfection, and neuroinflammation further promote the entry of activated T cells.

It appears that activated T cells specific for CNS antigens are capable of inducing the secretion of trophic factors by microglial cells that contribute to tissue repair and attenuate neurodegeneration (Schwartz and Kipnis 2005; Schwartz et al. 1999). For instance, differentiated T cells of the Th2 type secrete antiinflammatory cytokines and are potent inducers of insulin-like growth factor (IGF)1 secretion by microglial cells. In mouse models of AD disease, it was observed that the adoptive transfer of Aβ-specific T cells or GA (Copaxone)-treated T cells to diseased recipients could control the Aβ load, the neuron alteration, and ameliorate cognitive functions. The artificial induction of T cell entry within the CNS or immunization with Aβ could also limit/reduce Aβ accumulation (Butovsky et al. 2006; Ethell et al. 2006; Fisher et al. 2010; Frenkel et al. 2005).

9.3.2 Blood–Brain Barrier Functioning in AD

Sustained inflammation is commonly linked to pathologies characterized by vascular dysfunction. The occurrence of marked Aβ deposit could relate to an altered physiology of the BBB, the specialized anatomical structure associated with brain capillaries that controls molecular transfers in and out of the brain. Both vascular and BBB structures could be involved in the progressive extracellular accumulation of Aβ. Cerebral amyloid angiopathy (CAA) is often observed in AD and could play a role in its pathogenesis (Jeynes and Provias 2006; Weller et al. 2009). CAA is caused by Aβ deposits either within the cerebral vessels themselves or in the immediate perivascular spaces. Thus, under such conditions, Aβ accumulates at sites that should transport it into, or out of, the brain parenchyma or at sites that normally redirect Aβ into the systemic circulation or the CSF (Jeynes and Provias 2006). The question of how vasculopathy contributes to the pathogenesis of AD remains, nevertheless, open. Lipoprotein receptor-related protein (LRP), RAGE, and P-glycoprotein (P-gp) that are among the main protein transporters associated with both endothelial cells and BBB capillaries are studied in the context of a possible role for capillary CAA in sporadic AD pathogenesis. In fact, all three transporters are involved in bidirectional Aβ exchange: LRP and P-gp directs an efflux out of the brain, possibly on a cooperative mode, and RAGE permits an influx into the brain from the vascular compartment (reviewed in Jeynes and Provias 2011). Inhibition of P-gp increases $A\beta_{1-40}$ and $A\beta_{1-42}$ deposits in mice (Cirrito et al. 2005). Abnormal transporter activity may directly alter the exchange of Aβ across the BBB (Donahue et al. 2006; Pietrzik et al. 2004). In addition, LRP interacts with ApoE, a factor that is abundant in the CNS and plays a role in the clearance of Aβ (Corder et al. 1993). Dysregulation of ApoE expression could impact Aβ removal from brain parenchyma. In AD, it is plausible that neuron degeneration and/or microglia activation affects vasculature permeability and the efficiency of BBB functioning (Farrall and Wardlaw 2009; Popescu et al. 2009), for instance, via induction of NO or vascular growth factor (VEGF) that both influence microvasculature permeability.

9.3.3 Genetic Susceptibility to AD

In the case of familial AD, it has been established that various mutations of the genes encoding the precursor protein APP and the presenilin 1 and 2 γ-secretases cause AD through altered APP processing and deleterious Aβ deposition (Rovelet-Lecrux et al. 2006; Selkoe and Podlisny 2002). Some APP mutations directly promote Aβ production or increase the $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio, while others appear to augment the aggregation potential of Aβ and/or make it more resistant to clearance. It remains possible that some of the mutations also contribute to familial AD through alteration of the physiological roles of APP which are not elucidated. Presenilin gene mutations are also associated with a perturbed $A\beta_{1-42}$ to $A\beta_{1-40}$ ratio and facilitate early Aβ deposition. In fact, particular mutations affecting presenilin-1 represent a major cause of familial AD.

In the case of sporadic AD, a large part of the risk to develop disease is linked to genetic factors as well. Particular genes suspected or established to be genetic risk factors have been identified. Among these, the highest association was observed for the gene encoding ApoE. A particular allele (ApoE ε4) is associated with an increased risk for Aβ deposition in the brain, for exacerbated microglia activation and for AD development (Barger and Harmon 1997; Stefani and Liguri 2009). Indeed, ApoE is involved in the LRP-associated transport of Aβ through the BBB (Deane et al. 2008). The dysregulation of ApoE in the brain could thus impact on Aβ accumulation and contribute to plaque formation. A now enlarged list of additional risk factor genes include genes encoding ApoJ/clusterin, complement receptor CR1, Sorl1 (a regulator of the interaction of APP with secretases and a partner for ApoE), Tomm40 (a mitochondrial translocase that interacts with APP), Dyrk1a (that phosphorylates tau and is functionally regulated by Ab itself) Gsk3b (that is activated upon APP processing and further phosphorylates tau), tau itself (that shows altered capacity to bind microtubules upon mutation), phosphatidylinositol-binding clathrin assembly protein (PICALM), bridging integrator protein (BIN1), the sialic-acid-binding immunoglobulin-like lectin CD33, membrane-spanning 4A gene cluster (MS4A4A), CD2-associated protein (CD2AP), Ephrin receptor A1 (EPHA1), and ATP-binding cassette transporter (ABCA7) (Hollingworth et al. 2011; Naj et al. 2011 and for recent review see Ballard et al. 2011; Holtzman et al. 2011). Finally, polymorphisms in proinflammatory genes (IL-1, IL-6, TNF-α, and α1-antichymotrypsin) might also represent a risk factor, especially in association with ApoE ε4 (Alvarez et al. 2002; Kamboh et al. 1995; McCusker et al. 2001; Nicoll et al. 2000; Papassotiropoulos et al. 1999). How genetic factors combine with environmental factors to increase the risk for developing AD remains largely unknown.

9.3.4 Factors of Risk for AD

Besides family history, other parameters constitute risk factors (Ballard et al. 2011). Aging represents an obvious factor of risk. Among peoples older than 65, nearly 5% show symptoms consistent with sporadic AD development. Environmental risk factors are thought to play an important role in the occurrence of sporadic AD in aging

individuals (Khachaturian et al. 2004). Head injury with consciousness loss, chronic inflammatory diseases and infections (see below) can represent risk factors for AD development and in the case of infection, the risk becomes higher with increasing age (Casserly and Topol 2004). As suggested by studies in animal models, peripheral inflammation is susceptible to further increase the proinflammatory status of microglial cells that confront plaque formation and neuronal death (Perry et al. 2007). Thus, the concomitance of peripheral inflammation and other risk factors is likely to increase the risk. It is even possible that the various episodes of acute and/or chronic inflammation as well as of oxidative stress occurring during life set a particular threshold for inflammatory reaction that plays a role in the probability for aged individuals to develop AD. Peripheral blood mononuclear cells (PBMCs) from AD patients show an increased secretion of proinflammatory cytokines upon stimulation (Huberman et al. 1995; Reale et al. 2004) and subjects lacking signs of cognitive defects but displaying a high IL-1β/TNFα secretion profile have an increased risk of developing AD (Tan et al. 2007).

9.3.5 Infectious Agents Associated with AD

Infection of the CNS by microorganisms that are potent inducers of inflammation could play a role in the pathogenesis of sporadic AD that is thought to be multifactorial in nature. The analysis of postmortem brains revealed that the intracellular bacterium *Chlamydia pneumoniae* was associated with late-onset AD (see Balin et al. 2008 for review). *C. pneumoniae* was found within neurons, astrocytes, and microglia from AD patients, and the number of infected cells was elevated in *APOE* ε4 carriers relative to ε4-allele negative patients. The bacterium load was also increased in the frontal cortex area and hippocampus compared to the temporal cortex. Such an association suggests that *C. pneumoniae* infection could confer a higher risk for developing AD in individuals bearing the *APOE* ε4 allele. Interestingly, in the brain of nonmanipulated young mice, activated astrocytes are found at sites of amyloid deposits upon *C. pneumoniae* infection. *Helicobacter pylori* was also reported to present an association with AD development: its prevalence in the gastric mucous was higher in AD patients than in controls, and the titer of anti-*H. pylori* immunoglobulin G in the serum was also elevated (Kountouras et al. 2006). A third type of gram-negative bacteria, spirochetes, was suspected of an association with AD development (reviewed in Miklossy 2008). Spirochetes can be observed, sometimes in close contact to amyloid deposits or NFTs, in the brains of AD patients. *Borrelia burgdorferi* antigens were found to colocalize with plaques. In vitro, *B. burgdorferi* facilitates βAPP and hyperphosphorylated tau accumulation in glial and neuronal cells. *Treponema pallidum* was detected in a high number of AD patients. Other unidentified types of spirochetes were detected in the brain, CSF, and blood of AD patients. However, spirochetes were not observed in all patients tested and could be a contributing factor to AD only in some cases.

Herpes simplex virus type 1 (HSV1) can infect the human CNS latently and is capable of replicating in the absence of overt neurological lesions. Upon acute infection, the regions that are more affected by HSV1 are, like in the case of AD, the

hippocampus, and the frontal and temporal cortices. HSV1 DNA was, indeed, detected prominently in these same regions. With some exceptions, a number of studies pointed to an association between HSV1 and AD (reviewed in Itzhaki and Wozniak 2008). Elevated titers of antibodies directed to HSV1 were observed in patients with neurological disorders, and the HSV1 genome could be detected in the brain of patients with dementia or AD. In animal models, HSV1 infection was associated with sustained inflammation and oxidative stress in the brain regardless of whether the infection was acute or latent. Latent infection with HSV1 could thus contribute to the chronic inflammatory context that is associated with senile plaque formation in the brain of patients with AD. Indeed, in mice, Aβ deposits are detected in the brain after HSV1 infection, and the levels of BACE and nicastrin (a γ-secretase component) were found increased in neuronal and glial cells in vitro. Other studies support a link between HSV1 and AD pathogenesis. In tissue culture, HSV1 infection triggered tau phosphorylation at sites that are known to be phosphorylated in AD, and both glycogen synthase 3β and protein kinase A enzymes were found upregulated (Wozniak et al. 2009). In addition, HSV1 DNA localized precisely within amyloid plaques in brain sections (Wozniak et al. 2009). It is possible that the aging immune system becomes more permissive for CNS infection by HSV1 because HSV1 DNA was rarely detected the brain or CSF in young individuals. HSV1 infection is in fact considered a risk factor for AD development in *APOE* ε4 allele positive individuals (Honjo et al. 2009). In such individuals, latent forms of HSV1 could, upon reactivation, promote amyloid plaque formation through exacerbated inflammation. Thus, HSV1 by itself, does not appear to constitute an independent factor of risk for late onset AD. Nevertheless, in conjunction with other factors, HSV1 might play a role in AD pathogenesis by initiating/sustaining inflammatory processes within the hippocampus and the frontal and temporal cortices.

Although it might not represent an independent cause of AD, HHV6 is another pathogen capable of promoting brain inflammation and lesions. HHV6 is detected in a sizable proportion of AD patients and is codetected with HSV1 in a majority of cases. CSF antibodies directed to HHV6 were detected in a substantial fraction of AD patients (Honjo et al. 2009).

Finally, *Toxoplasma* infection that can be found associated with various types of neurological disorders could also contribute to AD pathogenesis. This is suggested by the elevated levels of anti*Toxoplasma gondii* IgG present in the serum of AD patients (Kusbeci et al. 2011).

9.3.6 *Attempts to Modulate β-Amyloid Deposition by Means of Immuno-Intervention*

It has been hypothesized that removal of the Aβ load from the brain by the immune system could help to limit neuron loss in AD (antiamyloid vaccine approach). The vaccination of human APP transgenic mice with $A\beta_{1-42}$ efficiently induced antibodies against Aβ and reduced Aβ deposition (Schenk et al. 1999). Passive immunization with antiAβ antibodies also lowered Aβ deposit suggesting that vaccine effects

involved the induction of Aβ-specific antibodies (Bard et al. 2000; Levites et al. 2006). Both Aβ opsonization by specific IgG and phagocytosis by microglial cells could operate in the control of Aβ deposit. Due to occurrence of meningoencephalitis symptoms, an antiAβ vaccine trial in humans had to be stopped. Postmortem examination revealed marked plaque clearance within the brain. Microglial cells did contain Aβ material suggesting active phagocytosis. Nevertheless, no positive impact on cognitive decline was noted and the almost complete plaque elimination did not prevent evolution to marked dementia (Bayer et al. 2005; Gilman et al. 2005; Holmes et al. 2008; Nicoll et al. 2003, 2006). Although antiAβ antibodies induced by $A\beta_{1-42}$ vaccination were associated with a Th2 response in animal models, the postmortem examination of vaccine-treated patients revealed that proinflammatory Th1 T cell reactions could be detected in the CNS in some cases and could be involved in meningoencephalitis induction (Nicoll et al. 2003, 2006). Choosing $A\beta_{1-42}$ domains unlikely to be targeted by T cells or adjuvant that do not promote Th1 type of responses might help to design safer vaccines. Passive immunization might also help to prevent Th1-associated inflammation (Bard et al. 2000). In vitro, opsonization of Aβ with antiAβ IgG increased microglial cell chemotaxis and Aβ phagocytosis. The NSAID indomethacin reduced the concomitant secretion of proinflammatory factors without affecting Aβ phagocytosis suggesting that Aβ vaccine might benefit from the addition of antiinflammatory compounds. Hence, clinical trials are currently less promising than expected possibly because neurodegeneration is already advanced when dementia is observed and the removal of Aβ remains without effect on cognitive deficit. An alternative approach could rely on the infusion of natural antiAβ antibodies isolated from healthy donors. In phase I clinical trials, such transfers attenuated the alteration of cognitive functions for most AD patients (Dodel et al. 2004; Relkin et al. 2009).

9.4 Summary

MS, like many autoimmune diseases is complex and is filled with more questions than answers. MS dates back to the fourteenth century. Yet to date, we are no closer to identifying the events that initiate this disease mainly due to its heterogenous nature. Importantly, we are also no closer to finding curative treatments that will halt disease progression and/or initiate remyelination or axonal repair. The EAE model, although not perfect has been instrumental in providing a better understanding of the pathogenic mechanism of the immune response in neuroinflammation and neurodegeneration of MS. EAE has also led to several treatment modalities currently used in MS therapy. One consistent theme of this disease is that inflammation is an important pathogenic factor.

Regarding AD, it becomes clear that neuroinflammation can both positively and negatively influence the pathogenesis of AD, in particular, and of neurodegenerative disorders, in general. While inflammation can acutely clear neuroinfections and promote CNS tissue repair, it can promote neurodegenerative processes when

maintained at enforced levels. In this context, the activation of microglial cells play a particularly important role. Such an activation is triggered by aggregated proteinaceous material and cellular debris and is further exacerbated by various factors such as prostaglandins, proinflammatory cytokines, chemokines, proteases, or free radicals. As to activation of the complement system, studies in AD mouse models also point to a complex role in neurodegeneration. Opsonins can drive effective plaque clearance but the proinflammatory effects of both anaphylatoxins and MAC appear to exacerbate neurodegeneration as the disease progresses. Thus, uncontrolled activation of the complement system enhances microglia cell activation, proinflammatory factor secretion, and induction of oxidative products.

9.5 Recommendations

There is no doubt that the immune response plays a critical role in MS pathogenesis. Some emerging studies aimed at modulation of the immune response show promise. Previous studies in mice show that modulation of the local immune response in the skin can induce tolerance in myelin antigen-specific T cells (Bynoe et al. 2003). Recently, similar studies were performed in MS patients in a small clinical trial in Poland that led to remission of MS symptoms in a significant number (80%) of MS patients. These studies show promise in proving an alternative safe, noninvasive, and effective treatment for MS patients in the future. Another area of emerging research indicates that adenosine receptor modulation may be a viable alternative in blocking immune cells entry into the CNS. Likewise, this approach may be a one-size-fits-all MS patients, as the major pathogenic factor in MS is the entry of immune cells into the brain and the pathology they cause.

We have seen that, although no individual pathogen appears able to directly cause sporadic AD, a combination of neuroinfection with genetic factors or other risk factors may contribute to sporadic AD pathogenesis through sustained inflammatory and oxidative responses. Further investigations are needed to dissect the suspected association between infectious agents and AD. If infections can play a key role, understanding such a role would certainly impact the prevention and/or treatment of AD-associated neuropathology. Therapeutic approaches for the modulation of complement system-mediated inflammation in AD patients warrant further studies. Ideally, acting on downstream events might help to attenuate the deleterious effects of full complement activation while sparing the neuroprotective effects of certain factors such as C1q and C3. Although the status of T cells in AD pathogenesis is poorly characterized, studies from animal models of AD suggest that T cell adoptive transfer could represent a novel approach for immuno-intervention in AD and should be explored as well.

In conclusion, various components of the immune system appear to modulate both positively and negatively the cascade of events involved in AD pathogenesis. Along with other aspects not covered in this chapter (such as neuronal calcium homeostasis or autophagic pathway), the complex role of the immune system in AD

certainly represents a pertinent target for therapeutic interventions aimed at the prevention and treatment of the disease.

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Chapter 10
Immune Dysfunction in Autism Spectrum Disorders

Milo Careaga, Judy Van de Water, and Paul Ashwood

Abstract Autism spectrum disorders (ASDs) are complex and heterogeneous with a spectrum of diverse symptoms. Current estimates suggest that ASD affects approximately 1% of children and is a major public health issue. Despite decades of research, little is known about factors that contribute to the pathogenesis of the disorder, although both genetic and environmental factors have been implicated. A number of published findings have identified numerous immune abnormalities in subjects with ASD and their families, both at the systemic and cellular levels. In addition, genetic studies have also uncovered a number of candidate genes that link immune function with ASD. Collectively these findings point to a pivotal role for immune-dysregulation in the pathogenesis of ASD. A better understanding of the involvement of the immune response on early brain development and how this is altered in ASD will have important therapeutic implications.

Key Points

- Strong epidemiological evidence suggests a link between immune dysregulation and autism spectrum disorders (ASDs).
- Studies show persistent immune dysregulation in children with ASD relating to abnormal behaviors.
- Genetic studies in ASD point to a number of genes as risk factors for ASD involved in immune regulation.

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10.1 Historical Links of Immune Dysfunction and ASD

The etiology of autism spectrum disorders (ASDs) has remained a mystery since Leo Kanner first described the syndrome in 1943 (Kanner 1968). However, as early as 1971, a relationship between ASD and immune dysfunction was proposed by Money et al. (1971) who reported a familial link of polyendocrine autoimmune disorder with ASD. Since these early observations, however, the role for immune dysfunction in ASD has remained controversial both in terms of its existence as well as its role in both the etiology and pathology of the disorder.

Several epidemiology studies have sought to determine if a familial link exists between ASD and immune dysfunction. These studies have ranged from small cohort studies to large population-based studies and have relied on varying methods, including both self-report and medical record searches. The specific nature of the immune disorder assessed in these studies has varied greatly and has made direct comparisons between the various studies difficult. In addition, the relatively low rate of autoimmune disorders in the general population (Cooper et al. 2009) makes it difficult to link specific autoimmune diseases to ASD in smaller studies. Despite these limitations, a number of immune disorders and autoimmune diseases have been repeatedly reported in families of subjects with ASD including: asthma and allergy (Croen et al. 2005), celiac disease (Atladottir et al. 2009; Valicenti-McDermott et al. 2008), type-1 diabetes (Atladottir et al. 2009; Croen et al. 2005; Keil et al. 2010; Mouridsen et al. 2007), autoimmune thyroid disease (Molloy et al. 2006; Sweeten et al. 2003a), rheumatoid arthritis (Atladottir et al. 2009; Comi et al. 1999; Valicenti-McDermott et al. 2008), and rheumatic fever (Keil et al. 2010; Comi et al. 1999). Together, these studies point to abnormal immune function in the families of children with ASD, suggesting a probable role for immune dysfunction in the pathology ASD.

Surprisingly, many of the studies showed increased rates of immune dysfunction in both mothers and fathers of children with ASD (Comi et al. 1999; Sweeten et al. 2003a; Molloy et al. 2006; Atladottir et al. 2009; Keil et al. 2010). This would suggest that immune dysfunction in parents alludes to shared genetic or physiological pathways between these disorders and ASD, or specific endophenotypes of ASD. For example, children diagnosed with a regressive form of ASD compared with children who exhibited an early onset form of ASD have increased rates of familial autoimmune thyroid disease (Molloy et al. 2006). Further identification of specific endophenotypes of ASD that are linked with autoimmunity/immune dysfunction will help to elucidate the mechanisms involved in the disorder.

10.2 Maternal Immune Activation

Pregnancy involves a unique and complex dynamic for the immune system: protection of the mother from pathogens/infections, as well as support and acceptance of fetal tissue that contains many "nonself" paternal antigens. Uterine NK cells (uNKs) together

with macrophage and dendritic cells predominate the human maternal–fetal interface and promote fetal development (Mor and Cardenas 2010). However, because of the complexity of regulating this balance between immune tolerance and protection from pathogens, pregnancy represents a period of vulnerability to immune insult not only for the mother but also for the developing fetus. Both epidemiological and animal studies suggest immune dysregulation during this vital period can result in altered development and lead to an increased risk for the development of neurodevelopmental disorders such as ASD.

One such risk factor that would lead to an altered gestational immune profile is that of maternal infection during pregnancy, which has been suggested as a risk factor for ASD. Early studies showed casual links between maternal infection and ASD through case reports and small comparative studies (Chess 1977; Libbey et al. 2005; Sweeten et al. 2004). A recent population-based study using Denmark's medical registry, from the period of 1980–2005 and consisting of over a million children, found that an increased rate of mothers of children with ASD were hospitalized for viral infections in the first trimester and bacterial infections in the second trimester compared with mothers of typically developing children (Atladottir et al. 2010). Most maternal infections, however, do not result in the development of ASD in offspring, suggesting other factors such as a genetic susceptibility may be involved. In individuals with known genetic disorders that are associated with high rates of ASD, such as tuberous sclerosis (TCS) which has a 25–50% rate of ASD (Wiznitzer 2004), associations are found with season of birth and the incidence of ASD such that the peak flu season during the later stages of pregnancy is associated with the development of ASD (Ehninger et al. 2012).

Animal models support the theory that maternal immune activation can alter neurodevelopment and promote the development of neurodevelopment disorders (Smith et al. 2010). Using both viral and bacterial analogs, researchers have demonstrated that maternal immune activations result in behavioral changes in offspring and that a generalized immune response rather than a pathogen-specific response is involved (Borrell et al. 2002; Gilmore et al. 2005). Although it is not fully understood how immune activation affects fetal development, it is believed that activation of the maternal immune system leads to changes either in the placenta or directly in the fetal brain, which alter neurodevelopment (Hsiao and Patterson 2011; Meyer et al. 2008), potentially affecting progenitor cells. Although numerous cytokines are induced during maternal immune activation, interleukin (IL)-6 appears to be one of the major mediators of these changes in the animal models. Using IL-6 knock out mice or blocking IL-6 with an antibody has been shown to be protective in maternal immune activation models (Smith et al. 2007). Genetic factors may further contribute to the neuropathology in offspring during maternal immune activation. Mice with disruptions to genes associated with altered neurodevelopment, such as DISC1 or TSC1, appear to show a more exaggerated behavioral phenotype with greater impairments in social interaction after maternal immune activation (Abazyan et al. 2010; Ehninger et al. 2012). The effects of maternal immune activation are not limited to altered neurodevelopment in offspring and appear to result in ongoing immune dysfunction in the offspring as well (Lasala and Zhou 2007; Surriga et al. 2009;

Mandal et al. 2010). It is currently unclear whether the immune dysregulation contributes to the behavioral endophenotype seen in offspring, but increased dysregulation of immune cells as well as altered cytokine and chemokine profiles, have been linked to more severe behaviors in children with autism (Ashwood et al. 2011a–c; Enstrom et al. 2010; Heuer et al. 2008; Sandler et al. 2000).

10.3 Maternal Antibodies

The maternal transfer of antibodies from the mother to child during pregnancy is well documented and confers protection from a wide range of infectious agents to the fetus. However, along with antibodies that provide immunoprotection, antibodies that are immunoreactive to fetal "self" proteins will also cross the placental barrier and can affect neonatal outcome (Tincani et al. 2005). For example, the transfer to the fetus of maternal anti-Ro/SS-A and anti-La/SS-B antibodies in mothers with systemic lupus erythematosus (SLE) cause neonatal lupus syndrome, often leading to congenital heart block (Lee et al. 2009; Neri et al. 2004; McAllister et al. 1997; Tincani et al. 2006). Neurotoxic autoantibodies from patients with SLE transferred into pregnant mice result in abnormal brain development in offspring (Lee et al. 2009). In addition, abnormal thyroid function caused by placental transfer of maternal antithyroid antibodies is often seen in infants born to mothers with Hashimoto's thyroiditis or Graves' disease (Fu et al. 2005), and some cases of neonatal antiphospholipid syndrome (APS) are thought to occur due to the transfer of maternal autoantibodies from mothers with primary APS (Rolim et al. 2006). The presence of autoantibodies directed against critical neuronal components of fetal brain extracts in a subset of mothers of ASD children suggests that a possible similar mechanism may occur in ASD (Braunschweig et al. 2008; Silva et al. 2004; Zimmerman et al. 2007). The reactivity of these antibodies is to fetal and not adult brain proteins and is observed in approximately 12% of mothers of children with ASD, but not mothers of children who are typically developing or mothers of children who have developmental disorders other than ASD (Braunschweig et al. 2008).

The ASD-specific nature of these antibodies is intriguing and warrants further study. One possibility is that they bind to their neuronal targets during development, thereby interfering with, or altering neurodevelopment. Although it is hard to recapitulate exactly the core features of ASD in animal models, certain behaviors associated with ASD such as repetitive behaviors, difficulty in learning, and hyperactivity can be replicated (Klauck and Poustka 2006). In one model the transference of IgG isolated from mothers with children with ASD into Rhesus macaque monkeys during mid-gestation resulted in increased stereotypical behavior and hyperactivity in the offspring, which were not observed in monkeys that received IgG from mothers of typically developing children or monkeys that were saline treated (Martin et al. 2008). Evidence of a potential role for these antibodies and altered neurodevelopment was also observed in a murine model (Singer et al. 2009). Whether these

behavioral changes are directly related to ASD or are themselves distinct phenomena is not clear. These models suggest that antibodies isolated from mothers with children with ASD may alter the course of early neurodevelopment leading to changes in behavior in the offspring. Future studies will need to better characterize the behavioral changes evoked by these antibodies and their relevance to core features or symptoms of ASD as well as the potential cellular targets of the antibodies. Although the fetal reacting autoantibodies are present in only a subset of mothers who have children with ASD, the significance of carrying these antibodies may be substantial (Braunschweig et al. 2008) and presents an exciting avenue for screening and/or therapy.

10.4 Altered Cytokine Production

The immune system interacts with the central nervous system (CNS) during development and continues to do so throughout life. Cytokines, small molecules used as the messengers of the immune system, have been shown to interact with neurons and help to shape their differentiation, proliferation, migration, and synaptic plasticity (Deverman and Patterson 2009). This interaction occurs either directly or through endocrine intermediates (Silverman et al. 2005). The neuroimmune systems work by maintaining an intricate balance, and dysregulation in one system often results in dysregulation of the others (reviewed by Schwartz and Kipnis 2011).

Increased immune activation is associated with a number of neurodegenerative disorders and is speculated to play a role in psychiatric disorders such as schizophrenia (Strous and Shoenfeld 2006), obsessive compulsive disorder (Murphy et al. 2010), depression (Dantzer et al. 2008), bipolar disorder (Eaton et al. 2010), Gilles de La Tourette syndrome (Murphy et al. 2010), and ASD (Careaga et al. 2010; Enstrom et al. 2009c; Goines and Van de Water 2010). Elevated levels of inflammatory cytokines in the CNS could reflect ongoing inflammatory processes affecting neuronal function. Increased levels of proinflammatory cytokines such as IL-6, TNF-α, and MCP-1 in brain specimens and cerebral spinal fluid (CSF) obtained from young and older individuals with ASD (age range 5–44 years) suggest that an active neuroinflammatory process is ongoing in ASD (Vargas et al. 2005; Li et al. 2009). Studies that have assessed cytokine levels in the periphery have often shown similar increases in proinflammatory cytokines (Ashwood et al. 2004, 2011a; Jyonouchi et al. 2001; Singh et al. 1991, 1996). Inflammatory processes are kept in check by the release of cytokines that have antiinflammatory or regulatory properties such as IL-10 and TGFβ1. In ASD, production of IL-10 (Ashwood et al. 2004; Ashwood and Wakefield 2006) and circulating levels of TGFβ (Ashwood et al. 2008; Okada et al. 2007) are decreased. These data suggest that a shift has occurred toward a more proinflammatory state in ASD. Furthermore, in a recent, large population-based case–control study increased plasma cytokines levels were associated with late onset of symptoms and more impaired communication and aberrant behaviors (Ashwood et al. 2011a). However, the literature on cytokines and ASD is not always

consistent and likely reflects complex patterns of immune activation among different subgroups of individuals with ASD. The data is further complicated by the use of siblings of children with ASD as controls as these siblings are themselves often on a broader autism phenotype (Constantino et al. 2010). In addition to behavioral similarities in siblings of children with ASD, researchers have demonstrated that unaffected sibling often have immune profiles more similar to children with ASD than controls (Saresella et al. 2009).

10.5 Innate Immune Abnormalities

The immune system is generally described as having two arms: the innate immune system, which uses genetically encoded receptors and nonspecific mechanisms to defend the host, and the adaptive immune system, which responds to a particular threat and develops memory for future insults. These two systems work together to protect the host and maintain proper homeostasis.

In ASD, a number of studies have demonstrated abnormalities in innate immune function. Notable findings include alterations in natural killer (NK) cell activity. NK cells play an important role in viral defense, tumor surveillance, and help maintain the uterine environment during pregnancy. Early observations showed that NK cell-mediated killing of the target cell K562 were lower in some individuals with ASD (Warren et al. 1987). Similar results were found in a larger multisite study, with nearly half of the ASD subjects being found to have low NK cell cytotoxicity activity against the target cell (Vojdani et al. 2008). A more in-depth investigation into NK cell function in ASD demonstrated that NK cells from subjects with ASD are actually more activated in ASD, potentially reflecting their in vivo status, but when these cells are stimulated in vitro in cultures with K562 cells, they fail to respond (Enstrom et al. 2009b). This feature has also been described in several autoimmune diseases and suggests that the immune cells may be maximally stimulated in vivo and cannot respond further once challenged. In addition to NK cells, several studies have reported differences in monocytes and macrophages in ASD. Sweeten et al. reported increased numbers of circulating monocytes in subjects with ASD (Sweeten et al. 2003b). Moreover, plasma cytokine levels in children with ASD compared with controls show higher levels of IL-1β, IL-6, and IL-12, cytokines produced predominantly by cells of the myeloid lineage, i.e., monocytes and dendritic cells (Ashwood et al. 2011a). Jyonouchi and colleagues found that peripheral blood mononuclear cells (PBMC) stimulated with the innate immune activating TLR-4 agonist LPS released more IL-1β in ASD, further suggesting involvement of monocytes and or circulating dendritic cells in ASD (Jyonouchi et al. 2002). In a subset of ASD patients with recurrent infections, altered responses to a number of TLR ligands were also demonstrated (Jyonouchi et al. 2008). These studies, however, used mixed cultures of peripheral cells, and it is unclear if the altered response was from monocytes alone or from a more complex interaction between monocytes and other cells including adaptive immune cells. By using only isolated monocytes,

Enstrom et al. attempted to better elucidate innate immune findings in ASD and found that IL-1β, IL-6, and TNF-α responses were increased following TLR-2 stimulation, and IL-1β production was increased following TLR-4 stimulation in children with ASD, but not typically in developing controls. However, monocytes isolated from subjects with ASD showed a reduced production of IL-1β, IL-6, GM-CSF, and TNF-α after TLR-9 stimulation (Enstrom et al. 2010). This abnormal innate immune response might explain the increased rate of early childhood (first 30 days) infections seen in children with ASD compared with typically developing controls (Rosen et al. 2007). During this early postnatal period, the adaptive immune system is still being educated and the body is most reliant on the innate immune system for host defense. This inappropriate immune response to pathogens might contribute to symptomology of ASD through direct interactions between immune messengers and neuronal or neuroendocrine targets, or more circuitously by modulating the adaptive immune response.

10.6 Adaptive Immune Abnormalities

Cellular studies in ASD have also demonstrated functional alterations in the adaptive immune response. Early work by Stubbs was the first to suggest irregularities in cellular immune function in ASD; however, this early study was small and poorly controlled (Stubbs 1976). Further work was performed by Warren et al., who found T-cell abnormalities including skewed CD4:CD8 T-cell numbers and altered antigen recall responses (Warren et al. 1986). More recent work has shown the same decreases in T-cell numbers, but only in children with ASD that also have gastrointestinal issues (Ashwood et al. 2003) that may reflect the efflux of T cells from the periphery into the mucosa in this subset of ASD individuals. Cellular markers show that there is an altered T cell activation in children with ASD, suggesting an incomplete or altered activation profile (Ashwood et al. 2011b; Ashwood and Wakefield 2006; Plioplys et al. 1994). A skewing towards a T_H2 cytokine profile has been hypothesized for ASD (Gupta et al. 1998). However, as is true for most human research, the pattern of cytokines produced in T cells in ASD is complex and does not easily fit into the traditional T_H2 classifications. In a recent study, dynamic T-cell function following stimulation with PHA was assayed in 63 children with ASD and 73 age-matched typically developing controls. After stimulation, levels of GM-CSF, TNF-α, and IL-13 were increased, and IL-12 (p40) was decreased in subjects with ASD compared with controls. Furthermore, the induced cytokine production was found to be associated with altered behaviors in children with ASD, such that increased proinflammatory or T_H1 cytokine production was associated with greater impairments in core features of ASD as well as aberrant behaviors (Ashwood et al. 2011a). By contrast, production of GM-CSF and T_H2 cytokines was associated with better cognitive and adaptive function (Ashwood et al. 2011b). Taken together, these findings suggest that altered T-cell activation and function are present in ASD and likely play a role in the ongoing pathophysiological process.

Currently, no studies exist that have directly studied B-cell function in ASD; however, there are several lines of indirect evidence that suggest altered B-cell dysfunction is present in individuals with ASD. The primary role of B-cells is the production of immunoglobins against pathogens. In order to best protect the host, B-cells produce a variety of immunoglobins, each with a dedicated role and in some cases specificity to a particular tissue. IgA is present mainly in mucosal tissue where it serves as a sentinel against pathogens. Deficiencies in IgA are associated with increased infections (Aghamohammadi et al. 2009). In ASD, lower circulating levels of IgA have previously been reported (Warren et al. 1997); however, more recent studies have not validated this finding (Enstrom et al. 2009a; Croonenberghs et al. 2002; Heuer et al. 2008). Instead, reduced production of the IgM and IgG classes of immunoglobins has been reported, with lower levels also found to correlate with more aberrant behaviors (Heuer et al. 2008). Analysis of specific IgG subclasses in children with ASD has found that although total levels for IgG are low, the less abundant IgG2 subtype (Croonenberghs et al. 2002) and IgG4 subtype (Croonenberghs et al. 2002; Enstrom et al. 2009a) are elevated in ASD compared to controls. Interestingly, IgG2 is important for immune responses to carbohydrate antigens and increases in this subtype of IgG might suggest increased exposure to specific pathogens. IgG4 functions as a blocking antibody and is associated with chronic infection. It has also been shown to function as a pathogenic antibody in certain autoimmune disorders (Qaqish et al. 2009). In addition to altered immunoglobulin levels, a number of autoantibodies reactive to brain and CNS tissue have been identified in ASD.

10.7 Autoantibodies in ASD

The presence of antibodies directed against fetal brain or CNS tissue but not adult brain tissue has been repeatedly reported in children with ASD (reviewed in Enstrom et al. 2009c; Wills et al. 2007). Several studies have reported that subjects with ASD exhibit autoantibodies directed against specific targets, including serotonin receptors (Todd and Ciaranello 1985), brain-derived neurotrophic factor (BDNF) (Connolly et al. 2006), myelin basic protein (MBP) (Connolly et al. 2006; Singh et al. 1993), neuron–axon acidic protein (NAFP) (Singh et al. 1997), glial fibrillary acidic protein (GFAP) (Singh et al. 1997), and gliadin (Vojdani et al. 2004). A number of unidentified neuronal targets have also been detected and appear to be associated with certain behavioral endophenotypes in ASD. Antibodies specific to a 45-kDa cerebellum protein were found more frequently in children with ASD and were associated with lower adaptive and cognitive function, as well as increases in aberrant behaviors (Goines et al. 2011; Wills et al. 2007). However, while it is unclear if any of these autoantibodies have direct pathogenic relevance, or are secondary to previous cellular damage or inflammatory reactions, it is clear that these antibodies are not present in all individuals with ASD (Morris et al. 2009). Moreover, the specificity of these antibodies is not the same across studies,

and there has been difficulty in replicating studies showing antibodies to specific targets such as MBP or GFAP (Libbey et al. 2008; Kirkman et al. 2008). This lack of specificity and the diverse array of antibody specificities may suggest that the antibodies are generated as a consequence of some previous indiscriminate damage that reveals different brain or CNS targets leading to the generation of different antibodies that varies from individual to individual. Alternatively, the presence of these autoantibodies could reveal a subgroup of children with ASD that are more prone to immune dysfunction.

10.8 Mucosal Immunity in Autism

Reports of gastrointestinal (GI) symptoms in a significant number of individuals in ASD are becoming more abundant. Among the more frequently reported GI symptoms are an increase in the number of food insensitivities/food allergies, which has led some investigators to focus on immunological responses to dietary proteins such as gluten and casein. Jyonouchi et al. (2002) found that PBMC from children with ASD responded by producing higher levels of inflammatory cytokines in response to gliadin, cow's milk protein, and soy compared with PBMC from typically developing children. However, children in this study were selected on the basis of having previously seen behavior improvements on a restricted diet and may translate to a specific subgroup of ASD that have specific GI symptoms. Other studies have shown that, in children with ASD who have GI symptoms, there is increased pan-enteric infiltration of T-cells, monocytes, NK cells, and eosinophils suggesting a subtle but extensive mucosal immune activation (Torrente et al. 2002, 2004; Ashwood et al. 2003; Furlano et al. 2001). Isolation of lamina propria mononuclear cells showed that under basal conditions there was T-cell activation with increased TNF-α but lower IL-10 production compared with noninflamed GI symptom controls and children with celiac disease or inflammatory bowel diseases (Ashwood et al. 2003, 2004; Ashwood and Wakefield 2006). Of note, antibodies directed against gut epithelium were also observed in children with ASD but not in controls (Torrente et al. 2002, 2004). These autoantibodies may represent nonspecific inflammation, but they may also represent an autoimmune response directed at the epithelial barrier that could lead to increased permeability. Other studies also suggest that the epithelial barrier may be perturbed and have shown increased gut permeability (de Magistris et al. 2010; D'Eufemia et al. 1996). However, there remains considerable uncertainty and controversy as to the prevalence of GI symptoms in individuals with ASD. Current estimates of GI symptoms in ASD range from 20 to 70%; however, whether the GI symptoms relate to clinically defined GI pathology in individuals with ASD, or how GI symptoms may relate to core features of ASD or associated behavioral symptoms is not known. A few small studies suggest that GI symptoms in ASD may be related to increased symptoms of aggression and hyperactivity and that in some cases individuals with ASD may benefit from immune therapies targeting GI issues in order to resolve activated mucosal immune responses (Adams et al. 2011; Buie et al. 2010).

In a recent case report, a case of celiac disease was mistaken for ASD. Upon removal of gluten from the patient's diet and implementation of a modified diet, behaviors improved (Genuis and Bouchard 2010). Studies like these suggest that an abnormal immune response/immune-mediated food sensitivity, in this case to a dietary protein, could contribute to behavioral abnormalities associated with ASD.

10.9 Immunogenetics in ASD

Numerous genes have been implicated in the development of ASD, and, not surprisingly, many of the genes are associated with the control of immune function. Among these, HLA genes have been implicated most often (Stubbs and Magenis 1980; Daniels et al. 1995; Lee et al. 2006; Torres et al. 2002; Warren et al. 1992). The HLA genes, found on chromosome 6, are located within a large genomic region referred to as the major histocompatibility complex (MHC). The HLA genes are involved in immune function, are among the strongest predictors of risk for autoimmune conditions, and have been associated with neurodevelopmental disorders such as schizophrenia and ASD (Fernando et al. 2008). Several studies have found that a number of HLA haplotypes, in particular HLA-DR4, occur more often in children with ASD compared to the general population (Daniels et al. 1995; Lee et al. 2006; Torres et al. 2002; Warren et al. 1992). However, a study by Guerini et al. (2009) found that there was no apparent HLA linkage, and, instead, there are several microsatellite linkages within the MHC which are associated with ASD. This suggests that genetic abnormalities in the MHC are not solely confined to HLA genes themselves and other genes in the region may be indicated.

Another gene associated with ASD in the MHC region is the gene coding for the complement protein C4. Complement is an important element of innate immunity and is vital in protecting the host from a number of infectious agents. In ASD, deficiencies in the C4B allele, as well as its protein product, have been reported (Odell et al. 2005; Warren et al. 1994, 1995). Furthermore, proteomic analyses of sera samples indicate that several complement proteins are differentially produced in ASD (Corbett et al. 2007).

In addition to the genes in or near the MHC region, a number of genes associated with immune function have been implicated in ASD as well. These include macrophage migration inhibitory factor (MIF) (Grigorenko et al. 2008), MET tyrosine receptors (Correll et al. 2004; Campbell et al. 2006), serine and threonine kinase C gene PRKCB1 (Lintas et al. 2009), protein phosphatase and tensin homolog (PTEN) (Herman et al. 2007), and reelin (Serajee et al. 2006; Zhang et al. 2002; Skaar et al. 2005). Many of these are involved in key pathways such as the mTOR/Akt pathway utilized by both the nervous and immune system. Several disorders with inordinately high rates of ASD have known gene mutations within this pathway, which could contribute to the immune dysfunction seen in ASD (reviewed by Careaga et al. 2010). However, it is currently unclear how dysregulation of these pathways contributes to the development of ASD, or to what level immune dysfunction is involved.

10.10 Summary

Our understanding of ASD is still very limited, but decades of research have given many clues to the pathology of ASD. Current research into the role of the immune system in appropriate neuronal development and function suggests that findings of immune dysregulation in ASD, both in the children and parents, indicate that there is a strong immune aspect of the disorder in at least a subpopulation of individuals. However, the exact role of the immune system in ASD remains to be defined. Epidemiological and animal model studies suggest that maternal immune activation might be involved in the initiation of the disorder, and evidence of persistent immune dysregulation in children with ASD indicate a possible role for continued immune involvement in the pathology of ASD. Future work will help further clarify the role of the immune system in ASD, as well as in identifying potential targets for therapies.

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Chapter 11
Atherosclerosis and Inflammatory Heart Disease

DeLisa Fairweather

Abstract Cardiovascular disease (CVD) is the leading cause of mortality worldwide, with atherosclerosis accounting for most deaths. Inflammation is a key component in the pathogenesis of CVDs such as atherosclerosis, stroke, myocarditis, dilated cardiomyopathy, and heart failure. A number of recent clinical and animal model findings suggest that the pathogenesis of autoimmune and CVDs may be linked. Many potentially modifiable risk factors like diet, tobacco smoking, and infections have been associated with increased cardiovascular risk for both atherosclerosis and several autoimmune diseases including myocarditis, systemic lupus erythematosus, and rheumatoid arthritis. Although many autoimmune diseases are more prevalent in women, the risk for developing CVD remains elevated in men. Future studies should examine sex differences in CVD biomarkers following infection and/or toxicant exposure.

Key Points

- Cardiovascular disease (CVD) is the number one cause of death worldwide, while autoimmune diseases as a group are the third highest cause of death.
- Most rheumatic autoimmune disease patients develop perimyocarditis and/or atherosclerosis.
- At least two autoinflammatory diseases, diabetes and rheumatoid arthritis, are risk factors for CVD.
- Common immunopathogenic mechanisms exist for CVD, heart failure, and autoimmune diseases like myocarditis, inflammatory dilated cardiomyopathy, systemic lupus erythematosus and rheumatoid arthritis.

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- Similar environmental agents have been associated with the development of cardiovascular and autoimmune diseases.
- Even though most autoimmune diseases occur more frequently in women, the risk for developing CVD is increased in men.

11.1 Inflammatory Heart Disease

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, with atherosclerosis accounting for most deaths. Inflammation is a key component in the pathogenesis of a number of CVDs including atherosclerosis, stroke, myocarditis, dilated cardiomyopathy (DCM), and heart failure.

11.1.1 Atherosclerosis

Approximately half of all deaths worldwide can be attributed to CVD and to atherosclerosis, or coronary artery disease (CAD), in particular (Roger et al. 2011). Atherosclerosis occurs frequently in developed nations, but is much less prevalent in Central and South America, Asia, and Africa. Diseases characterized by atheromas or fibrofatty plaques protrude in to obstruct vessels and compromise blood flow to the heart (and other organs) resulting in ischemic heart disease (IHD). Atherosclerotic plaques form primarily in three vascular beds: coronary arteries, lower extremities, and extracranial carotid arteries (Beckman et al. 2002). Myocardial infarction (heart attack), cerebral infarction (stroke), aortic aneurysms, and peripheral vascular disease are the major consequences of atherosclerosis. Myocardial infarction alone accounts for approximately 20–25% of all deaths in the United States (Kumar et al. 2005; White and Chew 2008). The key processes in atherosclerosis are inflammation, lipid accumulation, intimal thickening, and fibrosis (Chakraborty et al. 2010; Hansson and Hermansson 2011).

The prevalence and severity of atherosclerosis among individuals and groups is related to a number of major risk factors such as age, sex, and genetics, and other less well-defined influences such as diet, exercise, and infections (Table 11.1). Age is a dominant influence (Puntmann et al. 2011). Atherosclerosis is usually not clinically evident until the ages of 40–60 when the incidence of myocardial infarction (MI) doubles for men and increases fivefold for women (Shaw et al. 2009; Roger et al. 2011; Shih et al. 2011). Men are much more prone to atherosclerosis and other inflammatory forms of heart disease with the main exception being hypertension, which is more prevalent in women (Regitz-Zagrosek 2006; Vitale et al. 2009; Sakao et al. 2010; Roger et al. 2011). There is a well-known familial predisposition to atherosclerosis and IHD that is most likely polygenic and linked to certain risk factors such as hypertension and diabetes (Hansson and Hermansson 2011). Hypertension, or high blood pressure, is a major risk factor for atherosclerosis at all ages.

Table 11.1 Risk factors for atherosclerosis[a]

Major	Other
Nonmodifiable	
Increasing age	Obesity
Male gender	Postmenopausal estrogen deficiency
Family history	
Genetic abnormalities	
Potentially controllable	
Hyperlipidemia	Alcohol
Hypertension	Lipoprotein Lp(a)
Tobacco smoking	Diet/fat intake
Diabetes mellitus	Stress
Inflammation	Physical inactivity
Air pollution	Infections
	Chlamydia pneumoniae
	Herpes simplex virus
	Cytomegalovirus

[a]Adapted from Kumar et al. (2005)

Antihypertensive therapies (e.g., diuretics, angiotensin-converting enzyme/ACE inhibitors, calcium channel blockers, angiotensin II receptor antagonists, beta-blockers) reduce the incidence of atherosclerosis-related diseases, particularly stroke and IHD (Gaciong and Symonides 2010; Laffer and Elijovich 2010). Cigarette smoking is a well-established risk factor increasing the incidence and severity of CVD in men and women (Howard et al. 1998; Glantz and Parmley 2001; Samet et al. 2009). Although the increased incidence of CVD in men has long been attributed to gender differences in smoking habits, recent studies on the effect of sex hormones on CVD indicate that testosterone may directly increase inflammation and cholesterol levels in men while estrogen increases diabetes mellitus and hypertension in women (Vitale et al. 2009). Diabetes itself induces dyslipidemia, hypertension, hyperglycemia, and platelet activation, which work together to markedly increase the risk of CAD in diabetics 2–4-fold (Beckman et al. 2002). And finally, infections may initiate and/or promote atherosclerotic plaque formation, while markers of inflammation and thrombotic function, such as C-reactive protein (CRP) and plasminogen activator inhibitor-1 (PAI-1), are potent predictors of risk for major atherosclerotic events (Brevitti et al. 2010; Hansson and Hermansson 2011).

Most of our understanding on the role of inflammation in atherosclerosis comes from animal models (Libby et al. 2010; McNeill et al. 2010). The atherosclerotic plaque consists primarily of macrophages, T cells, mast cells (MCs), and dendritic cells (DCs) (Hansson and Hermansson 2011). It has a necrotic core of lipids and a fibrous cap of smooth muscle cells and collagen. In advanced stages of disease, immune cells may also accumulate on the lumen side of the plaque further disrupting blood flow. Elevated levels of circulating cholesterol and apolipoprotein B (ApoB) undergo oxidative modifications to form oxidized low-density lipoprotein (OxLDL) that is internalized by macrophage scavenger receptors to create macrophage foam

cells within plaques (Hulsmans and Holvoet 2010). Internalization of OxLDL has been shown to activate the inflammasome leading to secretion of interleukin (IL)-1β, chemokines, eicosanoids, proteinases, oxidases, and costimulatory molecules (Hansson and Libby 2006; Brevitti et al. 2010; Hansson and Hermansson 2011). Activation of Toll-like receptor (TLR)4, MyD88, and the inflammasome (i.e., NALP3) is critical for the production of IL-1β and IL-18, two proatherosclerotic cytokines critical to disease pathogenesis (Miller et al. 2009). $CD4^+$ and $CD8^+$ T cells are present in atherosclerotic plaques and a clear role for T helper (Th)1-type responses involving IL-12, IL-18, and IFN-γ has been described (Hansson and Hermansson 2011). However, contradictory results have been found in animal models regarding the role of Th2 (IL-4, IL-33) and Th17 responses in disease pathogenesis (Taleb et al. 2010; Hansson and Hermansson 2011).

The presence of autoantibodies and autoreactive T cells against ox-LDL/LDL and/or heat shock protein (hsp)60/65 has contributed to the idea that atherosclerosis may be an autoimmune disease (Shi 2010). T cell clones reactive to LDL have been isolated from human plaques and LDL autoantibodies are abundant in atherosclerosis patients (Hansson and Hermansson 2011). Regulatory T cells and B cells have been found to protect against atherosclerosis. However, some recent studies suggest that certain autoantibodies (e.g., anti-ox-LDL IgM and IgG antibodies against native ApoB) protect against atherosclerosis, while others (e.g., higher titer ox-LDL IgG antibodies) may promote disease (Nilsson and Fredrikson 2010). Infections that have been associated with autoimmune diseases have also been detected in atherosclerotic plaques, suggesting that infection may induce dysregulation of the immune response resulting in uncontrolled inflammation or that molecular mimicry may be involved in disease initiation or progression (Tables 11.1, 11.2, and 11.4) (Fairweather and Rose 2007a). Interestingly, case-control studies have shown that patients with the autoimmune diseases systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma and psoriasis, for example, have a higher incidence of CAD (Hansson and Hermansson 2011). Recent findings from two large population-based case-control studies found that RA develops prior to CAD, suggesting that RA may promote atherosclerosis (Holmqvist et al. 2009). Additionally, anti-TNF treatment of RA patients was not only found to reduce RA, but also to significantly reduce the incidence of MI associated with CAD (Dixon et al. 2007). These findings suggest that the pathogenesis of autoimmune and CVDs may be linked.

The ability of many of the same toxins, drugs, and chemicals to induce cardiac damage in atherosclerosis and autoimmune diseases provides a further link between these two types of chronic inflammation (Tables 11.1, 11.2, and 11.4). Bacterial lipopolysaccharide (endotoxin) is capable of indirectly disrupting heart function through activation of TLRs (TLR4 in particular) resulting in the production of TNF and IL-1β (Rubin et al. 2001). Many toxins alter heart function by interfering with ion channels or pumps (Sperelakis et al. 2001). However, two chemical exposures in particular, tobacco smoke and air pollution, have been linked with the development of MI in atherosclerosis patients. In both cases, the lung serves as a conduit with CVD outnumbering deaths from lung disease (Utell and Samet 2009). Exposure to increasing concentrations of particulate matter (PM), particularly fine PM

Table 11.2 Causes of myocarditis

Viruses/disorders	Bacteria/disorders	Cardiotoxins	Hypersensitivity
Adenovirus[a]	*Chlamydia*	Ethanol[a]	Cephalosporins
Cosackievirus B[a]	Cholera	Anthracycline drugs[a]	Clozapine
Cytomegalovirus[a]	*Mycoplasma*	Arsenic	Diuretics
Epstein-Barr virus	*Neisseria*	Carbon monoxide	Insect bites
Hepatitis C virus	*Salmonella*	Catecholamines	Lithium
Herpes simplex virus	*Staphylococcus*	Cocaine[a]	Snake bites
HIV[a]	*Streptococcus*	Heavy metals	Sulfonamides
Influenza virus	Tetanus	Copper	Tetanus toxoid
Mumps	Tuberculosis	Iron	Tetracycline
Parvovirus B19		Lead	
Poliovirus	Spirochetal		Systemic disorders
Rabies	Leptospirosis	Protozoa	Hypereosinophilia
Rubella	Lyme disease	Chagas disease	Kawasaki disease
Varicella zoster virus	Relapsing fever	Leishmaniasis	Sarcoidosis
Yellow fever	Syphilis	Malaria	Wegener granulomatosis

[a]Frequent cause of myocarditis

Table 11.3 Disorders associated with idiopathic giant cell myocarditis[a]

Inflammatory disorders/autoimmune diseases
Alopecia totalis/Vitiligo
Crohn's disease
Myasthenia gravis
Myositis
Pernicious anemia
Rheumatoid arthritis
Takayasu's arteritis
Thyroiditis
Ulcerative colitis
Hypersensitivity reactions
Anti-seizure medication
Silicone rubber
Tumors
Lung carcinoma
Lymphoma
Sarcoma
Thymoma

[a]Adapted from Cooper (2000)

(PM2.5), is associated with triggering CV events like MI, arrhythmias, and congestive heart failure (Samet et al. 2000). PM and other air pollution contaminants like ozone and diesel exhaust are believed to contribute to CVD by increasing blood pressure, inflammation, and fibrosis (Lippmann 2009a, b; Mauderly and Garshick 2009). Active smoking increases the risk for CV events twofold (Glantz and Parmley 2001).

Table 11.4 Environmental factors associated with autoimmune diseases

SLE	RA	Scleroderma
UV light	Mycobacteria	*Heliobacter pylori*
Epstein-Barr virus	Epstein-Barr virus	Epstein-Barr virus
Parvovirus B19	Parvovirus B19	Parvovirus B19
Cytomegalovirus	Cytomegalovirus	Cytomegalovirus
Mercury	Gold	Mercury
Silica	Silica	Silica
Tobacco smoke	Tobacco smoke	Tobacco smoke
Sulfonamides	Obesity	Pesticides/herbicides
Estrogen replacement therapy	Physical inactivity Hyperlipidemia	Estrogen replacement therapy
Drugs: thiazides, calcium channel blockers, ACE inhibitors	Hypertension	Chemicals: epoxy resins, solvents, trichloroethylene, formaldehyde, etc.

Tobacco smoke contains a complex mixture of gases and particles including PM, nicotine, polycyclic aromatic hydrocarbons, carbon monoxide (CO), acrolein, and nitrogen dioxide (NO). Some of these components (PM, CO and NO) are also found in air pollution (Samet et al. 2009; Utell and Samet 2009). Passive smokers are equally susceptible to the adverse cardiovascular effects of smoking (Glantz and Parmley 2001; Samet et al. 2009). Active smoking is considered to increase the risk for CAD by increasing inflammation, promoting atherosclerotic plaque formation, altering endothelial cell function, and increasing thrombosis and arrhythmias. Tobacco smoke is directly toxic to cells by oxidant-mediated mechanisms, activating inflammatory cells like macrophages to produce reactive oxygen species (ROS). One puff of cigarette smoke contains $10^{14–16}$ free radicals, which activate inflammatory cells and matrix proteases while inactivating anti-proteases (Kinnula 2005).

11.1.2 Stroke

Cerebrovascular disease, or stroke, is the third leading cause of death in the United States after CAD and cancer (Roger et al. 2011). The clinical designation of "stroke" includes three processes: thrombosis, embolism, and hemorrhage. Pathologically, stroke involves hypoxia, ischemia, and infarction resulting from the loss of oxygenated blood supply to the brain, and hemorrhage resulting from the rupture of vessels (Kumar et al. 2005). Thrombotic occlusions in the brain are primarily caused by atherosclerosis and are frequently associated with hypertension and diabetes (Beckman et al. 2002; Curb et al. 2003; Carter 2005). Brain embolism can arise from a wide range of sources, but cardiac mural thrombi (which frequently occur during myocarditis/DCM) are the most common source (Gottdiener et al. 1983; Kojima et al. 1988; Kuh and Seo 2005; Kumar et al. 2005; Antoniak et al. 2008; Abdo et al. 2010).

Basic and clinical research has provided evidence that inflammatory mechanisms play a central role in the pathogenesis and progression of thrombosis and stroke (Elkind 2010). The first immune cells to enter the brain following ischemia are eosinophils, followed by neutrophils and then other inflammatory cells (Kumar et al. 2005). Importantly, inflammatory biomarkers such as high-sensitivity CRP (hs-CRP) and lipoprotein-associated phospholipase-2 (Lp-PLA_2) have been related in epidemiologic studies with stroke severity and the risk of recurrent stroke, respectively (Curb et al. 2003; Persson et al. 2008; Emerging Risk Factor Collaboration et al. 2010; Elkind 2010). These biomarkers are commonly associated with chronic inflammatory diseases, such as cardiovascular and autoimmune diseases, but are also markers of infection. Infection may trigger strokes based on the findings that the risk of stroke is approximately threefold higher within 1 week of infection (Smeeth et al. 2004; Elkind 2010). Inflammation of small and large vessels (arteritis/infectious vasculitis) has been found to be associated with infections such as syphilis and tuberculosis, which increase the risk for stroke (Sheu et al. 2010; Chahine et al. 2011), while brain inflammation (meningitis) is most often due to bacterial or viral infections. In fact, 80% of viral meningitis cases are reported to be due to enteroviruses like coxsackievirus (Kumar et al. 2005; Wong et al. 2011). Thus, bacterial and viral infections have been associated with increasing the risk for both atherosclerosis and stroke (Elkind 2010).

The risk factors for stroke are essentially identical with those for atherosclerosis and include chronic inflammation, atherosclerosis, diabetes, tobacco smoking, and obesity (Table 11.1) (Drake et al. 2010). A strong association exists, for example, between smoking and stroke risk, with current smokers having at least a 2–4-fold increased risk of stroke compared with lifelong nonsmokers or individuals who had quit smoking more than 10 years prior. The risk increased to sixfold when this population was compared with nonsmokers who had never been exposed to environmental tobacco smoke (i.e., second-hand smoke) (Bonita et al. 1999). Tobacco smoke is believed to increase the risk of stroke by inducing carboxyhemoglobinemia, increasing platelet aggregation, increasing fibrinogen levels, reducing HDL-cholesterol, and by direct toxicity of tobacco compounds (Shah and Cole 2010). Impaired fibrinolysis and reduced blood flow in the brain secondary to smoking-induced vasoconstriction may also contribute to stroke. Ischemic stroke is not the only form of stroke associated with smoking; both intracerebral hemorrhage and subarachnoid hemorrhage risks are also elevated (Shah and Cole 2010).

11.1.3 Myocarditis and Dilated Cardiomyopathy

Myocarditis, or inflammation of the myocardium, leads to around half of all DCM cases in the United States (Roger et al. 2011). DCM is the most common form of cardiomyopathy requiring a heart transplant (Cooper 2009; Wexler et al. 2009). Additionally, DCM contributes to approximately one third of all instances of congestive heart failure (Jameson et al. 2005). The life expectancy after diagnosis of

myocarditis is 50% at 10 years and only 50% at 4 years after diagnosis of DCM (Grzybowski et al. 1996; Gupta et al. 2008). In many cases, cardiomyopathies (literally, heart muscle disease) are idiopathic or of unknown cause. However, viral infection is the most common cause of myocarditis in developed countries, with other etiologies including bacterial, protozoal, toxins, drug reactions, autoimmunity, and sarcoidosis (Table 11.2) (Gupta et al. 2008; Blauwet and Cooper 2010). The prognosis for patients with acute myocarditis varies but depends on ejection fraction, clinical presentation, and pulmonary artery pressure (Schultz et al. 2009). Over 20% of sudden deaths among young adults, the military and athletes are due to myocarditis (Gupta et al. 2008). Similar to atherosclerosis, myocarditis and DCM occur more frequently in men than women (Cooper 2009).

Infections are generally thought to result in a form of myocarditis that is termed lymphocytic myocarditis, with endomyocardial biopsy of patients revealing an inflammatory infiltrate composed primarily of $CD68^+$ macrophages, $CD3^+$ T cells, and $CD220^+$ B cells, with few or rare eosinophils (Cooper 2009; Blauwet and Cooper 2010). If inflammation is severe, the disease is termed fulminant myocarditis. Fulminant myocarditis is characterized by an acute onset of severe hemodynamic instability, often in previously healthy individuals, that usually results in cardiogenic shock and multiorgan failure (Gupta et al. 2008). Another form of myocarditis, termed eosinophilic myocarditis, may result from hypereosinophilic syndrome (HES), a rare disease of unregulated systemic eosinophilia, or from malignancies, parasitic infections, vasculitic diseases, tropical myocarditis, or drug hypersensitivity reactions (Klienfeldt et al. 2010; Rezaizadeh et al. 2010). CVD is the major cause of morbidity in patients with HES, and clinical features range from myocarditis to heart failure and arterial embolism. A dominant feature of this form of myocarditis is the presence of numerous eosinophils in the infiltrate, pericarditis, fibrosis, left ventricular mural thrombi, and an unfavorable prognosis (Klienfeldt et al. 2010). Eosinophilic myocarditis also occurs more frequently in men than women with a ratio of 9:1 (Klienfeldt et al. 2010). Idiopathic giant cell myocarditis (GCM) is a rare and usually fatal CVD that generally affects otherwise healthy individuals (Cooper 2000). The diagnostic criteria is the presence of multinucleated giant cells (macrophages), but numerous eosinophils are also present in the infiltrate as well as T and B cells (Cooper 2000; Cooper et al. 2008). GCM is often associated with other inflammatory and autoimmune diseases, particularly inflammatory bowel disease (Table 11.3). Myasthenia gravis and myositis, autoimmune diseases that occur more frequently in women (Fairweather et al. 2008), are associated with thymoma (cancer of epithelial cells of the thymus) and GCM individually and in combination (Cooper 2000). Interestingly, the association of GCM with thymoma occurs almost exclusively in women (Cooper 2000). When myocardial inflammation is present in patients that have been identified with DCM by echocardiography or magnetic resonance imaging, the disease has been named inflammatory DCM (iDCM) (Kallwellis-Opara et al. 2007; Schultz et al. 2009). However, it is not unusual for myocarditis and DCM to coexist because acute myocarditis is often clinically silent and only when signs and symptoms of DCM or heart failure appear do patients consult a physician.

There is evidence that both cellular and auto/antibody-mediated damage contribute to the progression from myocarditis to DCM (Cooper et al. 2007; Kallwellis-Opara et al. 2007; Fairweather et al. 2008; Schultz et al. 2009). One of the key events in this progression is the development of fibrosis (Fairweather et al. 2004a; Kania et al. 2009; Looi et al. 2010). In both animal and human studies of DCM, genes associated with extracellular matrix remodeling and fibrosis are upregulated with disease (Yung et al. 2004; Diedrich et al. 2007; Piro et al. 2010). Inflammation contributes to remodeling by the production of a number of profibrotic cytokines including TNF, IL-1β, IL-6, IL-17 and transforming growth factor-β (Fairweather et al. 2004a; Blyszczuk et al. 2009; Kania et al. 2009; Baldeviano et al. 2010). Most of our understanding of the progression from myocarditis to DCM comes from animal models. There are three categories of mouse models of myocarditis/DCM: viral-only models (purified, infectious virus), autoimmune viral models (infectious virus with heart proteins), and adjuvant models (cardiac myosin peptide with complete Freund's adjuvant and/or Pertussis toxin, cell transfer models) (Huber 1997; Fairweather and Rose 2007b; Esfandiarei and McManus 2008; Cihakova and Rose 2008; Leuschner et al. 2009). Similar to atherosclerosis, acute myocardial inflammation is associated with an elevated Th1 response in males (Huber and Pfaeffle 1994; Frisancho-Kiss et al. 2007; Nishikubo et al. 2007; Daniels et al. 2008). TLR4-deficient mice develop reduced acute inflammation and lower IL-1β and IL-18 levels in the heart (Fairweather et al. 2003; Nishikubo et al. 2007). IL-1β has been demonstrated to be critical in the development of acute myocarditis and in the progression to DCM, due to its direct effects on heart function as well as its ability to induce fibrosis (Fairweather et al. 2003, 2004a; Blyszczuk et al. 2009). IL-17 has also been shown to increase fibrosis leading to DCM in the experimental autoimmune myocarditis (EAM) model (Baldeviano et al. 2010). Interestingly, Th2 responses have also been implicated in the pathogenesis of disease (Afanasyeva et al. 2001; Fairweather et al. 2004a, b). IFN-γ–deficient mice, which have elevated IL-4 and Th2 responses, progress to severe DCM (Fairweather et al. 2004a; Afanasyeva et al. 2004). We were surprised to find that TLR4 was expressed on alternatively activated M2 macrophages (induced by Th2 cytokines) rather than classically activated M1 macrophages (induced by Th1 cytokines) during autoimmune coxsackievirus B3 (CVB3) myocarditis (Frisancho-Kiss et al. 2007, 2009; Fairweather and Cihakova 2009). We showed that an elevated Th1/IFN-γ response in male mice with acute autoimmune CVB3 myocarditis was due to IL-18, originally named IFN-γ-inducing factor, rather than a classical IL-12/STAT4-induced IFN-γ Th1 response (Fairweather et al. 2005; Frisancho-Kiss et al. 2006). This suggests that TLR4-mediated pathology may not be restricted to Th1/M1 responses, but may also be important for Th2/M2-mediated pathology. $TLR4^+$ macrophages in CVB3 myocarditis were identified as $CD11b^+GR1/Ly6G^+F4/80^+$, a common expression pattern observed for regulatory macrophages in many different chronic inflammatory diseases (Fairweather and Cihakova 2009; Frisancho-Kiss et al. 2009). Recently, $CD11b^+GR1/Ly6C^{hi}$ monocyte/macrophages were found to be critical to atherogenesis in mouse models of atherosclerosis (Woollard and Geissmann 2010). $Ly6C^{hi}$ macrophages are considered to be M1-like, expressing TLR and IL-1β, while $CD11b^+GR1^{lo}$ macrophages are considered to be M2-like, mediating tissue

repair (Libby et al. 2009; Pamukcu et al. 2010; Woollard and Geissmann 2010). Further work is required to determine whether these two macrophage subsets increase or regulate disease pathogenesis in atherosclerosis and myocarditis/DCM. However, the many similarities between the inflammatory and remodeling processes in myocarditis/DCM and atherosclerosis (i.e., TLR4, IL-1β, $GR1^+$ macrophages) suggest a common inflammatory pathway for these diseases.

With the many pathogenic similarities between atherosclerosis and myocarditis/DCM, one would expect that risk factors for atherosclerosis would have been examined in clinical and animal models of myocarditis, yet there are almost no studies examining the effect of chemical-infectious interactions on heart disease. One recent study found that acute tobacco smoke exposure of mice prior to and during encephalomyocarditis virus (ECMV) infection resulted in increased mortality and reduced heart function (Bae et al. 2010). Another study showed that Wistar Kyoto rats exposed to PM for 6 h, 1 day, or 16 weeks (no infection) developed cardiac inflammation, fibrosis, and MC degranulation (Kodavanti et al. 2003), suggesting that pollution may adversely affect myocarditis/DCM. More studies need to be done to understand the role of toxin and chemical exposures on the pathogenesis of myocarditis, DCM, and heart failure.

11.1.4 Heart Failure

Heart failure, or reduced cardiac output, is the end consequence of a number of inflammatory cardiovascular conditions including atherosclerosis, myocarditis, and DCM. Most cases of heart failure are caused by reduced myocardial contractile function (systolic dysfunction) as occurs during ischemic injury, pressure or volume overload, and during DCM. However, heart failure can also occur because of an inability to relax, expand, or fill the ventricle resulting in diastolic dysfunction observed during myocardial fibrosis and constrictive pericarditis (Afanasyeva et al. 2004; Kumar et al. 2005). A recent long-term study of myocarditis patients revealed that inflammation was the best predictor for the progression to heart failure (Kindermann et al. 2008). Viruses, like coxsackievirus B, adenovirus, parvovirus B19, and hepatitis C virus, are often detected in patient myocardial biopsies (Gupta et al. 2008; Cooper 2009). Antiviral treatments such as IFN-β reduce inflammation and heart failure in animal models and patients, implying that viral infections are an important cause of myocarditis cases that lead to heart failure (Kuhl et al. 2003; Wang et al. 2007). Inflammation appears to be etiologically linked with the development of heart failure, not only because heart failure is a consequence of inflammatory CVDs, but also because patients with chronic heart failure who have elevated levels of inflammatory mediators have a worse prognosis (Robinson et al. 2011). More males develop iDCM and the presence of eosinophils in the myocardium has been associated with a poor clinical status (Aguero et al. 2008; Cooper 2009). Risk factors for developing heart failure are essentially the same as for atherosclerosis and myocarditis (Tables 11.1 and 11.2).

11.2 Autoimmunity and Heart Disease

Autoimmune diseases are chronic inflammatory conditions that are defined by the presence of autoreactive T and B cells and elevated levels of autoantibodies (Fairweather and Rose 2007a). A number of autoimmune diseases, SLE, RA and scleroderma, are associated with the development of inflammatory heart disease, while autoimmunity is considered as an important pathologic mechanism for several CVDs (e.g., atherosclerosis, myocarditis, and DCM).

11.2.1 Systemic Lupus Erythematosus

SLE is a common multisystem autoimmune disease of unknown etiology that presents as a photosensitive rash, polyarthritis, and/or renal disease. SLE occurs more frequently in females than males at a ratio of 9:1 with the age of onset at 20–30 years of age (Fairweather et al. 2008). The role for genetic predisposition is low with only a 2% chance that a child whose parent has SLE will develop disease (Petri 2006). Numerous environmental agents have been associated with disease including ultraviolet (UV) light, antibiotics, anti-TNF therapies, drugs like thaizides and calcium channel blockers, and infections like Epstein-Barr virus, parvovirus B19, and cytomegalovirus (Table 11.4) (Petri 2006; Cooper and Miller 2007; Gualtierotti et al. 2010). There is a need for well-designed case-control and cohort epidemiologic studies to better determine the relationship of environmental agents to autoimmune diseases (Mayes 1999). SLE is a disease of multiple immune system abnormalities. Factors that increase the risk for SLE include defects in antigen presentation (HLA DR/DQ), poor clearance of immune complexes (IC) and/or apoptotic material by macrophages (CD11b, C1q, CRP, MBL, FcRs), transcriptional regulation of interferons (IRF3, IRF5), elevated proinflammatory cytokines like tumor necrosis factor (TNF), IL-6, and IFN, dysregulated B cells (BLyS), and elevated autoantibodies (anti-nuclear antibodies), for example (Petri 2006; Gualtierotti et al. 2010).

The heart is frequently involved in SLE with a prevalence of at least 50%, while postmortem studies of SLE patients revealed a prevalence of pericarditis of 63% (Doria and Petri 2004; Doria et al. 2005; Ashrafi et al. 2011). All major forms of inflammatory CVD can be found in SLE patients including pericarditis, myocarditis, endocarditis/valvular disease, conduction system abnormalities, and atherosclerosis (Table 11.5). Although pericarditis is the most common CVD in SLE patients and is part of the classification criteria for SLE, the major cause of death is premature atherosclerosis (Doria et al. 2005; Ippolito and Petri 2008). Vascular occlusion in SLE may be due to vasculitis, atherosclerosis, and/or antiphospholipid antibodies/syndrome (APS) (Table 11.5). Pericarditis and myocarditis in SLE are associated with complement C3 and IC deposition (Doria et al. 2005). Since the advent of steroid therapy, myocarditis associated with SLE has been reduced to around 10%, but postmortem studies indicate a prevalence of around 50% (Doria and Petri 2004).

Table 11.5 Cardiac involvement in autoimmune diseases[a]

SLE	RA	Scleroderma
Pericarditis	Pericarditis	Myocarditis
Myocarditis/DCM	Myocarditis/GCM	Atherosclerosis
Atherosclerosis	Atherosclerosis	Vasculitis
Valve disease/endocarditis	Valve disease/endocarditis	
Conduction system abnormalities	Conduction system abnormalities	
Hypertension		

[a]From Doria and Pauletto (2004)

Myocarditis associated with fibrosis in SLE patients may progress to DCM and heart failure. The risk of developing atherosclerosis/CAD in SLE patients is 4–8-fold higher than controls, and in young women the risk of MI is increased 50-fold (Doria et al. 2005). Traditional risk factors for atherosclerosis such as CRP and lipoprotein Lp(a) are also elevated in SLE patients (Table 11.1), but even after correcting for these factors an eightfold greater risk of MI exists with SLE (Esdaile et al. 2001; Petri 2006). A recent multicenter retrospective case-control study found that SLE patients who developed atherosclerosis were more likely to be male, had more exposure to classical CAD risk factors, and were more likely to have been treated with corticosteroids (Haque et al. 2010).

11.2.2 Rheumatoid Arthritis

RA is a common inflammatory form of arthritis affecting approximately 1% of the population. The incidence of RA is 2–3-fold higher in females than males (Fairweather et al. 2008). Genes are thought to account for as much as 50–60% of the susceptibility to develop RA. *HLA-DRB1* alleles are associated with more severe disease (Bartoloni et al. 2010). Additionally, polymorphisms in TNF and IL-6 have been associated with a twofold risk of CVD in RA patients, independently of traditional CVD risk factors (Panoulas et al. 2009). Elevated levels of these cytokines occur in RA patients and can contribute to increased inflammation, fibrosis, and thrombosis. A number of environmental chemicals, metals, and infectious organisms have been associated with RA (Table 11.4) (Cooper and Miller 2007), but similar to other autoimmune diseases, there are few well-designed epidemiological studies to confirm the relationships (Mayes 1999). Tobacco smoking is thought to be an important risk factor for RA. Smoking is associated with increasing anticycliccitrullinated peptide levels as well as joint erosion and extra-articular manifestation risk (Bartoloni et al. 2010). Anti-citrullinated protein antibodies (ACPA) are an early biomarker of RA. RA patients also have elevated levels of OxLDL in their sera (Steiner and Urowitz 2009). Inflammation in the synovium during RA includes $CD4^+$ T cells, macrophages and mast cells with increased levels of TNF, IL-1β, and IL-6 (Bartoloni et al. 2010; Robinson et al. 2011). Unregulated autoantibody production from B cells leads to IC deposition, which along with proinflammatory cytokines recruits more inflammation and promotes ECM remodeling of the joint.

With RA exhibiting so many of the traditional CVD risk factors, it is not surprising that RA is associated with an increased CV morbidity and mortality compared to the normal population, accounting for 30–50% of RA deaths (Wallberg-Jonsson et al. 1997; Robinson et al. 2011). There is a 2–3-fold increased risk for MI, congestive heart failure, sudden death and stroke in RA patients (Bartoloni et al. 2010). Because of the high prevalence of CVD in RA patients, the presence of RA probably should be considered a risk factor for CVD similar to diabetes (van Halm et al. 2009). Although overall mortality and death due to CVD and IHD were increased in both sexes, male sex predicted death and cardiovascular events in RA patients (Wallberg-Jonsson et al. 1997). Interestingly, this sex difference was also observed in SLE patients (Haque et al. 2010). Similar to SLE, nearly all structures of the heart can be affected during RA (Table 11.5). However, pericarditis is the most frequently reported cardiac manifestation (Goodson 2004). In pericarditis, inflammation gathers between the two cell layers on the outer surface of the heart and may cause accumulation of pericardial fluid resulting in pericardial effusion. If the pericardium becomes thickened, fibrotic, and rigid, then the individual can develop constrictive pericarditis (Afanasyeva et al. 2004; Fairweather et al. 2004a). Myocardial inflammation associated with RA is often granulomatous, resembling GCM and increasing the likelihood of heart failure (Tables 11.3 and 11.5). RA is likely to increase the risk for developing CVD by increasing vascular and myocardial inflammation and dyslipidemia. Evidence of the important role for elevated proinflammatory cytokines in the disease pathogenesis comes from studies showing that treatment of RA patients with anti-TNF drugs reduces the chance of developing heart failure by 50% (Dixon et al. 2007; Robinson et al. 2011).

11.3 Summary

Recent clinical and animal studies have revealed a remarkable degree of similarity in the pathogenesis, biomarkers, and potential etiologic agents associated with cardiovascular and autoimmune diseases. Although all of these diseases are inflammatory in nature, a pattern is emerging of important immunoregulatory pathways that are being altered resulting in CVD. Nonspecific markers of inflammation like TNF and CRP can be induced by chemicals and infections and then further amplified by autoinflammatory processes (Parke and Sapota 1996). Genetic polymorphisms in key inflammatory mediators like TNF and IL-1β may result in a dysregulated immune response to infections, toxins and/or chemicals, with environmental agents acting as triggers of disease.

11.4 Recommendations

Opportunities for future research include the need to conduct well-designed epidemiological studies to examine the relationship between infections, toxicants, and autoinflammatory responses. Studies need to consider autoimmune diseases as

groupings as well as individually, and to examine whether differences exist according to sex, rather than controlling for sex, since all of the major inflammatory diseases have dominant sex differences in expression (Fairweather et al. 2008; Dube et al. 2009). Increased use of genomics, proteomics, and exposomes should uncover important new information about the relationship between the environment and chronic inflammatory heart disease (Rappaport and Smith 2010).

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Chapter 12
Inflammatory Bowel Disease and Celiac Disease: Environmental Risks Factors and Consequences

Rodney R. Dietert

Abstract Gastrointestinal manifestations of autoimmune-inflammatory diseases represent a major category of immune dysfunction-based chronic diseases. Two prominent examples of these diseases are introduced in this chapter. Inflammatory bowel disease (IBD) is an inflammatory autoimmune disease that consists of two distinct conditions, Crohn's disease (CD) and ulcerative colitis (UC), while celiac disease (CED) is a gut-associated inflammatory disease with both allergic and autoimmune features. These diseases have been increasing in prevalence among the overall population including an expansion among the pediatric population. This chapter provides a foundational description of the suggested environmental risk factors, immune dysfunction, and comorbidities associated with IBD and CED.

Key Points

- Inflammatory bowel disease (IBD) and celiac disease (CED) have been increasing in prevalence.
- While genetic factors have been associated with these diseases, environmental risk factors are prominent in the induction of immune dysfunction and the subsequent tissue pathologies.
- Interaction with the gut microbiome is important in mucosal immune development and proper function, and a disruption in the microbiome represents a key component of these GI tract diseases.
- IBD and CED have numerous comorbidities that can arise in virtually any tissue and place a significant additional disease burden on patients.

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- Comorbidities of IBD and CED illustrate the key role that misregulated inflammation plays in promoting, if not causing, a host of impairments involving the neurological, reproductive, respiratory, dermal, hepatic, gastrointestinal, and cardiovascular systems.

12.1 Introduction

The present chapter considers two major chronic autoimmune-inflammatory diseases of the gastrointestinal tract, inflammatory bowel disease (IBD) and celiac disease (CED). Additionally, the two major forms of IBD, Crohn's disease and ulcerative colitis (UC), are discussed both under the rubric of IBD and as separate disease entities. IBD has been increasing in prevalence and, with its increased presence in various regions, it is now considered to be a global disease of significance (Molodecky et al. 2012). Disease classification and diagnostic challenges have restricted the availability of prevalence data. But it appears the disease may approach a prevalence of 0.4% globally (Lakatos 2006). By comparison, a 2003 publication (Fasano et al. 2003) estimated that CED affects approximately 1% of the U.S. population. As is discussed in more detail throughout the chapter, both diseases represent very serious immune dysfunction-based chronic conditions that require intensive medical treatment; however, they are also connected as comorbidities to numerous other autoimmune and inflammatory conditions further complicating the treatment and prognosis for patients (Dietert and Zelikoff 2009; Dietert et al. 2010; Kappelman et al. 2011; Pitocco et al. 2011).

These diseases are prime examples of the cause–effect relationship between environmentally affected immune dysfunction and tissue-directed chronic disease manifestations. Among the topics considered under the overall umbrella of immunotoxicity, immune dysfunction, relative risk, and consequences of IBD and CED are: (1) the nature of the immune dysfunction, (2) the reported comorbidities for these diseases, and (3) the risk factors for IBD and CED with an emphasis on the environmental risk factors.

Chronic inflammatory diseases may have overlapping characteristics, but the target and disease outcomes can take many different manifestations (Chiurchiù and Maccarrone 2011). The gastrointestinal tract is a primary target for immune dysfunction-based disease. This is not surprising since, like the respiratory and dermal systems, it represents a major portal for environmental exposure. In the gut, specialized immune cell populations interact with gut microbiota and a diversity of environmental factors taken up via the oral route. Both of these interactions affect immune development (Kaplan et al. 2011) and resulting homeostatic regulation within the gut.

Immune dysfunction-based gastrointestinal diseases have been increasing and much of this increase is attributable to changes in our environment and lifestyle (Hall and Crowe 2011). Many of these gut-targeted inflammatory conditions were first observed to have particular geo-associated prevalences (e.g., associated more with developed countries and with northern latitudes Logan and Bowlus 2010). Various theories were proposed to explain these demographics including early-life

vitamin D intake, specific crop production, and geo-distributed microbial diversity and exposures. But, as discussed by Logan and Bowlus (2010), recently increased migrations of people have tended to blur these more historic distinctions.

Immune- and inflammation-based diseases and conditions can take several forms. Many of these have overlapping symptoms and risks, usually involving inflammatory dysfunction, tissue swelling, malabsorption, and elevated later-life risk of gut-associated diseases (e.g., colon cancer). Additionally, the immune dysfunction with these gut-inflammatory diseases falls into the category of inappropriately enhanced immune responses as opposed to immunosuppression. However, there are also important distinctions among the various manifestations of GI tract inflammatory diseases. These distinctions can involve the area of the GI tract that is affected, the impact and nature of various genetic and environmental risk factors, and the precise spectrum of immune dysfunction that is associated with each disease. It is useful to recognize that while IBD with the subcategories of CD and UC is defined by gastrointestinal inflammatory misregulation and destruction, the immune dysfunction basis of this disease can result in extra-intestinal manifestations. Tissue involvement beyond the gastrointestinal tract may affect the course and potential effectiveness of various treatments (Veloso 2011).

Unlike the IBD conditions, celiac disease (CED), sometimes called "sprue," is a gut-associated inflammatory disease with a very specific and pivotal instigator, gluten (a common grain-based protein found in many foods) (Lionetti and Catassi 2011). Gluten peptides and/or posttranslationally modified gluten products produce both innate and adaptive immune responses in gluten-sensitive individuals (Rossi and Bot 2011). While the disease has an initiating food allergic-like component, it seems clear that autoimmune responses play an important part in later disease processes (Tommasini et al. 2011). Diagnosis can involve not only biopsies, but also detection of anti-tissue transglutaminase as well as anti-gliadin antibodies (Matthias et al. 2010). There are several different manifestations of the disease. Malnourishment due to improper absorption of nutrients is a common symptom (García-Manzanares and Lucendo 2011). However, even an asymptomatic "silent" form has been defined in children and adults (Bardella et al. 2007). In fact, it was recently suggested that gluten sensitivity itself and CED are actually two distinct clinical entities (Sapone et al. 2011). The former is suggested to involve gluten-sensitive innate immune activation in the absence of adaptive immune responses and loss of mucosal barrier function. In contrast, CED involves both branches of the immune system with eventual loss of mucosal barrier and increased gut permeability. Additional investigations have suggested that gluten sensitivity can be separated from CED in some patients (Massari et al. 2011).

12.2 Crohn's Disease vs. Ulcerative Colitis

Inflammatory bowel disease (IBD) refers to inflammation and swelling of the intestines that usually features a chronic relapsing course of symptoms. IBD has two primary disease manifestations within the gastrointestinal system: Crohn's disease (CD) and

ulcerative colitis (UC). While CD can affect any site in the gastrointestinal tract from the mouth to the anus, the most common area affected is the ileum, the lower part of the small intestine. The inflammation of CD involves the lining of the affected area and can cause pain, frequent emptying of the bowels and diarrhea. The health status of the bowel is very heterogeneous with CD where healthy areas may be found interspersed between areas of diseased tissue. Because loss of function can occur in the diseased areas affecting nutrient absorption, children with CD can exhibit stunted growth and delays in development. Animal models of CD vs. UC suggest that it is not only the affected area of the gastrointestinal tract that differs between CD and UC, but also the precise cascade of inflammatory mediators as well as the specific targets of actions (Shi et al. 2011).

In contrast to CD and the targeting of the ileum, UC usually is associated with inflammation that causes swelling of the colon and/or rectum. While it can strike at any age, age-of-onset data suggest that there is a focal range in the late adolescent-young adult (approximately 15–40) and again in the elderly (approximately 50–80) (Langan et al. 2007; Ha et al. 2010). Age-of-onset differences in disease phenotype have been reported (Guariso et al. 2010). Therefore, it is not surprising that prognosis may be quite different based on the age of onset (e.g., in the young-adult-onset vs. late-adult-onset groups) (Ha et al. 2010; Lee et al. 2010).

Environmental factors affecting the risk of UC can exert an effect from early childhood (Sonnenberg 2008). First signs of disease are often seen in the rectum and then spread to parts of the large intestine as the disease progresses. The symptoms of gastrointestinal bleeding, pain, diarrhea, weight loss (growth impairment in children) can range from mild (about half of patients) to quite severe and frequent among those with the disease. Episodes or relapses of UC show seasonality (Karamanolis et al. 1997) and are often triggered by immune-challenging conditions such as infections (Friswell et al. 2010) or stress (Maunder and Levenstein 2008). Treatments of the disease often involve immunomodulatory drugs (Hoentjen et al. 2011). As with most chronic inflammatory diseases of the GI tract, there is an elevated risk of colon cancer among UC patients (Lukas 2010).

12.2.1 Differential Prevalences and Susceptibilities

Genetics, epigenetics, and lifestyle-driven differences in environmental exposures all play a role in IBD. This is particularly true in a comparison of indigenous populations within larger countries. For example, Canada's First Nation indigenous population is known to have a relatively low rate of IBD compared with the remainder of the country's population (Blanchard et al. 2001). While investigators had thought this might relate to differences in antibody production against infectious agents, such a distinction was not found (Bernstein et al. 2011).

It is recognized that the developing immune system is a sensitive target for immune dysfunction and the emergence of later-life immune-mediated chronic disease (Dietert 2009). Additionally, children are also susceptible to the onset of IBD. Benchimol et al. (2011) reported that both developed and developing countries are contributing to globally rising rates of pediatric IBD.

12.2.2 Immune Dysfunction

Inflammatory bowel disease stems from dysregulation of mucosal immunity involving both innate and acquired immune components and the cross-regulation between them (Siegmund and Zeitz 2011). One of the factors that appears to be important in risk of IBD is the maturational pathway of colonic Tregs. These cells appear to use a TCR repertoire that is distinct from that of other Tregs in other locations. Importantly, the interaction of these cells with commensal microbiota in the periphery is needed to generate antigen-specific Tregs that are tolerant against an individual's normal flora (Lathrop et al. 2011). Relative to causative factors for IBD, this raises the question of which comes first, immune dysfunction that affects the interactions of the immune system with GI tract microbiota or disruptions in GI tract microbiota that interrupt the normal maturation course of immune development?

While the two forms of IBD have many distinctions, some common overlapping features have been reported. For example, endoplasmic reticulum (ER) stress, an imbalance in the demand and capacity for ER protein modification, has been suggested to play a role in risk of both forms of IBD (Bogaert et al. 2011). These researchers reported a tight interconnection between inflammation, as measured by transcriptional interleukin-8 (IL-8) production, and endothelial-focused ER stress. Some distinctions in signal transduction-associated gene expression were made in the colonic forms of IBD vs. the ileal forms of this disease (Bogaert et al. 2011). An additional similarity has been reported among childhood CD and UC. Children with both forms of IBD have a strong overexpression of CXCR3 axis components: the CXCR3 receptor and its three ligands—CXCL9, CXCL10, and CXCL11 (Schroepf et al. 2010). The overexpression of the CXCR axis was directly correlated with local IL-8 production in inflamed tissues (Schroepf et al. 2010). The CXCR3-associated chemokines seem to have a role in the pathogenesis of IBD, and it has been suggested they may be involved in the recruitment of Th17 cells (Schroepf et al. 2010). It should be noted that not all studies of IBD in adults has reported this similarity (Manousou et al. 2008).

Some important distinctions exist between immune dysfunction involvement in CD vs. UC. Different populations of T lymphocytes appear to be aberrantly activated in CD vs. UC, resulting in different levels of T-cell-driven cytokine mediators (reviewed in Siegmund and Zeitz 2011). For example, an overabundance of Th17 cell-associated cytokines (I-17 and IL-23) have been reported with inflamed CD mucosa (Fujino et al. 2003).

Some authors have also reported an involvement of Th17 cells in UC (Kobayashi et al. 2008). In contrast, overproduction of IL-13 by natural killer T cells (NKT cells) appears to be a critical factor in UC-associated pathogenesis (Fuss et al. 2004; Heller et al. 2005). Additionally in UC, one of the hallmarks of the disease appears to be the dysregulation of the IL-1 family-related cytokine, IL-33 (Pastorelli et al. 2010, 2011). Interestingly, upregulation of IL-33 production is known to induce an increase in certain Th2-related cytokines such as IL-5 and IL-13 (Moro et al. 2010). This suggests that IL-33 overproduction may be a significant factor in the immune dysfunction associated with UC.

In CD, immune dysfunction and excessive unresolved inflammation appear to affect the intestinal epithelium causing a translocation of bacteria, reducing the bacterial shield and prompting chronic inflammation in the ileum. In reality, the immune problems associated with CD are more of a true dysfunction with both specific deficiencies and excesses of immune reactions rather than solely immune hyperresponsiveness. In fact, not all of the immune problems associated with CD appear to be explained by excessive inflammation. Recently, Rogler (2011) and Glocker and Grimbacher (2012) suggested that inadequate responses are an initial problem that then results in improperly controlled inflammation. Part of this inadequate or deficient response appears to involve the interaction of host immune cells with bacterial cell wall products. This results in impaired acute inflammation (Marks 2011). In fact, effective resolution of acute inflammation is a critical turning point in the consideration of inflammatory diseases like IBD (Campbell et al. 2011). Such resolution of inflammation and the avoidance of subsequent, inappropriate chronic inflammation plays a critical role. Recently, resolution of acute inflammation has been shown to be influenced by the production of specific lipid mediators (Campbell et al. 2011; Serhan et al. 2011).

Cell signaling at the mucosal surface—bacterial interface is also central in IBD. Function of the nucleotide-binding oligomerization domain 2 (NOD-2) receptor appears to be very important in these mucosal immune-intestinal microbiota interactions. NOD-2 polymorphisms for innate immune cell signaling and for other genes affecting autophagy of innate immune cells in response to bacteria are important (Fritz et al. 2011). Not surprisingly, improper NOD-2 function has been associated with IBD and in particular with CD (Biswas et al. 2012; Zhao et al. 2011). In the GI tract, one of the key interactions affecting NOD-2 signaling appears to involve Caspase activating and recruitment domain 8 (CARD8) (von Kampen et al. 2010).

Additionally, the enzyme, Caspase 8, is critical in the pathway to apoptosis and appears to be important in regulating intestinal homeostasis and protecting epithelial cells of the intestine from necroptotic cell death induced via TNF-α (Günther et al. 2011). These authors proposed that problems with Caspase 8 led to loss of Paneth cells by necrosis and allowed bacteria to penetrate epithelial barriers resulting in unresolved chronic inflammation. Problems with Paneth cell and globlet cell maturation and deficiencies in the defensins (e.g., HD-5 and HD-6) have been reported to be associated with risk of CD (Gersemann et al. 2011). However, distinctions may exist within CD. For example, Wehkamp et al. (2009) suggested that disturbed Paneth cell differentiation in a central part of ileal CD but an impaired induction of mucosal beta-defensins is featured in colonic CD.

12.3 Celiac Disease (CED) vs. IBD

In contrast with IBD, CED is characterized as a chronic, immune-mediated enteropathy that often results in damage to the lining of the small intestine. While CED usually begins with a hallmark allergic response to gluten, it also includes a significant autoimmune disease component (Catassi and Fasano 2008). CED onset

can appear as a wide variety of symptoms, which can mean that accurate diagnosis of the disease may be delayed in some patients. Screening for the disease can involve not only biopsy, but also measurement of IgA anti-tissue transglutaminase autoantibodies (Samaşca et al. 2011). However, diagnosis in pediatric populations remains a challenge (Brusca et al. 2011). For example, some children with CED may present with growth failure and/or short stature as the primary indications (van Dommelen et al. 2008; Nemet et al. 2009). Avoidance of dietary gluten remains a major management tool (Goh and Werlin 2011).

12.4 Chronic Disease Comorbidities of IBD and CED

Comorbidity patterns for IBD and CED suggest that there is a nexus of autoimmune, allergic-autoimmune, and inflammatory diseases where there are extensive elevated risks of additional chronic diseases that can manifest in many different tissues and organs. As suggested by Dietert et al. (2010), there is a significant overlap in chronic disease comorbidities when misregulated inflammation in tissues is involved. This usually leads to neurological–behavioral (Zois et al. 2010) problems as well as cardiovascular health concerns (Wei et al. 2008). Additionally, the emergence of one autoimmune-related condition appears to elevate the risk of additional later-life autoimmune diseases. This is certainly the case when one examines the patterns of comorbidities for IBD and CED.

Importantly, while both men and women patients with IBD or CED are at an elevated risk for several additional chronic diseases, sex is an important factor that appears to influence both the psychosocial concerns of patients with the primary disease (Maunder et al. 1999; Kane 2002; Tontini et al. 2010) as well as the specific spectrum of additional health risks that need to be considered in these patients (Söderlund et al. 2010; Sánchez et al. 2011; Sridhar et al. 2011; Stewart et al. 2011) Therefore, sex-specific management of these diseases with the aim of preventing additional specific comorbidities may prove useful.

12.4.1 IBD

Patients with IBD have an increased risk of colorectal cancer (Ullman and Itzkowitz 2011) that is thought to be related to the inflammatory dysfunction and prolonged tissue insult. Additional problems that are likely connected to inflammatory dysregulation include both depression (Häuser et al. 2011; Maes et al. 2011; Szigethy et al. 2011) and systemic fatigue (van Langenberg and Gibson 2010). Neurological problems, such as peripheral neuropathy, have also been reported to be common among IBD patients (Oliveira et al. 2008) Additionally, an increased risk for coronary heart disease, asthma, and connective tissue diseases has been reported among

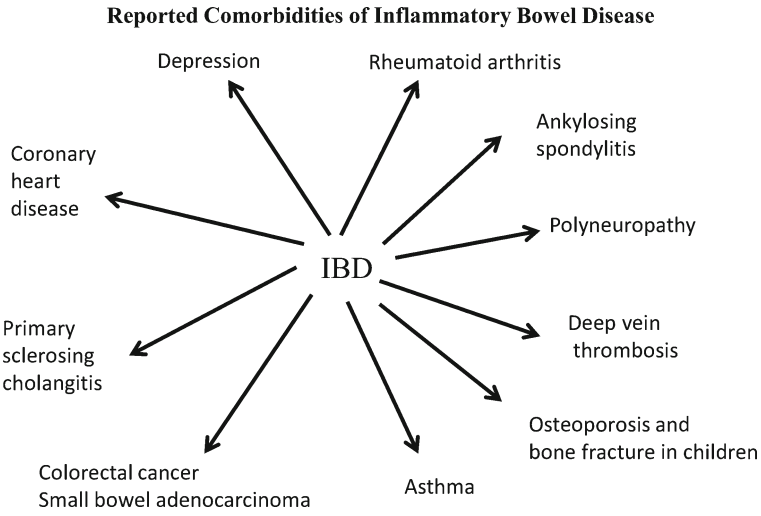

Fig. 12.1 Reported comorbidities for inflammatory bowel disease (IBD) are illustrated. Individuals with IBD are reported to have an elevated risk for these additional diseases and conditions

IBD patients (Haapamäki et al. 2011). Berstein et al. (2011) reported that the incidence of fracture was 40% greater for IBD patients than among the general population. It should be noted that certain treatment strategies such as the use of corticosteroids could augment these specific manifestations. Figure 12.1 illustrates the spectrum of reported comorbidities for IBD (i.e., including both those that may be CD- or UC-specific).

Children with CD are reported to be at an elevated risk of later-life rheumatoid arthritis, lupus, and hypothyroidism (Kappelman et al. 2011). Additionally, systemic inflammation associated with Crohn's disease in children is reported to increase their susceptibility for osteoporosis and bone fracture in later life irrespective of the extent of corticosteroid therapy (Hill et al. 2011).

12.4.2 CED

Given the complex profile of allergic, autoimmune, and inflammatory reactions involved with CED, it is not surprising that the disease has been associated with a wide range of comorbidities affecting a majority of tissues and organ systems. The pattern of extra-gastrointestinal health risks observed with IBD is also a concern among CED patients. The range of reported comorbidities for CED is shown in Fig. 12.2.

For CED, as was seen with IBD, autoimmune diseases represent a significant category of comorbidities. Risk of sarcoidosis has been reported to be elevated among CED patients (Hwang et al. 2008). As with most chronic gut-inflammatory

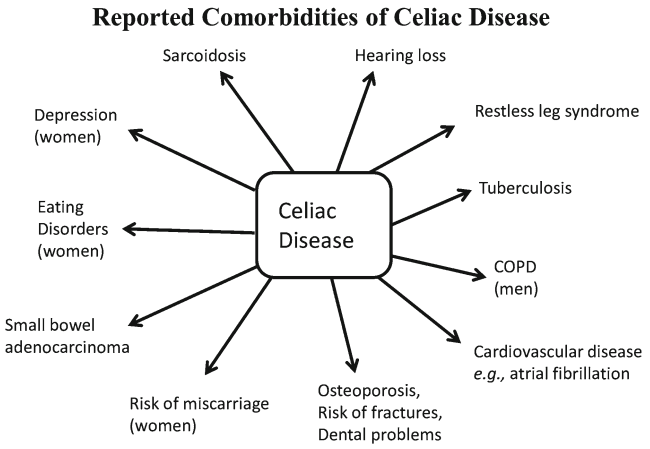

Fig. 12.2 Reported comorbidities for celiac disease (CED) are illustrated. Individuals with CED are reported to have an elevated risk for these additional diseases and conditions

diseases, there is an associated elevated risk of developing certain GI tract cancers among CED patients. One of the best studied associations is for celiac and small bowel adenocarcinoma (Bergmann et al. 2010). Additional associations were considered in depth in the context of autoimmunity, inflammation, and alimentary tract cancers by Landgren et al. (2011). Lymphomas, particularly T cell lymphomas, are an increased risk for celiac patients (Halfdanarson et al. 2007). Liver disease is also an anticipated comorbidity for CED (Prasad et al. 2011).

Recently, reports of an association with neurological symptoms have also emerged (Uygur-Bayramicli and Ozel 2011), and the association appears to exist not only in adults but also in children (Lionetti et al. 2010). Additional neurological comorbidities can take the form of psychiatric symptoms including depression and eating disorders that appear to be at an elevated risk particularly among women CED patients (Arigo et al. 2012). Among children with CED, there is an elevated risk of sensorineural hearing loss (Hizli et al. 2011).

In a community-based cohort study (Wei et al. 2008), a slightly increased risk for cardiovascular disease has been reported to be associated with celiac disease particularly when patients with prior hospitalization of cardiovascular diseases were excluded. This is consistent with the recent report of Emilsson et al. (2011) that among the Swedish population, biopsy-confirmed CD patients are at an elevated risk of atrial fibrillation (HR = 1.34, 95% CI = 1.24–1.44) compared against age-matched controls.

Lung inflammatory diseases are also at an elevated risk in CED patients. In an additional study from Sweden, Ludvigsson et al. (2011a) reported a moderately elevated risk for chronic obstructive pulmonary disease (COPD) particularly among men with CED (HR = 1.39; 95% CI = 1.18–1.62). There is a reported increased risk of tuberculosis among celiac patients that may be related to nutrient adsorption problems and/or underlying immune dysfunction (Ludvigsson et al. 2007, 2011b).

Another comorbid manifestation of celiac disease that appears to be linked with the nutrient absorption problems is the increased risk of restless leg syndrome among celiac disease patients (Moccia et al. 2010; Weinstock et al. 2010). In a study among patients in Italy, restless leg syndrome was reported to be more common among adult CED patients (31%) compared against a control population (4%) (Moccia et al. 2010). Weinstock et al. (2010) reported similar findings in a study among US celiac disease patients and their spouses. Based on their findings, these investigators suggested that the neuromuscular disorder may be associated with iron deficiency in celiac patients (Weinstock et al. 2010).

Bone problems can also develop, which include increased risk of osteoporosis (Katz and Weinerman 2010), fractures (Sánchez et al. 2011), and dental problems (Cheng et al. 2010). Oral mucosal characteristics are different among children with celiac (Mina et al. 2008). Therefore, it is not surprising that dental enamel defects and aphthous ulcers have been reported as comorbidities of CED particularly with early-life onset (Cheng et al. 2010).

Elevated risk for a wide range of reproductive disorders has been described among women with CED (Martinelli et al. 2010). Among the common problems is recurrent miscarriage (Soni and Badawy 2010). This is consistent with the general finding that endocrine conditions are prominent in children with CED (Buzby 2010).

12.5 Environmental Risk Factors: IBD and CED

Genetic predispositions for both IBD and CED are known to play a significant role. However, many twin studies are not able to distinguish between stable genomic heritable factors and epigenetically imprinted multigenerational susceptibility factors. For both IBD and CED, environmental factors are thought to play a significant role. In the case of IBD, twin studies suggest that environmental risk factors play a predominant role in IBD. In a study of German twins, Spehlmann et al. (2008) reported a concordance of 35% for CD and 16% for UC among monozygotic twins suggesting that environmental risk factors are likely to contribute to a majority of the risk for IBD. In contrast, for CED, heritable factors appear likely to constitute the majority of the overall risk. However, environmental factors still affect whether an individual will manifest the disease. While inheritance of the genetic susceptibility of celiac disease is multifactorial (Garner et al. 2009; Trynka et al. 2010), specific *HLA-DQ* alleles are known to be important in genetic predisposition (Freeman 2010; Clouzeau-Girard et al. 2011).

In general, research into environmental risk factors for autoimmune-inflammatory conditions has tended to lag behind when compared with research into the environmental causes of allergic diseases. Certainly that is the case for IBD and CED where more research is needed into the potential for environmental risk reduction. However, a cadre of suggested environmental risk factors has emerged based primarily on epidemiological research studies. Suggested environmental risk factors are shown in Table 12.1. It seems likely that interactions occur among genetic and environmental risk factors as

Table 12.1 Suggested environmental risk factors for inflammatory bowel disease and celiac disease

Inflammatory bowel disease (CD, UC)[a]	References	Celiac disease	References
Cesarean delivery	Bager et al. (2011)	Ingestion of gluten[b] (symptoms)	Barone et al. (2011) and Fasano and Catassi (2011)
Living in urban areas	López-Serrano et al. (2010)	Birth seasonality	Ivarsson et al. (2003) and Lewy et al. (2009)
Prior smoking (UC), smoking (CD)	Bastida and Beltrán (2011) (UC) and Ng et al. (2012) (CD)	Elective cesarean delivery	Mårild et al. (2011)
Maternal smoking during pregnancy (CD)	Roberts et al. (2011)	Lack of breastfeeding during gluten introduction	Akobeng and Heller (2006)
White collar employment	Sonnenberg and Walker (2011)	Altered diversity of gut bacteria	Sánchez et al. (2010)
Childhood antibiotic use (CD)	Hviid et al. (2011)	Neonatal infections	Sandberg-Bennich et al. (2002)
High dietary fat intake	Hou et al. (2011)	Infections as precipitating factors (i.e., triggers)	Plot and Amital (2009)
Lack of breastfeeding	Gearry et al. (2010)		
Lack of a childhood pet (CD)	Han et al. (2010)		
Oral contraceptives (strongest association for CD)	Cornish et al. (2008)		

[a]*CD* Crohn's disease; *UC* ulcerative colitis

[b]Ingestion of gluten and/or its components is associated with symptoms of celiac disease in susceptible individuals. They may or may not be a predisposing cause of the condition based on factors such as timing and maturation of exposure

well as between different categories of environmental factors (e.g., interactions among diet, infections, pets, hygiene, birth delivery mode, toxicants, drugs, stress). Such interactions are important in determining the ultimate risk of these diseases. For example, a focal point for environmental effects is the gut microbiome; this plays a key role in immune development and the risk these gastrointestinal-focused diseases (Siggers et al. 2011). Recent research suggests that a wide range of both genetic and environmental factors are likely to affect the spectrum of diversity and distribution of the gut bacteria across several stages of development (Spor et al. 2011). The extent of the potentially beneficial postnatal effect of breastfeeding on infant gut status may be affected by prior prenatal environmental conditions (e.g., maternal smoking, infections).

12.5.1 IBD

Molodecky and Kaplan (2010, 2011) recently reviewed studies involving several potential environmental factors (smoking, appendectomy, oral contraceptives, diet, breastfeeding, infections/vaccinations, antibiotics, and childhood hygiene) for IBD. These authors concluded that results are somewhat inconsistent and that more work is needed to identify and/or distinguish among priority environmental risk factors. A recent twin study in the UK suggested that smoking was associated with an increased risk of CD, but was protective against UC (Ng et al. 2012). There is a significant risk of CD in the children of mothers who smoked (Roberts et al. 2011). Exposure to smoking as a child or adolescent in New Zealand increased the risk of CD (Han et al. 2010). Current smoking increases the risk of CD (Cosnes 2010) yet is protective against UC (Carbonnel et al. 2009; Lakatos 2009).

Consistent with the idea that early-life factors can play a significant role in immune dysfunction-based diseases, birth delivery mode appears likely to affect the risk of later-life IBD, although the precise nature of the impact remains somewhat uncertain. Decker et al. (2010) reported that Caesarian delivery was as a risk factor for celiac disease but not for IBD (Decker et al. 2010). In contrast, a more recent study among a Danish population reported a moderately increased risk of IBD in children associated with Caesarian delivery (Bager et al. 2011). Preterm birth is associated with a significant increase in the risk of UC (Sonntag et al. 2007).

Interactions with normal gut microbes as well as with pathogenic infectious agents represent a risk factor for IBD. There is a suggestion that early-life infection may play a role in risk of IBD (Ruemmele et al. 2006). Chronic infections with bacteria such as *Campylobacter* spp. have been suggested to play a possible role in risk of CD. Keenan et al. (2011) suggested that a low rate of reported *Campylobacter* infections among Maori and Pacific islander populations may help to explain their relatively low prevalence of CD. Once IBD has been initiated, host resistance to certain infections can be affected. Patients with IBD have a higher risk of recurrent infection with *Clostridium difficile* (Kelsen et al. 2011).

Additionally, use of medications such as antibiotics and analgesics can be connected to infections as well. In studies of medications as risk factors, there is always

the consideration of whether an observed effect is the result of the medication itself or the infection that led to required use of antibiotics and/or analgesics. Shaw et al. (2010) found there is a greater likelihood that subjects with IBD were administered antibiotics in the first year of life. The investigators also reported that earlier use of antibiotics predisposed for a diagnosis of IBD. A dose–response relationship was reported between the number of antibiotic dispensations and risk of subsequent IBD (Shaw et al. 2011). The tetracycline class of antibiotics has been implicated with an increased risk of IBD (Margolis et al. 2010). In New Zealand, taking four or more antibiotics in a year as an adolescent increased the risk of CD (Han et al. 2011). In a prospective study, Hviid et al. (2011) found a significant association between childhood antibiotic use and risk of CD. In a prospective cohort study of adults associated with the European Cancer and Nutrition study, regular aspirin use was reported to significantly elevate the risk of CD (OR = 6.14, 95% CI = 1.76–21.35) but not UC (OR = 1.29, 95% CI = 0.67–2.46) (Chan et al. 2011).

Interactions between host immune cells and normal gut microbes are a significant factor in the risk of IBD. Factors that affect normal microbial diversity and load are important. IBD seems to have increased in prevalence very rapidly because of various environmental factors that have reduced the microbial diversity (dysbiosis) in the gut of children (Lakatos 2009). In contrast, factors that help to increase microbial diversity can be protective. The range of environmental factors that affect microbial diversity can be quite broad. For example, in New Zealand, having a pet as a child was reported to be a protective factor against CD (Han et al. 2010). Dietary regimes may also affect gut microbial diversity. Jantchou et al. (2010), using a prospective dietary study of 67,581 French middle-aged women, reported that both high total dietary protein and protein specifically from meat and fish was associated with an elevated risk of IBD. It seems likely that these various effects operate, at least in part, on the interactions between gut microbes and pattern recognition receptors associated with innate immune cells. For example, Scharl and Rogler (2010) discuss the interactions between NOD-2 receptor and TLRs in interactions with commensal bacteria and pathogenic bacteria in the pathogenesis of CD. The importance of these receptor-bacteria interactions is consistent with the report that the presence of NOD-1 and NOD-2 genes variants confers a different risk for CD (Vasseur et al. 2012).

Han et al. (2010) examined the potential role of 35 environmental factors in the risk of CD. In a North Island-based New Zealand study, previously discussed risk factors such as smoking, exposure to smoking as a child, prolonged use of antibiotics, and lack of a childhood pet in the home were found to be significant environmental risk factors. But one of the more intriguing observations from this study was that long-term use of oral contraceptives also increased the risk of CD (Han et al. 2010). The mechanistic basis for this latter interaction is not yet clear.

12.5.2 Celiac Disease

There are both familial-genetic (Freeman 2010) and environmental risk (Hall and Crowe 2011; Sanz et al. 2011) components to CED. Interestingly, the genetic factors

may overlap with others affecting other immune dysfunction disease outcomes (Gutierrez-Achury et al. 2011). Common genetic loci have been reported to be shared by CED and CD. These include IL-18RAP, TAGAP, PTPN2, and PUS10 (Festen et al. 2011).

An apparent shift in both prevalence and age of onset for children with CED in Sweden suggests that several risk factors might contribute to the disease (Olsson et al. 2008). This was further supported by geographic regional variation not explained by the known variables (Olsson et al. 2009). There is some indication that prolonged breastfeeding may reduce the risk of celiac disease (Ivarsson et al. 2000). In a study in Sweden, seasonality has also been reported to affect the risk of CED. Ivarsson et al. (2003) reported that children born in summer months were at en elevated risk for developing the disease compared with those born in the winter.

12.6 Conclusions

Inflammatory bowel disease, comprising both Crohn's disease and ulcerative colitis, and celiac disease are two major categories of immune-based chronic diseases targeting the gastrointestinal system. While they overlap in the significance of inflammation in these diseases, the influence of genetics, environmental risk factors, targeting within the GI tract, specific immune dysfunction and immune mediators or pathology, and comorbidities are quite distinct. Their prevalences have been increasing in recent decades beyond the contribution of improved diagnostics and more effective patient screening. One nexus point worthy of more attention is the capacity of early-life environment to dictate GI tract influences on immune development and later-life immune function. These chronic diseases demonstrate the need to effectively manage interaction of the microbiome and the developing immune system. The greatest window for managing the microbial modulation of immune development is during the prenatal-neonatal and adolescent stages of development,

While IBD and CED have a significant negative impact on quality of life, the likelihood of additional comorbid chronic diseases among patients is high. Associated diseases such as autoimmune conditions and cancer add to the health burden across a lifetime. For this reason, effective treatments that include risk reduction for possible comorbidities would be advantageous.

12.7 Recommendations

- Additional research is needed to better define the environmental risk factors for IBD and CED.
- A focus on gene–environment interactions is likely to be useful in examining these chronic diseases.
- Management of the early-life microbiome and its influence on immune development is one area of possible risk reduction.

- Treatment of inflammatory bowel disease and celiac disease should include a consideration of potential comorbidities.

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Chapter 13
COPD and Other Inflammatory Diseases of the Lung: Focus on AhR Signaling

Celine A. Beamer, Benjamin P. Seaver, and David M. Shepherd

Abstract Millions of individuals worldwide are afflicted with disorders affecting the lungs, resulting in difficulty breathing. Oftentimes, these diseases occur as a result of altered immune response in the lung. The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, acts as a regulator of mucosal barrier function and may influence immune responsiveness in lung diseases through changes in gene expression, cell-cell adhesion, mucin production, and cytokine expression. Of these cytokines, interleukin 22 (IL-22) signaling appears to be involved in lung inflammation and fibrosis, and both endogenous and exogenous AhR agonists promote IL-22 expression. Therefore, this chapter discusses the role of the AhR pathway and IL-22 signaling in lung diseases such as chronic obstructive pulmonary disorder, idiopathic fibrosis, Sarcoidosis, acute respiratory distress syndrome, and silicosis. Finally, we address the therapeutic potential of targeting the AhR—IL-22 axis in regulating immunity, inflammation, and tissue homeostasis in lung diseases.

Key Points

- The aryl hydrocarbon receptor (AhR) has a dual role as an activator of xenobiotic metabolism and as a participant in normal homeostasis, organogenesis, and immune activation. The AhR modulates the immune response in many respiratory diseases and the lung is sensitive to endogenous and exogenous AhR ligands.
- Interleukin 22 (IL-22) has emerged as an important cytokine in mucosal immunity and as a prospective mechanism to attenuate inflammation and fibrosis. Because AhR activation is required for IL-22 production by Th17

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cells, γδ T cells, and human Th22 cells, AhR ligands may represent a means to therapeutically manipulate IL-22 expression.
- While IL-22 expression is upregulated in chronic obstructive pulmonary disease (COPD) and correlates with disease progression, it is not clear whether IL-22 plays a causative or bystander role. Similarly, although the AhR may attenuate inflammation caused by cigarette smoke, the role of the AhR signaling pathway relative to IL-22 expression or function in COPD has yet to be examined.
- Data suggest that the AhR does not play a role in the generation of idiopathic fibrosis (IPF); however, the use of select AhR agonists to induce IL-22 may yet have therapeutic value in other types of lung fibrosis.
- To date, there exists a critical shortage of clinical and basic science studies examining the contributions of the AhR and/or IL-22 in Sarcoidosis. Although IL-22 is downregulated in Sarcoidosis patients, the significance of this finding remains unknown.
- The AhR contributes to immune regulation in models of acute respiratory distress syndrome (ARDS); yet to date, no studies have examined the contributions of AhR activation relative to IL-22 expression or function. Although patients with ARDS exhibit reduced levels of IL-22, the significance of this finding remains unknown.
- Although the AhR modulates acute and chronic inflammation in mouse models of silicosis and IL-22+ T cells accumulate in the lungs of silica-exposed mice, IL-22+ T cells do not appear to play a prominent role in chronic inflammatory and fibrotic process.

13.1 Introduction

Regulation of the immune response in the lung is a complex process that involves maintaining tolerance to endogenous self-antigens and innocuous particulates, while preserving the capability to appropriately respond to invading microorganisms and pathogens. Respiratory injury perturbs immune homeostasis by inducing an initial inflammatory response followed by a compensatory anti-inflammatory response. These opposing actions represent the behaviors of the innate and adaptive immune systems, respectively. The relative importance of the altered reactivity of these two systems in immune dysfunction observed in respiratory diseases is not yet defined and remains a subject of some controversy.

It has recently become evident that not only innate, but also adaptive, immune responses to pathogenic particles and microorganisms entering the lung are complex and dynamic events. To prevent invasion of pathogenic particles and microorganisms into the lower respiratory tract, the airways and lungs have innate defense mechanisms encompassing the epithelial barrier, mucociliary escalator, humoral factors (e.g., antimicrobial peptides, complement proteins, and surfactant proteins), and cells that participate in innate immunity: macrophages, dendritic cells, monocytes, neutrophils, natural killer cells, basophils, nuocytes, innate lymphoid cells,

and mast cells. In contrast, the adaptive immune response includes T helper (Th1, Th2, Th17, Th22, and Treg) lymphocytes, B lymphocytes, antibody-mediated responses, and granuloma formation. Innate inflammation is a key component of the host defense mechanism, but uncontrolled inflammation results in tissue destruction and disease. Anti-inflammatory responses regulate this process by blocking or suppressing the intensity of the inflammatory cascade, but unrestrained anti-inflammatory cascades contribute to abnormal wound repair and fibrotic disease. Hence, a balance between the effects of pro- and anti-inflammatory cells or soluble mediators (e.g., cytokines) is thought to influence the outcome of disease. Several forms of respiratory disease share the characteristic of accumulating inflammatory cells in the alveoli and in some cases fibrosis of the lung parenchyma and have been associated with a persistence of the inflammatory response, suggesting that a failure of normal homeostatic control occurs. Consequently, defining the cellular and molecular events involved in regulating inflammation and fibrosis will result in more effective diagnosis and treatment of respiratory diseases.

The recent identification of the aryl hydrocarbon receptor (AhR) as a master regulator of mucosal barrier function and the significance of AhR-mediated responses following activation by a variety of endogenous and exogenous ligands suggest that alterations in AhR expression or function may influence the inflammatory response in lung disease (Stejskalova et al. 2011). Similarly, components of interleukin 22 (IL-22) signaling appear to be involved in lung inflammation and fibrosis, and both endogenous and exogenous AhR agonists promote IL-22 expression. Therefore, we have organized this chapter relative to the relationship between a select group of inflammatory and fibrotic disorders of the lung: acute respiratory distress syndrome (ARDS), silicosis, Sarcoidosis, chronic obstructive pulmonary disorder, and idiopathic fibrosis (IPF), the AhR pathway, and IL-22 signaling. However, as the AhR is abundantly expressed throughout cells of the innate and adaptive immune systems, it is by necessity overly simplistic to attempt to explain its role in disease modification only through its affects on IL-22 biology. Moreover, a better understanding of the important functional AhR ligands (e.g., dietary substances, tryptophan photoproducts, and environmental pollutants) relative to the development of therapeutic and preventive approaches to inflammation and fibrosis is required. Thus, further investigation is necessary to understand the multifaceted role of the AhR in the context of acute and chronic inflammatory and fibrotic respiratory diseases.

13.2 Ligand-Dependent Regulation of the Aryl Hydrocarbon Receptor

Dioxins are a group of highly toxic chemicals known as persistent environmental pollutants found throughout the world. The term “dioxin” refers to a family of structurally and chemically related polychlorinated dibenzoparadioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and also includes certain dioxin-like polychlorinated

biphenyls (PCBs) with similar toxic properties. More than 400 types of dioxin-related compounds have been identified, yet only 30 of these exhibit significant toxicity, with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) being the most toxic. Due to their chemical stability and their ability to be absorbed into the fatty tissue, dioxins bioaccumulate and may persist in the body for a decade or longer. Dioxins are unwanted industrial by-products of waste incineration, chemical and pesticide manufacturing, and pulp and paper bleaching. Dioxins may also result from natural processes such as volcanic eruptions and forest fires. The most infamous dioxins were contaminants within the herbicide defoliant Agent Orange used during the Vietnam War, and more recently, the deliberate poisoning of Ukrainian president Viktor Yuschenko with TCDD in 2004 (Lee et al. 2010).

Experimental and epidemiological evidence has shown that dioxins, in many instances acting through the AhR, adversely affect a number of different organs and biological systems. The liver, adipose tissue, and skin are the major storage sites of dioxins and other AhR ligands in humans (Van den Berg et al. 1994). The AhR is highly conserved evolutionarily and is expressed in all major human cell types, including pulmonary tissue—although at differing levels (Ema et al. 1994; Kewley et al. 2004; Frericks et al. 2007; Kerkvliet 2009b; Mitchell and Elferink 2009; Chiba et al. 2011a, b). The AhR was first discovered as a mediator of dioxin toxicity in the early 1970s. Decades after its discovery and initial characterization, despite the fact that the mouse and human AhR genes have been cloned (Nebert and Karp 2008), the function(s) of the AhR remains a bit of a mystery (Whitlock 1999; Lee et al. 2010; Chiba et al. 2011a). Although the physiological role(s) of the AhR continues to be elucidated, it is clear that the AhR has a dual role as an activator of xenobiotic metabolism and as a participant in normal homeostasis, organogenesis, and immune activation (Kawajiri and Fujii-Kuriyama 2007; Chiaro et al. 2008; Esser et al. 2009; Mitchell and Elferink 2009; Marshall and Kerkvliet 2010). AhR-deficient mice, mice with a constitutively active AhR, and other AhR signaling mutants have been generated by various laboratories and used to analyze the physiological function of AhR, including its role in the immune system (Esser 2009). Moreover, numerous ligands have been discovered that bind to the AhR with variable affinity and potency, thereby providing important insight into the functions of the AhR (Thomas et al. 2002). These compounds contain tryptophan derivatives such as FICZ (6-formylindolo[3,-*b*]carbazole), IAA (indole-3-acetic acid), tryptamine, indirubin, ICZ (indolo[3,2-*b*] carbazole) and DIM (3,3′-diindolylmethane), prostaglandins such as PGG_2 and lipoxin A4, and heme metabolites including bilirubin and biliruverdin (Seidel et al. 2001; Fujii-Kuriyama and Kawajiri 2010). Another activator of the AhR, benzo(a) pyrene (BaP), is a component of cigarette smoke (Chiba et al. 2011a), whereas 17α-ketocholesterol is an AhR antagonist (Seidel et al. 2001). Although TCDD is widely used as the prototypical AhR ligand, its use can be problematic when studying the physiological function(s) of the AhR because of its extended half-life (~2 weeks in mice and 7–10 years in humans) (Miniero et al. 2001; Kerger et al. 2006). The effects of TCDD, and presumably AhR activation, on various biological responses have been studied for many years with much conflicting data in the literature. Indeed, diverse ligands produce differential outcomes depending on the context

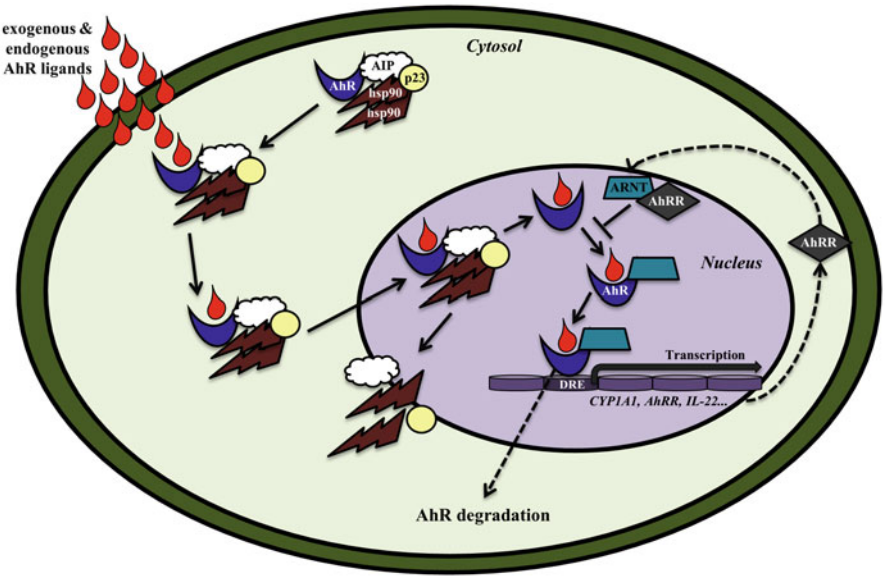

Fig. 13.1 Schematic representation of the canonical AhR signaling pathway. Cytosolic AhR is complexed by two molecules of heat shock protein 90 (Hsp90), AhR-interacting protein (AIP), and the cochaperone p23. Binding of endogenous and exogenous AhR ligands such as TCDD leads to a conformational change, thereby allowing nuclear translocation to occur. In the nucleus, the AhR dissociates from the complex and dimerizes with the AhR nuclear translocator (ARNT). The AhR–ARNT heterodimer then binds to dioxin-responsive elements (DREs) in the promoters of genes encoding for phase I and phase II metabolizing enzymes (e.g., CYP1A1), as well as the AhR repressor protein (AhRR) and interleukin 22 (IL-22). AhRR competes with AhR for binding to ARNT, and this AhRR/ARNT heterodimer binds to DRE leading to repression of transcription. Finally, after ligand-binding nuclear translocation-transcriptional activation, AhR is rapidly degraded

of the extracellular and intracellular environments (Esser et al. 2009). Therefore, a proper understanding of AhR biology must be context-dependent and differentiate between the dose- and cell-specific potential triggered by toxic ligands and the physiological effects triggered by endogenous ligands.

The AhR is a basic helix-loop-helix (bHLH), ligand-activated transcription factor that acts as a regulatory chemosensor (Sogawa and Fujii-Kuriyama 1997; Chiaro et al. 2008; Lee et al. 2010; Veldhoen and Duarte 2010). The AhR requires binding of an agonistic ligand to become functional and induce the activation of various signaling cascades (Fig. 13.1) (Seidel et al. 2001; Kawajiri and Fujii-Kuriyama 2007; Chiaro et al. 2008; Esser et al. 2009; Lee et al. 2010; Chiba et al. 2011a, b). In the cytosol, the AhR is chaperoned by heat shock protein 90 (Hsp90), p23, and AhR-interacting protein (AIP) (Rowlands and Gustafsson 1997; Kawajiri and Fujii-Kuriyama 2007; Chiaro et al. 2008; Esser et al. 2009). After ligand binding, the AhR dissociates from Hsp90 and p23 (Rowlands and Gustafsson 1997; Sogawa and Fujii-Kuriyama 1997; Whitlock 1999; Seidel et al. 2001; Chiaro et al. 2008), trans-

locates from the cytosol to the nucleus, heterodimerizes with the AhR nuclear translocator (ARNT), and binds to an enhancer sequence, referred to as dioxin response elements (DREs), within genes such as cytochrome P450 1A1 (CYP1A1) (Hankinson 1995; Sogawa and Fujii-Kuriyama 1997; Whitlock 1999; Seidel et al. 2001; Mimura and Fujii-Kuriyama 2003; Kawajiri and Fujii-Kuriyama 2007; Chiaro et al. 2008; Esser et al. 2009; Lee et al. 2010; Veldhoen and Duarte 2010; Chiba et al. 2011a, b). AhR/ARNT binding DRE results in the initiation of chromatin remodeling by disrupting the local structure without changing the remaining chromatin (Rowlands and Gustafsson 1997; Whitlock 1999), thereby increasing access to the promoter region (Seidel et al. 2001; Kawajiri and Fujii-Kuriyama 2007). The AhR/ARNT heterodimer forms a sort of scaffold for various coactivator complexes that affect transcriptional regulation (Hankinson 1995; Rowlands and Gustafsson 1997; Mitchell and Elferink 2009). It is the dual functions of chromatin unwinding and induction of gene expression that mediate downstream effects of AhR/ARNT transcriptional regulation (Rowlands and Gustafsson 1997).

As with most receptor systems, transcriptional or otherwise, signal termination provides another layer of regulation of receptor-mediated signaling. Prolonged receptor activity by AhR is linked to receptor degradation by ubiquination (Mitchell and Elferink 2009). Other means of terminating AhR signaling are the short half-life of the receptor (~8 h) (Hankinson 1995), depletion of ligand by gene products (enzymatic degradation), and the AhR repressor protein (AhRR) (Kawajiri and Fujii-Kuriyama 2007; Mitchell and Elferink 2009). AhRR is constitutively expressed in some tissues (heart and brain) and inducible in others (liver and lung) (Mitchell and Elferink 2009). The AhRR has nuclear localization signals and nuclear export signals that are similar to the AhR, yet wholly localized to the nucleus (Kawajiri and Fujii-Kuriyama 2007). AhRR competes with AhR for binding to Arnt, and this AhRR/Arnt heterodimer binds to DRE leading to repression of transcription (Kawajiri and Fujii-Kuriyama 2007; Mitchell and Elferink 2009). Finally, after ligand-binding nuclear translocation-transcriptional activation, AhR is rapidly degraded by a 26S proteasome mechanism (Kawajiri and Fujii-Kuriyama 2007).

The respiratory system is sensitive to AhR ligands (Chiba et al. 2011b). BaP and dioxin-like compounds may be converted by cytochrome P450 enzymes into toxic metabolites that may concentrate in bronchial epithelial cells and exert carcinogenic and immunomodulatory effects (Alexandrov et al. 2010). PCBs and dioxin-like compounds accumulate in the airways of exposed mice and can be detected in the sputum of Yusho oil disease patients following ingestion of contaminated rice bran oil containing dioxins, PCBs, and other organochlorine compounds (Brandt 1975). These noxious compounds are known causative agents of chronic bronchitis, allergic asthma, chronic obstructive pulmonary disease (COPD), and lung cancer (Nagayama et al. 1976; Chiba et al. 2011a, b). Recent reports have begun to investigate the role of the AhR in diverse pulmonary diseases using selective AhR agonists and antagonists, and various AhR-deficient mouse models. From these studies, it was revealed that the AhR functions to modulate the immune response in various respiratory diseases (Kimura et al. 2008; Chiba et al. 2011a, b; Rico de Souza et al. 2011).

Activation of the AhR by TCDD results in a range of toxic endpoints, including profound immunosuppression (Mandal 2005; Lawrence and Kerkvliet 2006; Kerkvliet 2009b). In vitro and in vivo immunotoxicological studies have revealed dramatic alterations in thymocyte lineage, shifts in immune-cell subset frequencies, and aberrant cytokine secretion among many other effects. A majority of the cells that participate in immune responses constitutively or inducibly express the AhR (Kerkvliet 2009a; Fujii-Kuriyama and Kawajiri 2010). Many genes that participate in immune responses have DRE sequences in their promoters and are responsive to AhR ligands (Kerkvliet 2009a; Stevens et al. 2009). Moreover, it is important to note that although antigen presenting cells such as macrophages and dendritic cells express AhR (Esser et al. 2009; Veldhoen and Duarte 2010), the AhR also prominently participates in Th cell differentiation, particularly with regard to FoxP3+ Tregs and IL-10 producing Tr1 cells (Esser et al. 2009; Marshall and Kerkvliet 2010; Stockinger et al. 2011), and Th17 and Th22 cells (Esser et al. 2009; Veldhoen and Duarte 2010). Deficiency in AhR or treatment with AhR antagonists impairs but does not ablate Th17 differentiation (Veldhoen et al. 2008, 2009). Indeed, AhR activation is required for IL-22 production in Th17 cells (Esser et al. 2009), γδ T cells (Martin et al. 2009), and human Th22 cells (Duhen et al. 2009; Trifari et al. 2009). Interestingly, Th cells from AhR-deficient mice, while still being able to develop Th17 responses, fail to produce IL-22 (Veldhoen et al. 2008). The exact mechanisms whereby AhR promotes IL-22 production remains unknown at the present time. Although DRE are present in the IL-22 gene locus, binding of the AhR to the IL-22 promoter or other regulatory elements has not been confirmed experimentally. Given the fact that TGFβ is a potent suppressor of IL-22, it is puzzling that the expression of AhR in Th17 cells requires TGFβ + IL-6 or TGFβ + IL-21 (Zheng et al. 2007; Kimura et al. 2008; Quintana et al. 2008; Duhen et al. 2009). While neither IL-6 nor IL-21 alone is sufficient to induce AhR, T cells cultured with either cytokine in the absence of TGFβ express the highest levels of IL-22 (Zheng et al. 2007). These data suggest that AhR is dispensable for high-level expression of IL-22, but rather functions to modulate the suppressive effects of TGFβ.

13.3 Regulation of Interleukin 22 Expression

Interleukin 22 (IL-22) was first characterized in 2000 in a screen to identify previously unknown cytokine transcripts in a mouse thymic T cell line (Dumoutier et al. 2000a). Mouse IL-22 shares structural similarities and 22% sequence homology with mouse IL-10; whereas human IL-22 shares 79% homology with mouse IL-22 and 25% sequence homology with human IL-10 (Dumoutier et al. 2000b). IL-22 was further classified as a class 2 α-helical cytokine of the IL-10 family, which consists of IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26 (Pestka et al. 2004). While IL-10 largely functions as an anti-inflammatory cytokine, IL-22 appears to mediate the cross talk between leukocytes and tissue epithelia, with its receptor being expressed mainly on epithelial cells. IL-22 signals through a

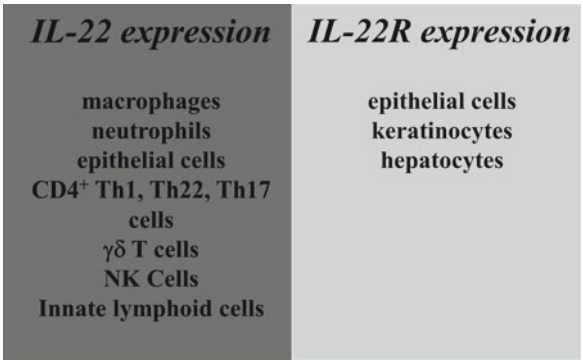

Fig. 13.2 Relationship between IL-22 and IL-22R expression. Expression of IL-22 appears to be restricted to cells of the innate and adaptive immune responses. This includes macrophages, neutrophils, epithelial cells, CD4+ T cells (Th1, Th22, Th17), γδ T cells, NK cells, LTi and LTi-like cells. In contrast, expression of the IL-22 receptor (IL-22R) seems to be restricted to nonhematopoietic cells of the skin, pancreas, intestine, liver, lung, and kidney, thereby providing signaling specificity

distinct class 2 receptor (IL-22R) consisting of IL-22R1 and IL-10R2 (Whittington et al. 2004), which are independently shared with IL-10, IL-20, IL-24, IL-26, and IL-28/29 (Sonnenberg et al. 2011; Zhang et al. 2011). First, IL-22 binds to IL-22R1, and then the IL-22/IL-22R1 complex binds IL-10R2 to propagate downstream signaling events (Logsdon et al. 2002; Li et al. 2004). Binding of IL-22 to its heterodimeric receptor results in the activation of the Jak-STAT signal transduction pathway, inducing phosphorylation of Jak1 and Tyk2 kinases, and STAT1, STAT3, and STAT5 transcription factors, as well as the Akt and mitogen-activated protein kinase (MAPK) pathways (Whittington et al. 2004; Louten et al. 2009; Sonnenberg et al. 2011). In turn, these signaling cascades result in induction of various tissue-specific genes, including serum amyloid A (SAA), antimicrobial proteins (e.g., β-defensins, Reg3γ, and lipocalin-2), and mucins, which exhibit both localized and systemic effects (Zenewicz and Flavell 2011). In addition to the cell surface IL-22 receptor complex, alveolar macrophages, alveolar epithelial cells (AECs), and neutrophils express a soluble secreted monomeric receptor of IL-22, called IL-22 binding protein (IL-22BP), which may function to sequester IL-22 and block interactions with IL-22R (Whittington et al. 2004; Zhang et al. 2011). In this manner, IL-22BP may act as an IL-22 antagonist to regulate inflammation (Whittington et al. 2004).

IL-22 expression appears to be restricted to cells of the innate and adaptive immune responses. This includes macrophages, neutrophils, epithelial cells, CD4+ T cells (Th1, Th22, Th17), γδ T cells, NK cells, LTi and LTi-like cells (Fig. 13.2) (Whittington et al. 2004; Di Stefano et al. 2009; Louten et al. 2009; Simonian et al. 2010; Zenewicz and Flavell 2011). In contrast, the IL-22R seems to be restricted to nonhematopoietic cells of the skin, pancreas, intestine, liver, lung, and kidney, thereby providing signaling specificity (Whittington et al. 2004; Lo Re et al. 2010; Sonnenberg et al. 2011;

Zenewicz and Flavell 2011). Regulation of IL-22 expression by these different subsets of immune cells has some commonalities such as similar activation receptors and transcription factors, but there are also some unique mechanisms. IL-22 is most highly expressed on Th17 cells (Liang et al. 2006). IL-22 expression differs from that of IL-17A and other Th17-associated cytokines. Conditions that promote IL-17 expressing T cells in vitro (e.g., IL-6+TGFβ) do not lead to optimal IL-22 expression, and in fact may repress IL-22 expression (Zenewicz and Flavell 2011). Furthermore, IL-17A production may occur independently of TGFβ signaling by differentiating naïve T cells in the presence of IL-1β, IL-6, and IL-23—conditions that also lead to enhanced IL-22 expression (Ghoreschi et al. 2010; Zhang et al. 2011). Finally, during infections in vivo, IL-22 expression is dependent on IL-23, but IL-17 is less so (Volpe et al. 2008; Munoz et al. 2009; Siegemund et al. 2009). IL-17A is highly dependent on the nuclear hormone receptor transcription factors retinoic acid-related orphan receptor γt (RORγt) and RORα for its expression, whereas IL-22 is less so. In contrast, IL-22 expression requires the AhR. AhR ligands such as environmental toxins and endogenous breakdown products of aromatic amino acids augment IL-22 expression during immune responses both in vitro and in vivo (Veldhoen et al. 2008, 2009; Veldhoen and Duarte 2010). Furthermore, Notch signaling induces production of endogenous AhR ligands resulting in enhanced IL-22 expression (Alam et al. 2010).

Recently, a subset of human CD4+ T cells that specifically expresses IL-22 was distinguished from Th17 and other known T cell subsets with regard to gene expression and function (Eyerich et al. 2009). Th22 cells do not express IFNγ or IL-17A, or the transcription factors T-bet or RORγt (Duhen et al. 2009); however, Th22 cells do produce IL-22, IL-26, and IL-13, of which IL-22 is the most important functional cytokine. Th22 cells express the chemokine receptors CCR4, CCR6, and CCR10, allowing for localization to the skin (Duhen et al. 2009; Trifari et al. 2009). Similar to murine Th17 cells, the AhR is the key transcription factor in Th22 cells (Trifari et al. 2009). Recent studies indicate that IL-6 and TNFα can promote the Th22 phenotype and that the addition of active vitamin D enhances IL-22 expression (Duhen et al. 2009). Interestingly, Langherhans cells found in the epidermis are able to induce Th22 cells, as were plasmacytoid dendritic cells (Duhen et al. 2009; Fujita et al. 2009). If cultured in the presence of Th1, Th2, Th17, or Treg polarizing conditions, Th22 clones continue to express IL-22 and not the prototypical cytokines associated with other Th subsets (Eyerich et al. 2009). Th22 cells appear to play a prominent role in skin homeostasis and inflammation as the Th22 cell population is increased in psoriasis patients (Kagami et al. 2010).

The γδ T cell subset is a unique subset of lymphocytes whose function is poorly understood. Based upon their location in the subepithelium of alveolar and nonalveolar regions of the lung (Wands et al. 2005), γδ T cells have been implicated in the regulation of the immune response generated against inhaled particles, microbial pathogens, and allergens (Born et al. 2007; Nanno et al. 2007). Unlike conventional CD4+ T cells, γδ T cells constitutively express IL-23R and, therefore, after IL-23 stimulation will express IL-17A and IL-22 (Martin et al. 2009; Zenewicz and Flavell 2011). As with Th17 and Th22 cells, the AhR is an important transcription factor for the expression of IL-22, but not IL-17A by γδ T cells (Martin et al. 2009;

Simonian et al. 2010). IL-22 expressing γδ T cells are particularly important in pulmonary immune responses (Zenewicz and Flavell 2011). γδ T cells isolated from the lung of mice chronically exposed to *Bacillus subtilis* express IL-22, and that expression of IL-22 is an important mechanism by which these cells attenuate inflammation and fibrosis (Simonian et al. 2010).

IL-22 has emerged as an important cytokine in mucosal immunity (Simonian et al. 2010). The mechanisms by which IL-22 protects epithelial structures in the lung, gut, and other organ systems from damage induced by numerous environmental insults are under intense investigation. Emerging data suggest that IL-22 may provide mucosal protection by inducing antimicrobial peptides from epithelial cells in the lung, gut, and skin as well as maintaining epithelial integrity either by preventing injury or accelerating epithelial repair after a variety of insults (Liang et al. 2006; Zheng et al. 2007; Aujla et al. 2008; Aujla and Kolls 2009).

IL-22 acts as an important regulator of tissue responses to inflammation expressed predominately by epithelial cells, but also by innate and adaptive immune cells by attenuating lung inflammation and decreasing collagen deposition. However, the functional importance of IL-22 is context-dependent. For example, in the lung, after intratracheal administration of bleomycin, IL-22 acts synergistically with IL-17A to promote pathological airway inflammation (Sonnenberg et al. 2010), yet IL-22 can promote the survival and repair of epithelial cells and limit excessive fibrosis (Aujla et al. 2008; Simonian et al. 2010). Immunoblotting demonstrated that lower levels of IL-22 were present in the bronchoalveolar lavage (BAL) fluid of patients with ARDS and Sarcoidosis relative to control subjects (Whittington et al. 2004).

IL-22/IL-22R interactions may induce the expression of genes encoding molecules involved in proliferation, anti-apoptotic pathways, tissue repair, inflammation, immune surveillance, and homeostasis (Zenewicz and Flavell 2011). Consistent with that, IL-22 is highly expressed in several inflammatory conditions including psoriasis, irritable bowel disease, and rheumatoid arthritis (Whittington et al. 2004; Zhao et al. 2010; Zenewicz and Flavell 2011). IL-22 expression is upregulated and correlates with disease progression; however, it is not entirely clear whether IL-22 plays a causative role in inflammation or occurs as a result of it. Using both gene-deficient mice and administration of neutralizing antibodies, investigators have demonstrated both harmful and protective functions of IL-22 in different models of inflammatory conditions. Future challenges include defining the context-dependent functions of IL-22 expression in tissue inflammation and repair and designing therapeutics targeting the IL-22/IL-22R pathway for the treatment of persistent infection, chronic inflammation, and autoimmune diseases.

13.4 Chronic Obstructive Pulmonary Disease

COPD affects over 200 million people worldwide and is the third leading cause of mortality in the United States (Lopez et al. 2006). Although the major environmental risk factor accounting for 80–90% of lung cancer and COPD cases is cigarette

smoking, only 10–20% of smokers develop COPD (Hukkanen et al. 2002; Pauwels and Rabe 2004; Postma and Boezen 2004; Cheng et al. 2009; Vibhuti et al. 2010). COPD has also been linked to long-term exposure to lung irritants such as second-hand smoke, biomass burning, air pollution, chemical fumes, or dust from the environment or workplace (Hukkanen et al. 2002; Postma and Boezen 2004; Rahman et al. 2006; Salvi and Barnes 2009; Alcorn et al. 2010; Uslu et al. 2010). COPD is preventable and treatable, but the pulmonary sequelae of COPD are generally not reversible (Uslu et al. 2010). Although there is no cure for COPD, treatments and lifestyle changes may alleviate symptoms and slow disease progression. Cessation of smoking, pulmonary rehabilitation, and supplemental oxygen therapy have proven of value, whereas there is often little to no response to high-dose corticosteroids. COPD treatment typically consists of bronchodilators in combination with β2 agonist, anticholinergic drugs, and/or Theophylline to further alleviate symptoms (Uslu et al. 2010).

Numerous mechanisms (e.g., genetic susceptibility, epigenetic changes, viral or bacterial infections, and oxidative stress) contribute to disease progression and have been linked to the pathogenesis of COPD. In much the same way as differences in genetic susceptibility in human smokers affect development of COPD, the induction of emphysema in mice is strain-dependent (Guerassimov et al. 2004). First, the hallmark of COPD is development of a progressive disease characterized by chronic inflammation in response to inhalation of cigarette smoke (Hogg and Timens 2009). Not only does induced sputum harbor neutrophils (PMNs), but eosinophils may also be present with either viral or combined viral/bacterial infections, and there are also increases in CD4+ Th1 and CD8+ lymphocytes (Fabbri et al. 2006; Papi et al. 2006). Airway hyperreactivity is a risk factor for developing COPD-like symptoms, and that risk is further increased if eosinophilia is present (Postma and Boezen 2004; Mirsadraee et al. 2005). Second, individuals with α1-antitrypsin deficiencies develop emphysema early in life due to an imbalance between proteinases and anti-proteinases resulting in a net increase in proteolytic activity (Laurell and Eriksson 1963). Third, in individuals with COPD an imbalance between oxidants and antioxidants leads to oxidative stress, airway inflammation, and cell death of alveolar epithelial and endothelial structural cells (Rahman and Adcock 2006). Disruption of the cycle of structural cell death and replacement contributes to the destruction of alveolar septa, leading to emphysema. Moreover, age-related changes in immune function and cellular senescence may further impede ongoing tissue repair processes in response to recurring cigarette smoke-induced injury (Tsuji et al. 2004). Lastly, autoimmunity has also been implicated as a pathogenic event in the progressive course of COPD (Cosio et al. 2009). Finally, it should be clearly stated that these diverse mechanisms do not operate independently in the pathogenesis of COPD, but are interconnected in a more complex manner. For example, oxidative stress may contribute an excess of apoptotic cells, which in turn may lead to secondary necrosis, which can exaggerate continuing pulmonary inflammation, and may contribute to autoimmunity (Demedts et al. 2006; Vandivier et al. 2006; Aoshiba and Nagai 2009).

Many inhaled chemical compounds, including components of cigarette smoke, are metabolized to reactive intermediaries by cytochrome P450 enzymes (Hukkanen et al. 2002; Schneider and Bernges 2009; Vibhuti et al. 2010). Clara cells, alveolar cells, type I and II, endothelial cells, macrophage, and ciliated bronchiolar cells express CYP enzymes (Hukkanen et al. 2002). Exposure to cigarette smoke including BaP and other dioxin compounds is a proven cause of COPD and lung cancer (Kuper et al. 2002; Stewart et al. 2008). PAHs are present in high levels in cigarette smoke and are primarily metabolized by CYP1A1, following AhR-dependent transcription of genes involved in detoxification (Cheng et al. 2009; Schneider and Bernges 2009; Rico de Souza et al. 2011). CYP1A1 produces the aromatic hydrocarbon hydroxylase, which is the first metabolic oxidation step in PAH, degradation, and CYP1A2 activates aromatic amines (Vibhuti et al. 2010). As a result, genetic polymorphisms in pulmonary cytochrome P450 enzymes play a prominent role in disease susceptibility (Hukkanen et al. 2002). Of the CYP1A2 forms, CYP1A2*F is associated with an increase in enzyme function, and *1C, 1K, 3, 4, 6–8, 11, 15, and 16 are associated with a decrease in enzyme activity (Uslu et al. 2010). Although CYP1A2*1C is correlated with low inducibility of CYP1A2 in smokers and CYP1A2*1F with high inducibility, neither polymorphism contributes to COPD (or asthma) (Takata et al. 2006). In Turkish COPD patients, a higher incidence of CYP1A2*1D and 1F was associated with a higher risk of the disease (Uslu et al. 2010).

Acute cigarette smoke exposure results in the activation of several pattern recognition receptors (PRRs) including toll-like receptors (TLRs), either directly by individual components or metabolites of cigarette smoke, or indirectly by causing injury to epithelial cells that release danger-associated molecular patterns or signals (DAMPs). After exposure to cigarette smoke, several mechanisms such as inhalation of toxic agents and irritants, oxidative stress, infections, and tissue hypoxia may all cause the release of DAMPs by injured airways. Activation of PRRs such as TLRs and RAGE leads to increased expression of pro-inflammatory cytokines and chemokines, such as IL-1β. Indeed, many cytokine and receptor-deficient mice including IL-1R-deficient mice show attenuated pulmonary inflammation after acute exposure to cigarette smoke and significant protection against emphysema after chronic exposure (Churg et al. 2009).

The pathological hallmarks of COPD are destruction of the lung parenchyma, which characterizes pulmonary emphysema; inflammation of the peripheral airways, which characterizes respiratory bronchiolitis; and inflammation of the central airways (Di Stefano et al. 2009). Activated PMNs, eosinophils, and macrophages have been well established in the resulting pathology of COPD (Alcorn et al. 2010), causing tissue destruction through the release of reactive oxygen species (ROS) and proteolytic enzymes such as elastase and matrix metalloproteinases (Chiba et al. 2011a). In addition, several subsets of T lymphocytes showed significant increases, which can be correlated with disease severity including CD4+ Th1 and Th17 cells, CD8+, and Tregs (Brusselle et al. 2011). Importantly, although patients with COPD accumulate activated T cells in the bronchial mucosa, immunodeficient mice lacking T and B cells develop cigarette smoke-induced inflammation and COPD

(D'Hulst et al. 2005). Patients with COPD also have increased numbers of IL-17A+, IL-22+, and IL-23+ cells in the bronchial mucosa compared to controls (Di Stefano et al. 2009). However, because production of these cytokines is linked to cells other than lymphocytes, sources other than T cells may be important. Overexpression of IL-17 in a mouse model produces COPD-like lung inflammation including CD4 recruitment, mucus hypersecretion, chemokine induction, and small airways fibrosis (Di Stefano et al. 2009). Consequently, IL-17 immune responses may play a role in COPD (Alcorn et al. 2010; Lo Re et al. 2010). Similar definitive data examining the contributions of IL22 to COPD are presently lacking. However, studies showing increased expression of IL-17A, IL-22, and IL-23 in the bronchial mucosa of stable COPD patients indicate that these Th17-related cytokines may be involved in the T cell and endothelial cell activation reported in patients with COPD. This, in turn, can play a role in inducing neutrophilia and tissue remodeling. However, their true relevance will await clinical studies with selective antagonists or monoclonal antibodies, some of which are already in clinical development.

Although recent publications indicate that the AhR attenuates pulmonary inflammation caused by cigarette smoke perhaps by regulating oxidant enzyme expression (Baglole et al. 2008; Rico de Souza et al. 2011), the role of AhR signaling relative to IL-22 expression or function in COPD has yet to be examined. COPD patients have increased numbers of IL-22+ cells in the bronchial mucosa compared to controls (Di Stefano et al. 2009). It is not yet known whether these cells are lymphocytes and whether the expression of IL-22 is dependent on ligand binding to the AhR. These results would provide substantial support for the hypothesis that the AhR is a novel and central regulator of immunopathogenic processes implicated in COPD etiology.

13.5 Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive and fatal disease characterized by ongoing dyspnea, bilateral interstitial infiltrates, and decreased pulmonary function testing. It is usually diagnosed in the sixth to seventh decade of life. Thus, IPF is a chronic lung disease with a slow progression that includes bursts of deterioration known as acute exacerbations (El-Chemaly et al. 2009; Corte and Wells 2010; Coward et al. 2010). Early symptoms are generally vague such as dyspnea, dry cough, and decreased exercise tolerance—symptoms often attributed to increasing age. This situation often results in a diagnostic delay leading to additional loss of lung function. Although previously uncommon, the incidence of this disease is increasing (Frankel and Schwarz 2009), and unfortunately, IPF causes irreversible loss of lung function (Simonian et al. 2010). Furthermore, there is no effective treatment for this devastating lung disease and a poor prognosis with the average survival being 3–5 years from diagnosis (El-Chemaly et al. 2009; Coward et al. 2010). Possible causes of IPF include respiratory infection, left heart failure, pulmonary embolism, pulmonary hypertension, fibrogenic xenobiotics (e.g., silica and asbestos), cigarette smoke, and idiopathic causes (Corte and Wells 2010; Boucher 2011).

Many cases of IPF are diagnosed based on clinical presentation and radiographic imaging. However, in a subset of individuals in whom a lung biopsy is performed, a characteristic pattern known as usual interstitial pneumonia (UIP) is observed (ATS and ERS 2002). UIP is characterized by fibrotic lung remodeling with temporal heterogeneity (a pattern in which normal lung, dense fibrosis and scar, and active areas of remodeling can be found simultaneously in the same lung specimen), hyperplastic type II AECs lining areas of fibrosis, presence of fibroblastic foci, minor amounts of inflammation, and thickening of the inter-alveolar septa. The traditional paradigm of IPF is one of chronic inflammation leading to fibrosis (El-Chemaly et al. 2009). The fact that anti-inflammatory agents do not treat IPF indicates that it is not an inflammatory pathology, but rather one of impaired epithelial injury/repair (Coward et al. 2010). Increasingly, IPF is being considered an abnormal healing response after repeated alveolar damage (Maher et al. 2010), and in this model repeated lung injury leads to a compromised alveolar-capillary basement membrane allowing entry of profibrotic cells (Coward et al. 2010). The genes involved are associated with AEC regulation of cell cycle and apoptosis (Boucher 2011). Taken together, these findings led to the overall model whereby a combination of genetic predisposition and environmental stressors results in a recurrent injury leading to ongoing lung damage and, in susceptible individuals, fibrosis (Boucher 2011).

Although basic and clinical research have yielded significant breakthroughs in our understanding of IPF, many aspects regarding the pathogenesis of this disease remain unknown. Oftentimes in basic research, the biggest obstacle is the creation of an appropriate animal model that is reflective of the disease process in humans, and IPF is no exception. To date, none of the current animal models adequately replicate the findings in IPF. Many different approaches to modeling pulmonary fibrosis have been used by investigators, including exposure to bleomycin, silica, or fluorescein isothiocyanate (FITC); irradiation; or expression of specific genes through delivery of a viral vector or utilization of a transgenic system. Each has served important purposes and contributed meaningfully to the body of knowledge about the disease process, yet each model has problems and pitfalls. This is most aptly demonstrated by the fact that >200 drugs have been shown to be beneficial in murine models, but essentially none have translated into therapeutic agents for IPF (Moeller et al. 2008; Moore and Hogaboam 2008).

Bleomycin continues to be the most frequently used agent in the laboratory and can be given directly into the airway by intratracheal (i.t.) or intranasal (i.n.) routes or systemically via subcutaneous, intraperitoneal, or intravenous injection. Bleomycin is a chemotherapeutic agent, inactivated thorough the enzyme bleomycin hydrolase, that causes pulmonary fibrosis as an uncommon side effect in patients undergoing therapy for various cancers (Muggia et al. 1983). The lung produces this enzyme in lower levels and, thus, is more prone to the toxic effects. Bleomycin is thought to cause lung damage through direct DNA strand breakage and the generation of free radicals, leading to the induction of oxidative stress (Moeller et al. 2008).

The major advantages of the bleomycin model are ease of drug delivery and short time to fibrosis manifestations. After drug delivery, intense inflammation and edema with an elevation in pro-inflammatory cytokines such as TNFα and IL-1β occur during the first week (Hoshino et al. 2009). By 2 weeks postexposure,

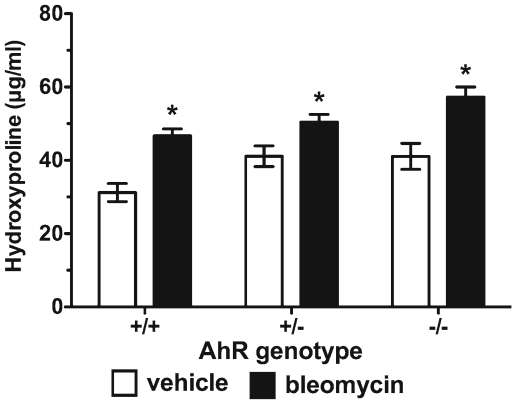

Fig. 13.3 Collagen deposition in AhR-deficient mice following exposure to bleomycin. Regardless of AhR status, all mice exposed to a single episode of 2U bleomycin exhibited significantly higher levels of hydroxyproline 14 days later compared to their respective vehicle controls. $n = 4–6$ mice/group. Values are means ± SEM; $^*p < 0.05$ compared to vehicle controls

expression of TGFβ peaks as well (Higashiyama et al. 2007; Moeller et al. 2008). By the third week, there is development of patchy isolated collagen deposits resembling fibrosis. Unfortunately, this model fails to recapitulate the important characteristics of UIP: fibroblastic foci, hyperplastic epithelium, and temporal heterogeneity. In addition, the PMN-dominated inflammatory response is more suggestive of acute lung injury (ALI) than fibrosis. Other major pitfalls include the fact that the murine lung is able to rapidly recover such that the disease findings are reversible to near normal by ~6 weeks postexposure (Chung et al. 2003), and that bleomycin-induced fibrosis is limited in certain mouse strains, due to strain-dependent variability in bleomycin hydrolase. However, despite these limitations, the bleomycin model of IPF has been enormously helpful in understanding critical aspects of pulmonary fibrosis and remains an important tool for ongoing and future use.

As previously introduced, IL-22 is an important cytokine in mucosal immunity (Aujla and Kolls 2009). Currently, the mechanisms by which IL-22 may protect epithelial structures in the lung from damage are under intense investigation. Emerging data suggest that IL-22 may provide mucosal protection by maintaining epithelial integrity either by preventing injury or accelerating epithelial repair (Liang et al. 2006; Zheng et al. 2007; Aujla et al. 2008). Although there was no change in levels of IL-22 in BAL from idiopathic pulmonary fibrosis patients (Whittington et al. 2004), other reports have suggested that IL-22 plays a critical role in regulating collagen deposition in the lung (Simonian et al. 2010). IL-17A is necessary for lung fibrosis in the bleomycin model either through IL-1β (Wilson et al. 2010) or interactions that regulate IL-22 expression and function (Sonnenberg et al. 2010). Recent results from our laboratory using the single episode bleomycin model indicated no difference in collagen deposition levels (measured by hydroxyproline analysis) (Fig. 13.3) and no difference in IL-22 levels in the BAL (data not shown) between AhR-deficient mice and wild-type controls. Although these data suggest that the AhR does not play a role in the generation of this form of fibrosis, the use

of select AhR agonists may yet have therapeutic value in other types of fibrosis through the induction of IL-22. AhR signaling is important for IL-22 expression by αβ and γδ T cells. In addition, neutralization of IL-22 by treatment with an anti-IL-22 mAb resulted in elevated numbers of CD4+ T cells and acceleration of lung fibrosis (Veldhoen et al. 2008; Martin et al. 2009). Fibroblasts express IL-22R, and IL-22 directly modulate fibroblast inflammatory functions by increasing IL-6 and IL-8, G-CSF, and matrix metalloproteinase production. Therefore, whether AhR activation and IL-22 protect or promote lung inflammation and fibrosis is likely dependent on the inhaled exposure.

13.6 Sarcoidosis

Sarcoidosis is a common, immune-mediated disease that produces noncaseating, epithelioid granulomas frequently manifesting in granulomatous pulmonary disease, yet lacking a defined etiology and origin (Prasse et al. 2000; Hauber et al. 2003; Whittington et al. 2004; Callejas-Rubio et al. 2008; Lodha et al. 2009; de Boer and Wilsher 2010). Although ~90% of patients demonstrate thoracic involvement, any organ may be affected. Sarcoidosis affects men and women of all ages and ethnicity (de Boer and Wilsher 2010). Mortality in Sarcoidosis is <5% (Hunninghake et al. 1999), with progressive pulmonary fibrosis being the most common cause of death. A combination of genetic susceptibility and a currently unknown environmental insult triggers development of Sarcoidosis (de Boer and Wilsher 2010). The two major exposures considered to play a role in the pathogenesis of Sarcoidosis are microbial organisms (e.g., tuberculosis) and noninfectious environmental agents. Due to the histological similarities between tuberculosis and Sarcoidosis, many attempts have been made to identify mycobacterial products from patients with Sarcoidosis, yet intact mycobacterium have not been conclusively identified as a causative agent. More recent work identified mycobacterial nucleic acids, proteins, and specific T cell immune responses to mycobacterial peptides in subjects with Sarcoidosis (Koth et al. 2011). Together, these data suggest that while an immune response to components of mycobacterium may play a role in certain individuals with Sarcoidosis, other events contribute to pathogenesis. These exposures include agricultural employment, mold or mildew, musty odors at work, pesticide-using industries, and dust from the fallen World Trade Center towers in New York City (Izbicki et al. 2007). Exposures negatively associated with Sarcoidosis include those allergic responses linked to household animal dust, feathers, or down pillows, and tobaccos use (Morgenthau and Iannuzzi 2011). In summary, no single cause of Sarcoidosis has been identified, suggesting that multiple triggers likely exist.

Many Sarcoidosis patients are asymptomatic and identified with a clinical diagnosis by incidental diagnostic tests, after exclusion of other possible pathologies. Currently, no reliable prognostic biomarkers have been identified. Sarcoidosis is a diagnosis of "exclusion" supported by: (1) compatible clinical and radiologic findings; (2) tissue biopsy specimen that reveals noncaseating epithelioid granulomas; and (3) the absence

of known granulomagenic agents (Hunninghake et al. 1999). Although multiorgan chronic disease is the feature clinical presentation, ~90% of Sarcoidosis patients exhibit manifestations of lung disease such as airway hyperreactivity, pulmonary inflammation, and fibrosis (Prasse et al. 2000; Hauber et al. 2003; Postma and Boezen 2004; Lodha et al. 2009; de Boer and Wilsher 2010). There is a differential risk/outcome severity correlated with ethnicity: erythema nodosum in females with Northern European ancestry, extra-pulmonary disease in African/Polynesian ancestry, and severe pulmonary disease in Indian/Asian ancestry (Lodha et al. 2009; de Boer and Wilsher 2010). Initial pharmacotherapy is with oral corticosteroids, although not all patients are treated due to spontaneous remission (de Boer and Wilsher 2010). In fact, more than 80% of patients experience mild symptoms or spontaneous recovery (Hauber et al. 2003). Given the mildness of most patient symptoms, a counter anti-inflammatory response has been proposed as the mitigating factor (Hauber et al. 2003).

During the past 2 decades, the immunopathogenesis of Sarcoidosis has been elucidated more clearly. Recent studies demonstrated that numerous cellular and soluble mediators of inflammation and immunity are involved in the immunopathogenesis of Sarcoidosis. Gene expression analyses clearly defined cytokine dysregulation in Sarcoidosis. IL-1, IL-2, IL-6, IL-8, IL-12, IL-18, IFNγ, and TNFα all contribute to cytokine cascades by promoting the recruitment, activation, and proliferation of mononuclear cells, as well as generating alveolitis and granulomas and affecting the final disease outcome (Hauber et al. 2003; Whittington et al. 2004; de Boer and Wilsher 2010). One of the current models suggests the alliance of CD4+ T cells, alveolar macrophages, and an interwoven cytokine signaling network creates the inflammatory process (Hauber et al. 2003). There are a profoundly increased number of activated CD4+ T cells infiltrating the pulmonary interstitium, compared to the blood, in Sarcoidosis (Prasse et al. 2000; Whittington et al. 2004; Koth et al. 2011). Up to 90% of BAL lymphocytes in Sarcoidosis stain positive for Th1 cytokines (Prasse et al. 2000). Very high numbers of activated CD4+ lymphocytes, in association with activated macrophages and IFNγ, participate in granuloma formation. IL-18 levels are increased in BAL fluid and plasma and have been associated with disease progression. IL-18, derived from monocyte/macrophages and airway epithelial cells, upregulates expression of IL-2 and supports IFNγ production (Whittington et al. 2004). Apparently, IL-4 does not contribute to disease, although IL-13 levels are elevated in bronchoalveolar lavage (BAL) fluid from Sarcoidosis patients (Hauber et al. 2003). The increase in IL-13 may be indicative of an anti-inflammatory role in the pulmonary inflammation processes of Sarcoidosis.

To date, there has been a shortage of clinical and basic science studies examining the contributions of the AhR to immune regulation in models of Sarcoidosis. Moreover, none have examined the role of IL-22 in vivo or in vitro, and/or the contributions of AhR signaling to IL-22 expression or function. IL-22 is downregulated in patients with Sarcoidosis (Whittington et al. 2004), although the significance of this finding remains unknown and has not been extended to studies of or in murine tissues. IL-22 may play either a protective or a pathogenic role in chronic inflammatory diseases depending on the nature of the affected tissue and the local cytokine milieu. The main biological role of IL-22 includes an increase in innate

immunity, protection from damage, and enhancement of regeneration. Additional studies will be required to establish the cellular source of IL-22 (e.g., Th22, Th1, and Th17 cells, classical and nonclassical (NK-22) NK cells, NKT cells, and lymphoid tissue inducer cells) and the role undertaken by IL-22 in Sarcoidosis patients (Zhang et al. 2011). Similarly, additional work will be required to establish what role, if any, activation the AhR plays in Sarcoidosis development and progression.

13.7 Acute Respiratory Distress Syndrome

ARDS and ALI represent potentially fatal diffuse pulmonary diseases characterized by severe dyspnea, tachypnea, and hypoxemia occurring within 24–48 h of the causative injury (Tsushima et al. 2009). Also known as noncardiogenic pulmonary edema, increased permeability pulmonary edema, stiff lung, shock lung and ALI, ARDS is typically concurrent with another pathology, illness, or injury (e.g., sepsis, pneumonia, trauma, or aspiration pneumonia). ARDS may also be triggered by noninfectious agents such as acid aspiration, hyperoxia, environmental toxins, high-pressure ventilation, pulmonary contusion, reperfusion, and bleomycin (Matute-Bello et al. 2008). Cigarette smoking and heavy alcohol consumption constitute additional risk factors. While these agents directly trigger lung injury, similar damage can arise indirectly following traumatic injury, pancreatitis, transfusion, or kidney disease. These indirect events initiate an inflammatory response called systemic inflammatory response syndrome that may lead to or exacerbate ARDS (Ware 2006). The chest X-ray and computed tomographic (CT) examination demonstrate bilateral infiltrates, alveolar consolidation, and “white out” of the lung. ARDS is perhaps best described as a syndrome occurring with an illness (or injury) that damages lung capillaries causing fluid leakage into the alveolar spaces. The pulmonary edema typical of ARDS prevents oxygen from passing into the bloodstream leading to oxygen deprivation, local or systemic hypoxia, and multiorgan failure. Despite innovations in intensive care medicine, the mortality of ARDS remains close to 40% (Ware and Matthay 2000). To date, no pharmacological therapy has emerged specifically for the treatment of ARDS patients, because the patients and the causes underlying the syndrome are very heterogeneous. Treating the fundamental pathology will often concomitantly resolve ARDS. Consequently, typical ARDS management is breathing support and oxygen supplementation during treatment of the underlying cause. Some ARDS survivors will regain lung function, while others exhibit permanent lung damage and fibrosis. Memory and/or cognitive deficits are also common as a result of brain damage stemming from prolonged oxygen deprivation.

In ARDS patients, lung edema, endothelial and epithelial injury are accompanied by an influx of neutrophils (PMNs) into the interstitium and bronchoalveolar space (Whittington et al. 2004). Epithelial injury and decreased barrier function facilitate influx of protein and fibrin-rich fluid and other macromolecules into alveolar spaces resulting in impaired cell fluid transport, reduced production of surfactant,

and alveolar collapse. The recruitment and activation of PMNs is a hallmark event in ARDS, although PMNs can migrate into the alveolar spaces without causing epithelial damage, and ARDS can occur in children and adults with neutropenia (Laufe et al. 1986; Ognibene et al. 1986; Martin et al. 1989; Sivan et al. 1990). While neutrophil activation is vital for host defense, overzealous activation leads to tissue damage by release of cytotoxic and immune cell-activating agents such as proteinases, cationic polypeptides, cytokines, and ROS, which further damage the alveolar barrier. In ARDS patients, PMN presence in the BAL fluid correlates with disease severity and outcome (Matthay et al. 1984; Parsons et al. 1985; Steinberg et al. 1994); while in mice, PMN depletion reduced the severity of lung injury (Abraham et al. 2000; Abraham 2003).

Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, provokes acute pulmonary inflammation (e.g., neutrophilia), pro-inflammatory cytokine/chemokine production, and alveolar-capillary leak in rodents, which mimics the pathophysiology of human ARDS and ALI (Matute-Bello et al. 2008). In animal models, recognition of bacterial LPS induces potent stimulation of toll-like receptor 4 (TLR4), leading to activation of the NF-κB pathway (Doyle and O'Neill 2006) and subsequent innate immune responses. The effects of TCDD (and presumably AhR activation) on LPS-induced inflammatory responses have been studied for many years with some conflicting data in the literature. Early studies reported that exposure to TCDD greatly enhanced LPS toxicity (Vos et al. 1978); yet $AhR^{-/-}$ mice are hypersensitive to LPS-induced inflammatory responses, including IL-1β secretion and neutrophilia—suggesting a critical function of the AhR in the acute inflammatory response (Kimura et al. 2008, 2009; Sekine et al. 2009). Similarly, AhR activation modifies the expression of LPS- and TLR4-regulated pro-inflammatory genes in a time- and tissue-dependent manner (Wu et al. 2011). Consistent with these in vivo effects, peritoneal macrophages isolated from AhR-deficient mice secreted higher levels of pro-inflammatory cytokines than those from wild-type mice in response to LPS. Furthermore, transfecting AhR null macrophages with an AhR expression plasmid suppressed this response (Kimura et al. 2009; Sekine et al. 2009). The AhR modulates NF-κB signaling pathways via physical interactions with RelA and RelB (Vogel et al. 2007a, b; Tian 2009; Vogel and Matsumura 2009). Furthermore, numerous studies have provided evidence that the AhR interacts with Stat1 and NF-κB and the AhR–Stat1 complex controls NF-κB-dependent pro-inflammatory responses by LPS. The AhR may also negatively regulate inflammatory responses mediated by LPS in macrophages through inhibiting the promoter activity of IL-6 (Kimura et al. 2008). On the other hand, ligand-activated AhR resulted in synergistic induction of IL-6 following cotreatment with IL-1β and TCDD (DiNatale et al. 2010). Together, these results indicate that the AhR suppresses the expression of inflammatory cytokines in response to LPS.

Although numerous studies have examined the contributions of the AhR to immune regulation in models of ARDS, less than a handful have examined the role of IL-22 in vivo or in vitro, and none have yet examined the contributions of AhR signaling to IL-22 expression or function. Interestingly, patients with ARDS exhibit

reduced levels of IL-22, yet elevated levels of IL-8 relative to control/normal subjects (Whittington et al. 2004)—an observation that is consistent with previous reports. Because these results may be due to either reduced IL-22 expression from the damaged epithelium or dilution of IL-22 in the lavage fluid due to pulmonary edema, additional studies will be required to establish the role of IL-22 in the pulmonary milieu of ARDS patients.

13.8 Silicosis

Silica crystals are ubiquitous and naturally occurring in the environment, even constituting a portion of the small particulate fraction of air pollution. Exposure to respirable crystalline silica (SiO_2) particles results from occupational (e.g., construction, mining, manufacturing), recreational (e.g., pottery), or environmental (e.g., soil, air pollution) contact. Silicosis results from inhalation of SiO_2 over prolonged periods of time, and once established, progresses even after exposure has ceased (Porter et al. 2004). Although significant efforts have been made through industrial hygiene standards to control ambient dust in the workplace, silicosis remains a prevalent health problem throughout the world, particularly in developing nations. Deposition of SiO_2 results in a granulomatous inflammatory response and progressive pulmonary fibrosis, as well as systemic immune deficits (Lynch and McCune 1997; Parks et al. 1999, 2002; Ding et al. 2002). Currently, there is no effective therapy for silicosis.

SiO_2-induced lung injury is characterized by the presence of abnormal numbers of immune cells and associated soluble mediators (Beamer and Holian 2007; Huaux 2007; Cassel et al. 2008; Beamer et al. 2010); however, the relationship between these cells, the sequence of their appearance and persistence, as well as their regulation is largely unknown. Alveolar macrophages and the IL-1β produced by these cells in response to SiO_2 plays a fundamental role in the development of acute and chronic inflammation and fibrosis (Cassel et al. 2008; Dostert et al. 2008; Hornung et al. 2008; Franchi et al. 2009a, b). Macrophages exposed to SiO_2 secrete IL-1β, and IL-1β-deficient and IL-1R-deficient mice are more resistant to developing silicosis, although IL-18R-deficient mice are not (Fig. 13.4) (Sarih et al. 1993; Srivastava et al. 2002). In addition, polymorphisms in the IL-1 receptor antagonist allele have been linked to human susceptibility to silicosis (Yucesoy et al. 2001). The inflammasome mediates caspase-1 cleavage of the inactive precursor pro-IL-1β, resulting in release of mature IL-1β (Sutterwala et al. 2006). In vitro, this process depends on signals that activate both TLRs and NOD-like receptors, such as Nlrp3 (Mariathasan et al. 2004; Franchi and Nunez 2008; Hornung et al. 2008). Nlrp3 is highly expressed in macrophages and is activated by many stimuli, including SiO_2 (Franchi et al. 2006; Mariathasan et al. 2006; Sutterwala et al. 2006; Dostert et al. 2008; Demento et al. 2009). These observations are noteworthy because numerous inflammatory diseases have been associated with dysregulated caspase-1 activation and IL-1β secretion (Dostert et al. 2008). Treatment with IL-1 receptor

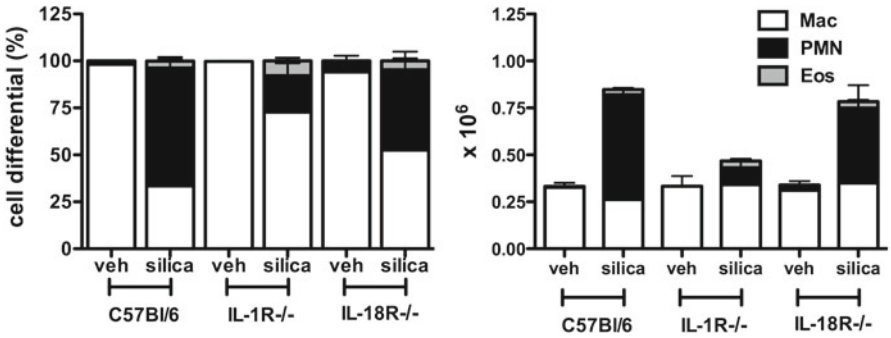

Fig. 13.4 Acute inflammation following instillation with silica. In C57Bl/6 wild-type mice, 24 h exposure to 1 mg crystalline silica increased the absolute number of cells recovered from the whole lung lavage over time compared to vehicle (veh) controls. Differential analysis revealed that this increase in cell number was largely due to an influx of neutrophils (PMNs) into the airways, although a small number of eosinophils (Eos) were also present. Similar exposures in mice deficient in the interleukin 1 receptor (IL-1R) and the interleukin 18 receptor (IL-18R) demonstrated that silica-induced neutrophil recruitment was dependent on IL-1/IL-1R, but not IL-18/IL-18R signaling. Values are means ± SEM; $n = 8$–10 mice per group, $^{*}p < 0.05$ compared to vehicle control

antagonist reverses clinical symptoms, suggesting a cause–effect relationship between inflammasome activation, IL-1β production, and disease pathogenesis (Hawkins et al. 2003, 2004; Dinarello 2011a, b).

Although animal models focused on early events in silicosis have yielded insight into underlying mechanisms that initiate injury, little is known about mechanisms that have the potential to resolve or regulate inflammation. Because the AhR plays a substantial role in the immune system and activation of the AhR by TCDD results in profound immunosuppression (Mandal 2005), we recently investigated AhR function in SiO_2-induced inflammation and fibrosis. In these studies, acute lung inflammation dominated by neutrophils (<1 month) was more severe in AhR-deficient mice; however, the fibrotic response was attenuated compared to wild-type mice. In a model of chronic SiO_2 exposure (3 months), AhR activation by TCDD resulted in reduced inflammation (e.g., neutrophilia), but did not change in the fibrotic response (Fig. 13.5). Bone marrow-derived macrophages (BMM) from $AhR^{-/-}$ mice also produced higher levels of cytokines and chemokines in response to SiO_2. Analysis of gene expression revealed that BMM derived from $AhR^{-/-}$ mice exhibit increased levels of pro-IL-1β, IL-6, and Bcl-2, yet decreased levels of STAT2, STAT5a, and Serpin B2 (Pai-2) in response to SiO_2. In addition, a recent report showed that acute inflammation caused by SiO_2 exposure is primarily directed by IL-17A producing γδ T cell, and driven by IL-23 secreting macrophages. Although this report also indicates IL-17A+ and IL-22+ lymphocytes accumulated in the lungs of SiO_2-exposed mice, neither cell type appeared to play a prominent role in the long-term inflammatory and fibrotic process (Lo Re et al. 2010). Together, these results suggest that the AhR signaling pathway may represent a promising novel therapeutic approach to treat patients with inflammatory disorders of the lung.

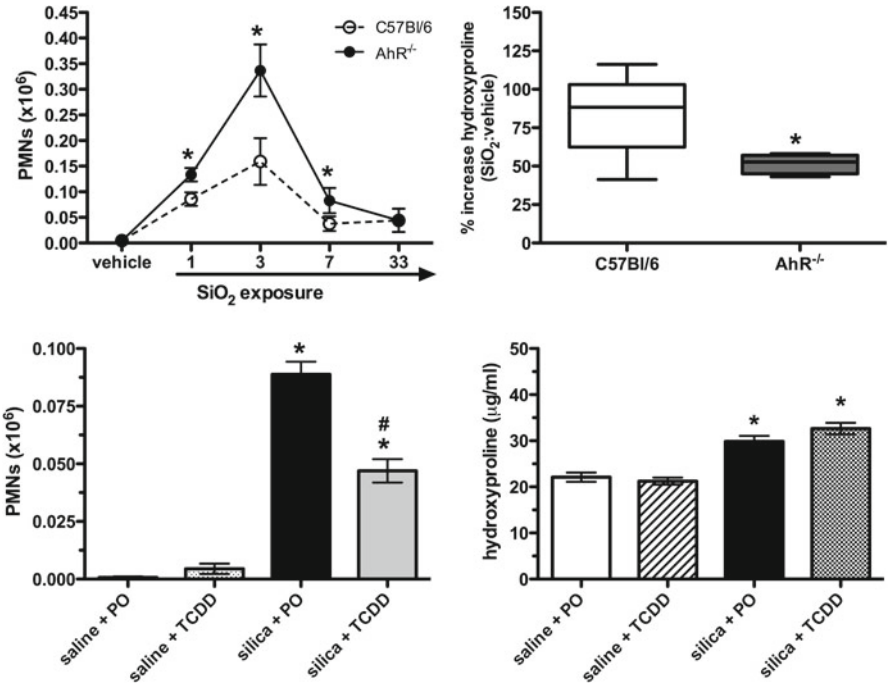

Fig. 13.5 Silicosis in AhR-deficient mice and TCDD-treated C57Bl/6 mice. Over time, neutrophil-dominated inflammation was observed in C57Bl/6 mice exposed to a single episode of silica—a response that was augmented in AhR-deficient mice. Although both C57Bl/6- and AhR-deficient mice exhibited increased collagen deposition at 1 month postexposure relative to their respective vehicle-exposed mice, this response was attenuated in AhR-deficient mice. $n = 5–7$, values are means ± SEM; $*p < 0.05$ compared to wild-type mice. Chronic SiO_2 exposure increased the absolute number of neutrophils (PMNs) in the whole lung lavage 3 months postexposure. Although TCDD-induced AhR activation attenuated silica-induced neutrophilia, it had no effect on collagen deposition in C57Bl/6 mice. $n = 5–6$, values are means ± SEM; $*p < 0.05$ compared to vehicle control, $\#p < 0.05$ compared to silica + peanut oil

13.9 Conclusions

Across the globe, respiratory diseases are among the leading causes of mortality and morbidity and consistently rank in the top tier in terms of mortality, incidence, prevalence, and associated treatment costs. A further increase in the prevalence of lung disease is projected, in particular for smoking-related diseases, such as COPD and lung cancer in women. Other risk factors, such as air pollution, allergens, infectious pathogens, and toxic occupational agents, also play an important role in the development (and exacerbation) of numerous lung diseases. Once lung injury occurs, it's generally irreversible. Medications may limit the damage and slow disease progression, but many people never regain full use of their lungs. Therefore, it is imperative that we better understand the causes and underlying

mechanisms involved in etiology of various lung diseases in order to develop more effective therapeutic interventions.

The immune system in particular plays a vital role in respiratory homeostasis by maintaining tolerance to endogenous self-antigens and innocuous particulates, while preserving the capability to respond to invading microorganisms and pathogens. Lung injury upsets this balance by provoking both inflammatory and compensatory anti-inflammatory responses. The relative contributions of these immune responses to the etiology of individual respiratory diseases are not yet fully defined. The AhR has a dual role as an activator of xenobiotic metabolism and a key contributor to immune regulation. Mice deficient of a functional AhR, those with a constitutively active AhR, and other AhR signaling mutations are currently available and have been used to analyze physiological functions associated with AhR activation, including its role in the immune system. Moreover, numerous endogenous and exogenous ligands have recently emerged that bind to the AhR with variable affinity and potency, thereby providing important insight into the functions of the AhR. One area of AhR biology that has recently become of significant interest focuses on how AhR activation regulates the production of IL-22. New data suggest that IL-22 provides mucosal protection; however, the functional importance of IL-22 appears to be context-dependent. Using both mice genetically deficient in IL-22 or the administration of IL-22 neutralizing antibodies, research has shown both harmful and protective functions of this cytokine in different models of inflammation. Future challenges in this field include defining the context-dependent functions of IL-22 expression in inflammation and repair and designing therapeutics targeting the IL-22/IL-22R pathway for the treatment of persistent infection, chronic inflammation, and autoimmune diseases of the lung. Using a combination of research approaches, we have recently begun to investigate how the AhR contributes towards the regulation of immune responses in respiratory disease with a focus on IL-22. Importantly, IL-22 expression requires the AhR, AhR ligands augment IL-22 expression during immune responses both in vitro and in vivo, and stimulating production of endogenous AhR ligands results in enhanced IL-22 expression. Thus, activation of the AhR (and subsequent effects on IL-22 levels) may represent a novel and highly significant therapeutic approach to the treatment of inflammatory and immune-mediated lung disease.

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Chapter 14
Environment, the Immune System, and Depression: An Integrative Review and Discussion of the Infection-Defense Hypothesis

Midori Tanaka, Sherry Anders, and Dennis K. Kinney

Abstract Major depressive disorder (MDD) is one of medicine's most prevalent and costly disorders, and it has high rates of comorbidity with a wide range of infectious diseases and serious medical disorders. MDD has been linked to immunologic alterations that include suppressed cellular immune function, excessive inflammatory response, and greater susceptibility to infection. In this chapter, we review research on environmental factors and immune factors associated with depression including: a clinical overview of MDD, current evidence on environmental risk factors and alterations in immune function, and the role of immune factors in the etiology of depression. In a concluding section, we discuss an integrative hypothesis—*The Infection-Defense Hypothesis*—that may help to resolve an important psychiatric puzzle, offering an explanation for why immune function is related to depression, and why depression remains common, despite its high evolutionary costs and evidence for its significant heritability.

Key Points

- Major depressive disorder (MDD) is associated with immune dysfunction, particularly an excessive inflammatory response and suppressed cellular immune function.
- Acute and chronic infections, as well as live-virus vaccinations, have been associated with an increased risk for depression.

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- A wide range of psychological, physiological, and environmental factors—such as chronic stress and sleep deprivation, cancer, autoimmune diseases, cardiovascular disease, seasonal changes, and pesticide pollution—are associated both with increased vulnerability to infection and with increased risk for depression.
- The role of cytokines has been identified as a key area of focus in understanding the neurobiological pathways by which depression and immune functioning are related, via both direct and indirect effects on hypothalamic–pituitary–adrenal (HPA) axis activation, and by altering monoamine neurotransmitters in multiple regions of the brain.
- Antidepressants, mood stabilizers, and other mood-enhancing interventions—such as physical activity, electroconvulsive therapy (ECT), and psychosocial support—show evidence of increasing immunologic defenses, with time courses of immune response often paralleling those for improvement in mood.
- An integrative hypothesis—*The Infection-Defense Hypothesis*—that we have described in detail elsewhere (Kinney and Tanaka 2009) offers an explanation for why immune function is related to depression, and why depression is so prevalent, despite the high evolutionary costs that it involves, and evidence for its heritability.
- A major clinical implication of our evolving understanding of the relationship between depression and the immune system is that depressed patients may be especially likely to have untreated infections or immune impairments, and addressing such underlying conditions may aid the treatment of depression.

14.1 Introductory Remarks

Major depressive disorder (MDD) is a prevalent and costly disorder. In addition to hallmark changes in affect and behavior, depression is associated with immunologic alterations that include immune suppression (marked by decreased lymphocyte proliferation and natural killer cell activity [NKCA]), excessive inflammatory response, and greater susceptibility to infection. Depression also has high rates of comorbidity with a wide range of serious and chronic medical illnesses such as cardiovascular disease, cancer, autoimmune disorders, and chronic pain. In this chapter, we present a focused review of research on environmental factors and immune factors associated with depression. Included in this review, we discuss key theories about biochemical pathways that may be involved in these associations. Of particular interest is the role of immune-activated inflammatory cytokines, which have been noted to affect multiple neurological domains, including neurotransmitter metabolism and activation of the hypothalamic–pituitary–adrenal (HPA) axis.

We also address an important psychiatric puzzle: How can a heritable disorder such as depression, with so many apparent adverse consequences for survival and reproduction, be extremely common and even increasing in prevalence? *The Infection-Defense Hypothesis*, which we have presented elsewhere (Kinney and Tanaka 2009), offers an integrative explanation for why immune function is related to depression, and why depression is so prevalent, despite the high evolutionary costs that it involves.

We close the chapter with a discussion of the clinical and scientific implications of our evolving understanding of the relationship between depression and the immune system, foremost among which are suggestions that (1) mood regulation and the immune system may function in tandem to protect against infection and disease, (2) depressed patients may be especially likely to have untreated infections or immune impairments, and (3) addressing underlying medical conditions and environmental factors that contribute to immune vulnerability may aid the treatment of depression.

14.2 Epidemiology and Clinical Manifestations of Major Depressive Disorder

14.2.1 Prevalence and Burden of Major Depressive Disorder

MDD is a major public health concern affecting more than 100 million people worldwide (World Health Organization 2001). The World Health Organization (WHO) projects that by the year 2020 depression will be the second leading cause of disease burden around the globe (Murray and Lopez 1996). The estimated lifetime risk of a major depressive episode has risen to 23% in the United States (Kessler et al. 2005), and there is evidence to suggest that the incidence of MDD may be increasing (e.g., Compton et al. 2006). Depression is associated with a wide range of personal and social problems, such as unemployment and divorce (Weissman et al. 1996). It is also the leading cause of disability and decreased productivity during the peak years of work and childbearing (Broadhead et al. 1990). A high mortality rate is associated with MDD because it is a risk factor for many disease-related causes of death, as well as for suicide (Mykletun et al. 2007). There are negative consequences for the families of depressed patients, as well. For example, children of depressed mothers show poorer outcomes on a wide range of developmental indices (Cummings and Davies 1994), with adverse consequences reported even in cases where there has only been prenatal exposure to maternal depression (Davis et al. 2007).

14.2.2 Diagnosis and Course of Major Depressive Disorder

MDD is part of a broader spectrum of mood disorders that includes *dysthymia* (a chronic, less severe form of depression) and *bipolar disorder* (elevated mood alternating with periods of depression). According to the DSM-IV-TR classification system (American Psychiatric Association 2000), symptoms of MDD include low mood, loss of interest and pleasure in nearly all activities, changes in appetite (overeating or undereating), sleep disturbance (insomnia or hypersomnia), psychomotor agitation or retardation, low energy, feelings of worthlessness or guilt, impaired concentration or indecisiveness, and thoughts of death or suicide.

A diagnosis of MDD requires five or more of the above symptoms lasting 2 weeks or longer, where at least one of the symptoms includes low mood or loss of interest. Other notable symptoms that may be subsumed under the above include social withdrawal and reduced sexual drive (American Psychiatric Association 2000). Depression can also take on a primarily somatic presentation, often noted in primary care settings, with complaints of weakness, nausea, gastrointestinal problems, headaches, or other aches and pains (Katon and Russo 1989).

MDD may occur as a single episode or it may be recurrent. First episodes are most likely to occur between the ages of 30 and 40, with a second, smaller peak of incidence between ages 50 and 60 (Eaton et al. 1997). Recent studies have shown that 50% of those suffering from their first major depressive episode will suffer from at least one more during their life (Eaton et al. 2008) with a lifetime average of four episodes. Depression is more likely to recur if symptoms have not fully resolved with treatment. Patients with MDD often do not experience a complete resolution of symptoms with conventional antidepressant treatment, and it has been reported that 10–20% of patients have depression that is refractory to all currently available treatment modalities (Greden 2001).

14.2.3 Genetics and Familial Risk

The heritability of depression is well-established. Heritability estimates based on twin, adoption, and genetic molecular studies, consistently fall at about 40% (Sullivan et al. 2000), and genetic association and linkage studies have discovered alleles that increase risk for depression (see review by Goldberg 2006). "Affective spectrum disorders," such as fibromyalgia and eating disorders, also run in the families of depressed patients (Hudson et al. 2003), suggesting that the genes that increase risk for depression may also increase risk for other medical disorders. The potential genetic risk of depression indicates that depression likely involves biological pathology in brain functioning. Psychological, physiological, and other environmental stressors are also likely to play a role in the etiology of depression. For example, in several studies investigating specific gene–environment interactions, it has been found that carriers of the 5-HTTLPR short allele of the serotonin transporter (SERT) gene show an elevated risk for depression that is further increased as a function of environmental stress levels (Eley et al. 2004; Kendler et al. 2005).

14.2.4 Brain Dysfunction and the Biochemistry of Depression

Vulnerability to depression is increased by psychological and physiological stress. Numerous studies have begun to investigate the biological backdrop by which such etiological factors can induce depressive symptoms. One of the most well-established hypotheses is the monoamine hypothesis (Hirschfeld 2000). According to this theory, depression is caused by a deficiency in synaptic concentrations of the

neurotransmitters serotonin and norepinephrine. The monoamine hypothesis suggests that this deficit can be treated and corrected by administering drugs. Many antidepressants have been developed and used in the treatment of depression based on this theory; however, between 30 and 50% of individuals treated with an antidepressant do not show sufficient response (Schatzberg 2000). This insufficiency of current pharmacological treatments for depression provides an impetus for expanding our knowledge of the biochemical pathways that are involved in depression. Emerging research on the neuroendocrine system and its role in the pathophysiology of depression is an especially promising area for study, with the potential to lead to novel interventions for the treatment and prevention of depression.

14.3 Infections, Immune Status, and Mood Alterations

14.3.1 Sickness Behavior, Depression, and Their Advantages for Fighting Infection

In 1988, Hart used the term "sickness behavior" to describe a constellation of somatic and behavioral changes that occur in response to infection. These changes include fever, together with the behavioral symptoms of weakness, malaise, listlessness, hyperalgesia (heightened sensitivity to pain), and an inability to concentrate. Hart and others (Hart 1988; Kluger 1991; Segerstrom 2010) have theorized that sickness behavior is a highly organized strategy for fighting infection by conserving energy and prioritizing resources. Several authors have suggested that depressive symptoms, like sickness behavior, may serve to conserve metabolic resources for fighting infection (Raison et al. 2006; Kinney and Tanaka 2009). For example, bodily aches and fatigue, hypersomnia, and psychomotor retardation (i.e., slowed speech, thinking, body movements, and decreased speech), all tend to reduce social activity and encourage rest, thereby conserving energy. Energy conservation is crucial for defending against infection because acute-phase responses to infection, such as fever, are costly. A 1°C elevation in body temperature requires a 10% increase in metabolic activity (Kluger 1991).

Studies involving both human and laboratory animals have demonstrated that sickness behavior can be induced by direct injection of proinflammatory cytokines such as interferon alpha (IFN-α), or of substances known to stimulate proinflammatory cytokines, such as lipopolysaccharides (LPSs). Although sickness behavior and depression are not equivalent, significant overlapping features have led to a psychoimmunological theory of depression known as the "cytokine hypothesis."

14.3.2 The Cytokine Hypothesis

The cytokine hypothesis—which suggests that proinflammatory cytokines play a key role in the pathophysiology of depression—was developed based on the results

of numerous studies finding elevated levels of certain proinflammatory cytokines in patients with MDD (Smith 1991; Maes 1999; Schiepers et al. 2005; Raison et al. 2006). In addition, interferon therapy—which is used in the treatment of cancer and hepatitis C (HCV) and involves the direct administration of a proinflammatory cytokine by injection—has been found to induce sickness behavior and depression (Capuron et al. 2002). There is evidence that the induced symptoms of depression are attenuated by antidepressant therapy (Capuron et al. 2002), suggesting that common neurobiological pathways may be involved. In fact, a growing body of research now suggests that depression may be a direct consequence of excessive cytokine production and chronic inflammation associated with certain medical illnesses (Evans et al. 2005). Notably, depression is also associated with a worse course and outcome for many of these illnesses (Evans et al. 2005).

In addition to evidence that cytokines are involved in the pathophysiology of numerous medical illnesses, there is also evidence to suggest that cytokines alter mood-regulating pathways such as monoamine neurotransmitter metabolism and HPA-axis function (Benton et al. 2009), offering further support to the cytokine hypothesis. The cytokine hypothesis has generated considerable interest in recent years, and it continues to develop as emerging evidence identifies links among neurotropic and neurotoxic factors related to HPA-axis function, cytokines, and other immune factors that are described below.

14.3.3 Infectious Diseases and Depression

14.3.3.1 Acute or Subacute Infections

Many infectious diseases are not only related to "sickness behavior" (Hart 1988) but also to depression. Increased rates of depression have been found following a number of acute or subacute infections, such as herpes simplex encephalitis (Fazekas et al. 2006) and West Nile virus (WNV) (Murray et al. 2007). For example, Murray et al. (2007) retrospectively investigated mood changes in 65 patients who contracted WNV. They found that over 31% of these patients experienced the onset of a depressive episode within 1 year of their infection. Those who had been diagnosed with the more severe form of WNV, *neuroinvasive disease*, had an even greater risk for developing depression. In addition, Berg et al. (2010) found little correlation between depression and physical fatigue indicators following WNV infection, suggesting that depressive symptoms from WNV are independent of more general symptoms of fatigue.

14.3.3.2 Chronic Infections

Evidence for an increased risk of depression among those who suffer from chronic infections comes from studies involving both humans and laboratory animals. Depression-like behavior has been observed in mice infected with Bacille Calmette-Guerin (BCG), a bacterium related to the one that causes tuberculosis

(O'Connor et al. 2009a). Humans suffering from chronic infections such as human immunodeficiency virus (HIV), HCV, and genital herpes (HSV-2) have also been found to have an increased risk for depression (Yates and Gleason 1998; Lipkin and Hornig 2004).

A meta-analysis reanalyzing ten published studies on HIV and depression concluded that the frequency of MDD was nearly two times higher in HIV-positive subjects than in HIV-negative comparison subjects (Ciesla and Roberts 2001). In addition, a large-scale study following over 1,300 HIV-seropositive gay men without the acquired immunodeficiency syndrome (AIDS) over 8 years found that 21.3% could be classified as depressed at the first follow-up assessment (Lyketsos et al. 2009).

Among HIV patients, high viral loads are associated with higher depression scores on the Hamilton Rating Scale for Depression (Cohen et al. 2002). Moreover, multiple studies—including several large-scale, longitudinal studies—have found evidence that depressive symptoms are associated with a greater decline in CD4+ T lymphocyte counts (Ickovics et al. 2001, reviewed by Leserman 2008). In another longitudinal study following 63 HIV-seropositive and 30 HIV-seronegative women, symptoms of depression and anxiety were significantly associated with higher viral loads and higher CD8+ T lymphocyte counts (Evans et al. 2002).

Depression worsens the mortality and morbidity of HIV (Ickovics et al. 2001; Cook et al. 2004; Leserman 2008). A review of over 20 longitudinal studies examining depression and HIV disease course found consistent evidence that chronic depression is associated with the clinical and immunological progression of HIV/AIDS (Leserman 2008).

Numerous studies also suggest that patients with HCV have a significantly higher prevalence of depression, with prevalence rates ranging from 20 to 60% (Yates and Gleason 1998; Beloborodova et al. 2010; Weinstein et al. 2011). HCV patients are more likely to develop depression than patients with other types of chronic hepatitis, such as hepatitis B (HBV). In addition to the severe stress of having a potentially life-threatening illness, heightened risk for depression among patients with HCV may also be due to therapeutic treatment with interferon, which can induce clinical depression (Capuron and Miller 2004). A recent study following 27 HCV patients for 12 weeks following peripheral administration of IFN-α found that cerebrospinal fluid (CSF) IFN-α levels were associated both with increased levels of activated indoleamine 2,3-dioxygenase (IDO) neurotoxic metabolites and with depressive symptoms (Raison et al. 2010).

14.3.3.3 Live-Virus Vaccines

Vaccines containing live viruses, which are known to trigger an immune response, can also cause depressive symptoms. For example, in a prospective double-blind study (Yirmiya et al. 2000), vaccination with live attenuated rubella virus resulted in depressive symptoms that lasted up to 10 weeks. In another study, Afsar et al. (2009) found increases in symptoms of depression related to antibody response following HBV vaccination in hemodialysis patients. Among certain subgroups of older adults, influenza vaccination has also been linked to increased depressive

symptoms, accompanied by a rise in serum levels of the proinflammatory cytokine interleukin-6 (IL-6) (Glaser et al. 2003).

14.3.3.4 Other Microbes with Known Psychotropic Effects

A growing number of studies suggest that other microbial infections can have psychotropic effects (Lipkin and Hornig 2004). *Toxoplasma gondii (T.gondii)*, the microscopic parasite that causes Toxoplasmosis, for example, has been found to induce self-destructive behavior in infected rodents. Numerous animal studies have found that rats and mice infected with *T. gondii* lose their fear response—and even develop an attraction—to the scent of a cat, causing them to move within close proximity despite the risk of being killed (Berdoy et al. 2000; Vyas et al. 2007; Webster 2007). In humans, studies show a significantly increased frequency of having traffic accidents among people with higher *T. gondii* antibody titers, indicating that *T. gondii* infection may have negative effects on cognitive functioning (Flegr et al. 2002; Yereli et al. 2006). Elevated levels of *T. gondii* antibody titers have also been found among individuals who have attempted suicide (Arling et al. 2009). Notably, valproic acid and haloperidol—drugs often used to treat mood and psychotic disorders—have been found to inhibit *T. gondii* growth in vitro (Jones-Brando et al. 2003).

Elevated rates of infection by Borna virus have been reported in individuals with schizophrenia and mood disorders. Borna disease virus (BDV) is a ribonucleic acid (RNA) virus with potent neurotropic properties, producing changes in mood, behavior, and cognition. Although the results of earlier studies were inconsistent (e.g., see review by Schwemmle 2001), more recent evidence suggests that BDV may indeed play a pathogenic role in psychiatric illness (see Bode and Ludwig 2003; Nunes et al. 2008). Heinrich and Adamaszek (2010), for example, investigated the persistence of BDV seropositivity and levels of antibody titers over the course of psychiatric illness in patients with schizophrenia and mood disorders. The results indicated that BDV was chronically present in many individuals and accompanied by predictable changes in antibody titers over the course of the psychiatric illness. Moreover, in patients with affective disorders, the point prevalence of BDV was associated with severity of mood symptoms (see Bode and Ludwig 2003). Interestingly, a number of animal studies have found elevated levels of proinflammatory cytokines—including interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ)—in BDV-infected mice (Shankar et al. 1992; Morimoto et al. 1996).

14.3.4 Immune Status and Depression

Early studies investigating immune alterations during depression focused almost exclusively on markers of immune suppression, such as decreased lymphocyte proliferation and NKCA. A number of studies have found associations between

depression and decreased lymphocyte proliferation, an in vitro correlate of T- and B-cell antigen response, although the findings have not been consistent (e.g., see Stein et al. 1991; Weisse 1992; Herbert and Cohen 1993). In an attempt to examine factors that might help to explain the inconsistency of previous findings, Schleifer et al. (1989) looked at age, gender, sex, and severity of depression as potential moderating factors. In their study, they found that among depressed subjects, there was a decreased proliferative response with advancing age, in contrast to an increased response in controls. They also found that within depressed subjects, suppression of lymphocyte proliferation was associated with the severity of depressive symptoms. More recent studies have also found a reliable association between depression and decreased proliferative responses to the three nonspecific mitogens phytohemaglutinin, concanavalin-A, and pokeweed (Zorilla et al. 2001).

Studies investigating associations between depression and NKCA have yielded more consistent and robust findings. The results of many investigations, as well as of meta-analytic studies, indicate that major depression is associated with impaired NKCA (Zorilla et al. 2001; Reiche et al. 2004). NK cells play a crucial role in defending against infections, especially those involving viruses; NK cells combat infections via their cytotoxic effects, as well as by their production of cytokines, including IFN-γ and TNF-α, which can help control viral infections (Orange and Ballas 2006).

More recently, focus has shifted from studies of suppressed cellular immunity to an emerging interest in the role of inflammation, with increasing numbers of studies reporting elevated levels of inflammatory cytokines in depressed individuals (Zorilla et al. 2001; Irwin and Miller 2007). Evidence is mounting that inflammatory cytokines act as neuromodulators of behavioral, neuroendocrine, and neurochemical features of depression by affecting multiple neurological systems that include neurotransmitter metabolism and activation of the HPA-axis (Miller and Raison 2008).

As noted previously, certain proinflammatory cytokines that are used to treat infectious diseases and cancer have been found to induce sickness behavior and depression (Capuron and Miller 2004). Therapeutic administration of cytokines (IFN-α and IL-2) has been found to induce a two-phase response; a few days following administration many patients experience sickness behavior, marked by neurovegetative and somatic symptoms (fatigue, aches, loss of appetite, sleep disturbance), with up to 50% of those treated going on to develop symptoms of major depression including depressed mood, feelings of worthlessness, guilt, and even suicidal ideation (Capuron et al. 2002).

14.4 Immune-Compromising Conditions Associated with Depression

Notably, many conditions in humans that are associated with increased vulnerability to infection are also associated with increased risk of depression. For example, considerable research, including some prospective studies, indicates that *chronic*

infections, as well as many conditions associated with increased *vulnerability to infection*—such as chronic stress and sleep deprivation, seasonal factors, exposure to environmental toxins, nutritional deficiencies, cancer, cardiovascular disease, and autoimmune diseases—are all associated with elevated risk for depression (Lustberg and Reynolds 2000; Mundt et al. 2000; Kiecolt-Glaser et al. 2002; Reiche et al. 2004; Evans et al. 2005; Horesh et al. 2008).

14.4.1 Psychological Stress

It is well-established, based on numerous studies that include both retrospective and prospective research designs, that psychological stress plays an important causal role in the etiology of depression (Kendler et al. 1999; Hammen 2005). For example, young mothers in adverse environments have a significantly increased rate of depression (Brown et al. 1986). In animal studies, exposure to a variety of stressors has been shown to induce depressive-like behaviors in rats, with evidence that these behavioral changes respond to antidepressant treatment (Sillaber et al. 2009). Many studies have found that depressed patients tend to have elevated levels of the human stress hormone *cortisol*, and experiments with mice have found that chronic exposure to elevated levels of *corticosterone*—the rodent analog of *cortisol*—increased anxious and depression-like behaviors in the mice (Ardayfio and Kim 2006).

Psychological stress is also related to immune system functioning. Psychosocial stressors can increase circulating numbers of proinflammatory cytokines (Bierhaus et al. 2003; Wolf et al. 2009). Increased cytokines sensitize the HPA-axis contributing to the hypersecretion of cortisol (Turnbull and Rivier 1995). This interaction has been identified as a critical pathway by which the central nervous system (CNS) is negatively impacted under psychological stress.

Cortisol can suppress cellular immune response that is critical in defending the body against viral infections (Maes et al. 1994). Many studies indicate that chronic stress increases vulnerability to infection (e.g., Cohen 1995; Cohen et al. 1999; Mundt et al. 2000; Kiecolt-Glaser et al. 2002; Horesh et al. 2008). For example, it has been found that stressed caretakers are more vulnerable to infectious diseases (Kiecolt-Glaser et al. 2002). Populations that experience higher levels of social stress also tend to have higher rates of mortality and morbidity from infectious disease (Weiss and McMichael 2004).

14.4.2 Insomnia

Chronic insomnia is a major risk factor for depression, and it may also be a prognostic indicator of depression relapse or recurrence (see review by Lustberg and Reynolds 2000). Chronic sleep deprivation can cause immune vulnerability (Irwin 2002a), and the severity of insomnia is negatively correlated with NKCA in both depressed and

nondepressed groups (Zorilla et al. 2001; Irwin 2002a). Poor sleep quality and insomnia can precipitate inflammatory processes, raising plasma cortisol, C-reactive protein (CRP), and proinflammatory cytokines (Motivala et al. 2005). In experiments with rats, chronic restriction of sleep causes depression-like changes in both the sensitivity of neurotransmitter receptors and neuroendocrine reactivity to stress (Novati et al. 2008). Recent studies have identified sex differences in immune alterations due to sleep loss (Suarez 2008; Irwin et al. 2010). For example, Suarez (2008) found that poor sleep quality was associated with increases of CRP and IL-6 in females, but not males. This finding may have important implications for understanding the increased risk of inflammatory diseases and major depression among women.

14.4.3 Seasonal Risk Factors

Major depression tends to be worse in the winter, and *seasonal affective disorder* (SAD), characterized by hypersomnia, fatigue, and carbohydrate cravings, has its peak in winter (Magnusson 2000). Winter has several immuno-compromising effects. First, cold and dry weather increases viral activity. This may happen, in part, because people tend to stay indoors under more crowded conditions, increasing viral transmission. In addition, certain pathogens, such as the influenza virus, have evolved so that they thrive in cold, dry air that promotes low absolute humidity (Shaman et al. 2010). Cold stress can increase susceptibility to infection (Johnson and Eccles 2005; Mourtzoukou and Falagas 2007), and reduced daylight hours impact melatonin secretion and Vitamin D synthesis, both of which have been associated with changes in immune function and mood (Lam et al. 2004; Ganji et al. 2010; Milaneschi et al. 2010; Borges et al. 2011).

14.4.3.1 Cold Stress

Research investigating the impact of cold stress on immune function predominantly suggests a suppressive effect. For example, Johnson and Eccles (2005) found that having subjects put their feet in cold water increased their susceptibility to viral infection. There is also considerable evidence that exposure to cold air can increase susceptibility to respiratory infections in winter (Mourtzoukou and Falagas 2007). Studies that have attempted to delineate the ways in which cold thermal stress alters immune function have shown inconsistent results. While animal studies have demonstrated that cold exposure induces a reduction in NK cell function, lymphocyte proliferation, and increased production of proinflammatory cytokines (Goundasheva et al. 1994; Won and Lin 1995), human studies have shown enhanced NK function in response to cold exposure (Brenner et al. 1999). The difference may be due to the duration of the exposure. While animals are likely to be exposed to prolonged cold stress, human studies that show enhanced immune function are more likely to involve brief bouts of cold exposure.

14.4.3.2 Melatonin

Seasonal changes in melatonin secretion due to reduced daylight hours also affect depression and immune status. Melatonin is a hormone secreted by the pineal gland in the brain, which helps regulate other hormones and is responsible for maintaining the body's circadian rhythms. In winter, reduced exposure to daylight can disrupt the body's normal melatonin function and increase nocturnal melatonin secretion. During winter months, a disrupted melatonin secretion cycle is likely to be found among patients with SAD and MDD (see Lam et al. 2004). Notably, light therapy—which has been established as an effective treatment for SAD—decreases melatonin production (see Lam et al. 2004).

Melatonin also exerts an influence on immune function. In general, melatonin has an immuno-enhancing effect. In winter, increased melatonin secretion prompted by longer nights may have evolved to enhance immunity against adverse conditions associated with winter, such as decreased availability of food, cold temperatures, and increased vulnerability to infection. Melatonin appears to activate immune function by elevating NKCA, as well as proinflammatory cytokines such as IFN-α, IFN-γ, and IL-6 (see Lam et al. 2004; Mediavilla et al. 2010). For example, results from animal studies suggest that melatonin increases immune macrophage activity, antibody response, cytokine production, and Th1 lymphocytes (see Lam et al. 2004). In human studies, administration of melatonin elevates proinflammatory cytokine production, shifting the Th1/Th2 balance toward the left (see Lam et al. 2004).

Taken together, these data suggest that mood alterations can be affected by melatonin secretion and associated changes in immune function. Of note, the newly developed antidepressant Agomelatine, a melatonin receptor agonist, has demonstrated significant antidepressant effects for MDD when compared to placebo (Zajecka et al. 2010). Agomelatine may exert its beneficial effects on mood by normalizing melatonin secretion and circadian rhythms.

14.4.3.3 Vitamin D

Vitamin D, also affected by climate and seasonal factors, plays an important role in both immune function and depression. Because vitamin D is synthesized in the skin by exposure to sunlight, colder temperatures and fewer daylight hours during the winter months—which can be especially marked in areas of high latitude—contribute to decreased levels of vitamin D. Vitamin D deficiency has been linked to a wide range of medical and psychiatric illnesses, such as cancer, cardiovascular disease, type II diabetes, infectious diseases, autoimmune diseases, neurological dysfunction, depression, and schizophrenia (see reviews by Borges et al. 2011; Nimitphong and Holick 2011). Vitamin D has modulating effects on the immune system, with one pathway being induction of antimicrobial peptide gene expression, which is known to aid the treatment of tuberculosis and other infectious diseases (reviewed by Borges et al. 2011). Vitamin D also contributes to the prevention of excessive inflammation by way of an adaptive immune response that decreases the cytokines IFN-γ and IL-2,

both of which have been implicated in the pathophysiology of depression. There are a number of studies that have found a correlation between low levels of vitamin D and depression. In a large-scale, population-based study, for example, Ganji et al. (2010) found that young adults with vitamin D deficiency were more likely to be depressed than those without a deficiency. In another large-scale study that used a prospective design, low levels of vitamin D in older adults was associated with a greater risk of developing depression over time (Milaneschi et al. 2010).

14.4.4 Exposure to Immuno-Compromising Chemicals

Pesticide exposure is associated with increased risk for suicide and mental illnesses, especially among agricultural workers. Multiple epidemiological studies, including several recent large-scale studies, suggest that both acute and chronic exposure to certain pesticides results in an increased risk for depression and other neurological disturbances (van Wijngaarden 2003; Alavanja et al. 2004; London et al. 2005; Beseler and Stallones 2008). Meyer et al. (2010) found that residents in a pesticide-intensive area of rural Brazil had higher rates of hospitalization due to suicide attempts and mood disorders. Meyer and his colleagues also found that, among agricultural workers, risk of mortality by suicide was increased in areas with higher rates of pesticide expenditure per individual. While social support and other life circumstances among farmers must also be taken into consideration as risk factors for mental disorders, a large-scale epidemiological survey among agricultural workers concluded that pesticide usage was associated with fatigue and insomnia, as well as with adverse life circumstances and decreased social support (Kallioniemi et al. 2009). Studies with laboratory animals also suggest a link between pesticide exposure and depression. Lima et al. (2009), for example, found that subchronic exposure to commonly used pesticides can induce depressive-like behavior in adult mice.

Organophosphates (OP), a widely used type of insecticide, have been thought to exert neurotoxic effects on the serotonergic system by inhibiting acetylcholinesterase (AChE) activity, although Lima et al. (2009) found that serotonergic influence could not explain behavioral changes in mice resulting from controlled exposure to OP. Recent evidence suggests that immune factors may play a role. Both experimental and observational studies have found that pesticides such as OP can have immunotoxic effects in animals and humans. Over the past 20 years, for example, evidence has accrued suggesting that pesticide exposure reduces host resistance against both viral and bacterial infections, and may contribute to the development of cancer (see Galloway and Handy 2003; Dallaire et al. 2004). Pesticide exposure is associated with decreased NKCA and increased cytokine production (see Corsini et al. 2008; Udoji et al. 2010), although cytokines appear to be differentially affected in response to pesticide exposure. OP and organochlorine increase IL-2, IL-4, and TNF-α, for example, but decrease IFN-γ in serious cases of human poisoning (Seth et al. 2005, 2008). Occupational exposure and bystander exposure also alter serum level of cytokines, although the results vary (Corsini et al. 2008). These variations

may depend on a number of factors, including pesticide type, type of exposure, and host conditions that affect immune vulnerability.

14.4.5 Other Physical Conditions and Medical Illnesses Associated with Impaired Immune Function

A number of other physical conditions and medical illnesses are associated both with impaired immune functioning and depression. These conditions and illnesses include cancer, cardiovascular disease, autoimmune diseases, chronic pain, changes in the menstrual cycle, the postpartum period, and omega-3 fatty-acid deficiency.

14.4.5.1 Cancer

Patients with cancer experience symptoms of depression, fatigue, sleep disturbance, and cognitive dysfunction that often persist throughout the course and treatment of the disease (Raison and Miller 2003; Evans et al. 2005; Miller et al. 2008). A recent systematic review of 31 studies that used standardized diagnostic procedures based on the Structured Clinical Interview for DSM disorders (SCID) found the prevalence of depression to be 10.8% among cancer patients (Ng et al. 2011).

Recent advances in the understanding of cancer-related depression has focused attention on the role of immunologic processes, such as inflammation, and their effects on mood and behavior by way of several key neuroendocrine pathways. For example, studies have shown evidence for reduced sensitivity to glucocorticoids and altered patterns of cortisol secretion in response to immunologic changes among cancer patients with depression (see Miller et al. 2008).

The presence of depression and psychological stress among cancer patients appears to be related to both the course and outcome of the disease, with evidence for increased rates of mortality. Stress appears to promote tumor progress, while psychological interventions that reduce stress benefit long-term survival. In fact, a number of studies involving both humans and laboratory animals have found that depression and psychological stress compromise the most critical elements of immune defense against tumors by reducing NKCA, phagocytosis, cytotoxic T-cell activity, and the DNA repair system (reviewed by Reiche et al. 2004).

Somewhat paradoxically, studies of cytokines have revealed dual roles for the immune system in both suppressing and promoting cancer formation. It has been established that certain viral infections, including Epstein–Barr, human herpes, papilloma, *Heliobacter pylori*, and HBV and HCV, are linked to cancer formation. During the course of infection, unresolved inflammation elicits cell turnover and can eventually lead cellular transformation to cancer. Dysregulation in the signaling and production of cytokines appears to promote the progression from chronic infection to cancer (Dranoff 2004). For example, proinflammatory cytokine IL-1 gene

polymorphisms are associated with an increased risk for gastric cancer. On the other hand, cytokines such as IFN-α have therapeutic effects that inhibit cancer growth, and they are commonly used as therapeutic agents to treat tumors (Dranoff 2004).

Taken together, chronic inflammation and cytokine activity appear to be linked to cancer pathology, and the high prevalence of depression among cancer patients may result from concomitant inflammatory processes. It is also important to note that the enzyme, IDO—known for its association with cytokine activity and its production of neurotoxic tryptophan metabolites that have been implicated in the pathophysiology of depression—also provides immune tolerance to tumors (Mellor and Munn 2008).

14.4.5.2 Cardiovascular Disease

Considerable evidence supports the idea that there is an intimate connection between depression and cardiovascular disease, and that this association can be largely explained by factors related to immune dysregulation. Epidemiological studies have identified the importance of psychosocial factors in increasing the risk of developing coronary artery disease (CAD), cardiac dysfunction, and cardiac events. Both acute and chronic psychological stress increase haemostatic factors. For example, chronic psychological stress increases acute-phase proteins such as fibrinogen. Depressive symptoms such as fatigue and irritability have been identified as precursors to both first and recurrent myocardial infarction (MI) (Barefoot and Schroll 1996; Frasure-Smith and Lespérance 2003). Depressive symptoms also appear to raise the risk for onset of CAD, as well as stroke, and may also affect the mortality of MI (see review by Echols and O'Connor 2010).

A recent review has identified a number of potential mechanisms that help explain associations among stress, depression, and cardiovascular disease. These mechanisms include hyperactivity of the HPA-axis and the sympathomedullary pathway, platelet mechanisms, inflammation, and reduced heart rate variability. These factors interact to produce a state of persistent inflammation. Inflammatory processes, including increased activity of proinflammatory cytokines such as IL-6, TNF-α, and CRP, are associated with both vascular disease and depression (Cesari et al. 2003; Kling et al. 2007).

14.4.5.3 Autoimmune Diseases

Decreased NKCA, as well as an increased risk of depression, have been observed in patients with autoimmune disorders such as systemic lupus erythematosus (SLE) (Struyf et al. 1990; Waterloo et al. 1998; Riccieri et al. 2000; Nery et al. 2008). Common physiological pathways, by which immunologic response and inflammation play a key role, have also been identified in the overlapping development of pain and depression in rheumatic diseases, such as rheumatoid arthritis and fibromyalgia (Goldenberg 2010).

14.4.5.4 Chronic Pain

Depression is common among patients who experience chronic pain (Campbell et al. 2003; Evans et al. 2005), and rates of suicidal behavior among those who experience chronic pain are high (Fishbain et al. 1997). Hyperalgesia (heightened sensitivity to pain) is one of the constellation of symptoms that occur during sickness behavior when an immune response is triggered, and it may have adaptive value by encouraging rest and inactivity that reduces exposure to further stressors and conserves an organism's energy and resources for the immune system to use in fighting infection. The up-regulation of proinflammatory cytokines during an immune response has been described as playing the most important role in the development of chronic pain (Poole and Woolf 1999). Proinflammatory cytokines mediate pain states at multiple levels of the central and peripheral nervous systems (Watkins and Maier 2000).

14.4.5.5 Menstrual Cycle

Hormonal changes associated with women's menstrual period also may affect immune factors and mood. Premenstrual syndrome (PMS), and in its more extreme form premenstrual dysphoric disorder (PMDD), is characterized by psychological symptoms such as irritability and depressed mood, as well as physical symptoms such as pain, fatigue, and hypersomnia that peak during the luteal phase of the menstrual cycle. The exact mechanisms are still unclear, but a number of potential causal factors have been identified. These include changes in gonadal steroid levels—especially changes in the estrogen–progesterone ratio—hypothyroidism, and neurobiologic factors such as decreased serotonin availability and lower GABA receptor activity levels (see review by Rubinow et al. 2009). Disturbed and blunted HPA-axis response to progesterone has been found among women with PMS and PMDD (Rubinow et al. 2009). Cortisol and its neuromodulative effects have also been investigated as a potential causal mechanism (Rubinow et al. 2009).

The luteal phase of the menstrual cycle is also associated with changes in immune function. Increased susceptibility to several types of infections, including genital herpes, fungi, and herpes zoster, has been observed (reviewed by Doyle et al. 2007). This is consistent with evidence of reduced NKCA during the luteal phase when compared to the follicular phase (Souza et al. 2001). The normal menstrual cycle is also associated with increased production of IL-6, IL-4, and TNF-α in the luteal phase when compared with the early follicular phase (O'Brien et al. 2007). O'Brien et al. (2007) found that IL-4 was positively correlated with estrogen levels, while TNF-α was positively correlated with progesterone levels, helping to explain changes in mood and behavior during the menstrual cycle from a psychoneuroimmunological context.

14.4.5.6 Postpartum Period

Postpartum depression (PPD) is common. Prevalence estimates range from 10 to 20% during the first year after giving birth (Gavin et al. 2005). PPD negatively

impacts both mothers and infants (Beck 1995). It affects attachment and bonding between mother and child, and it can have negative effects on children's development that persist beyond infancy (Hay et al. 2003). Etiological factors that have been proposed to play a role in the development of PPD include radical hormonal changes following birth, thyroid dysfunction, and immune alterations involving HPA-axis hyper- or hypoactivity (Groër and Morgan 2007).

To maintain a fetus despite its antigenic incompatibility, a woman needs to suppress immune function during her pregnancy. Several studies suggest that maternal anti-inflammatory cytokines are elevated, and proinflammatory cytokines are down-regulated, during pregnancy (Saito et al. 1999; Veenstra van Nieuwenhoven et al. 2003; Ostensen et al. 2005). With the cessation of pregnancy and delivery, the anti-inflammatory milieu abruptly shifts toward a proinflammatory state. This change can provide protective advantages during the postpartum period, which has increased vulnerability to infections because of (a) elevated rates of hemorrhaging, (b) exhaustion associated with labor and delivery, and (c) significant perineal tissue injury. Multiple studies have described an increase in proinflammatory cytokines in healthy women with the onset of labor, an increase that continues for up to 1 month (see review by Corwin and Pajer 2008).

Associated with this change in cytokine levels, HPA-axis suppression also occurs during pregnancy. Maternal levels of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol increase during late pregnancy, drop in the early postpartum period, and then normalize within several weeks (Corwin and Pajer 2008). Although investigations of women with PPD have yielded inconsistent results regarding HPA-axis status (Pedersen et al. 1993; Bloch et al. 2003), increased levels of proinflammatory cytokines such as IL-6 and TNF-α have been found repeatedly in serum as well as CSF. They are also correlated with scores on measures of depression (Maes et al. 2000; Boufidou et al. 2009). In another study, lower serum levels of IFN-γ and lower IFN-γ/IL-10 ratios were found among depressed mothers, although they were also found to have elevated levels of IL-6 (Groër and Morgan 2007). This suggests that an over-suppression of HPA-axis activity—caused by an over-adjustment to normalize elevated HPA-axis activity during late pregnancy—may be a causal factor in PPD. Groër and Morgan (2007) found HPA-axis hypoactivity to be common in both atypical depression and PPD. Taken together, this evidence suggests that interactions with the immune system and hormonal changes that occur over the course of pregnancy and delivery activate proinflammatory cytokines and contribute to the development of PPD. Future studies are needed to delineate more precisely the ways in which HPA-axis activity plays a role in the development of PPD.

It is of interest to note that *breastfeeding* appears to be protective against PPD and contributes to immune recovery after delivery (Groër et al. 2005). Breastfeeding also appears to prevent the over-activation of proinflammatory cytokines (Groër and Davis 2006). This evidence supports the idea that neuroimmunological factors play a causal role in PPD.

14.4.5.7 Omega-3 Deficiency

A number of studies have found associations linking omega-3 deficiency with inflammation and depression. In an experimental study using rats, omega-3 deficiency resulted in increased proinflammatory cytokine production and increased central serotonin (5-HT) turnover, both of which have been implicated in the pathophysiology of depression (McNamara et al. 2010). Epidemiologic evidence suggests that diets rich in omega-3 fatty acids are associated with a decreased risk for MDD (Noaghiul and Hibbeln 2003; Golding et al. 2009), whereas omega-3 deficiency has been associated with increased depressive symptomatology (Parker et al. 2006; Hibbeln 2009) and an increased risk of suicide (Tanskanen et al. 2001; Huan et al. 2004; Sublette et al. 2006; Lewis et al. 2011). Omega-3 supplementation—particularly in combined supplements with high eicosapentaenoic (EPA) to docosahexaenoic (DHA) ratios—have been found effective in reducing depressive symptomatology (see Freeman and Rapaport 2011). Kiecolt-Glaser et al. (2011) found that, even among healthy adults, omega-3 supplementation resulted in overall decreases in levels of both inflammation and psychological symptoms.

14.5 High Rates of Infection and Immune Alteration in Depressed Patients

Not only have high rates of depression been found among those who suffer from infections or other conditions of compromised immunity, but immune alteration (e.g., decreased NKCA and responses to interleukins) and chronic inflammatory response have been found repeatedly in depressed patients over several decades (see review by Irwin and Miller 2007).

Many observational studies have found increased levels of proinflammatory cytokines in patients with MDD or bipolar disorder (Brietzke and Kapczinski 2008; Dawlati et al. 2010). Depressed individuals are also more likely to contract certain infections, such as upper respiratory tract infections (Cohen 1995). Among HIV-infected patients, depressive symptoms are associated with higher viral load and lower NKCA (see review by Kopinsky et al. 2004). Depressive symptoms are also significantly associated with recurrence of herpes simplex infection (Zorilla et al. 1996) and reduced cellular immunity to varicella-zoster (Irwin 2002b).

14.6 Mechanisms That May Link Immune Alterations to Depression

The role of proinflammatory cytokines has been identified as a key area of focus in understanding the neurobiologic pathways by which depression and immune functioning are related (Raison et al. 2006; Dantzer et al. 2008). Based on mounting evidence that patients with depression show increased levels of inflammatory

biomarkers, Smith proposed *The macrophage theory of depression* (Smith 1991), which was further developed by Maes (Maes et al. 1995; Maes 1999). According to this theory, the production of proinflammatory cytokines by macrophages during the acute-phase of an immune response plays a causal role in depression. In the face of infection or injury, macrophages create and release proinflammatory cytokines, which are responsible for orchestrating the early immune response, including sickness behavior. Several lines of evidence suggest that the proinflammatory cytokines responsible for this acute-phase reaction may also mediate the effects of environmental factors on depression, via connections with the neuroendocrine axis and the central and sympathetic nervous system (Irwin and Miller 2007).

Several mechanisms have been proposed to account for the role of inflammatory immune factors in depression. First, there is growing evidence to suggest that proinflammatory cytokines alter monoamine neurotransmitters in multiple regions of the brain, with considerable focus on the direct and indirect effects of cytokines on serotonin metabolism (Raison et al. 2006; Dantzer et al. 2008; Miller and Raison 2008). Cytokine receptors are expressed in neurons throughout the CNS, also raising the possibility that cytokines themselves act as neurotransmitters (Bartfai and Schultzberg 1993). In addition, cytokines may contribute to depression via activation of the HPA-axis either directly, through their effects on CRH and vasopressin (AVP), or indirectly through cytokine-induced glucocorticoid-receptor resistance (Holsboer 2000; Pace et al. 2007).

14.6.1 The Effects of Cytokines on Neurotransmitter Availability and Metabolism

Proinflammatory cytokines exert both direct and indirect effects on monoamine neurotransmitters in the brain (Dunn and Wang 1995). Cytokines administered to laboratory animals and humans have been shown to alter the metabolism of serotonin, norepinephrine, and dopamine in regions of the brain associated with mood regulation (Rivier et al. 1989; Shuto et al. 1997; Capuron et al. 2003). A considerable amount of attention has been focused on the effects of cytokines on serotonin metabolism. Serotonin deficiency has long been implicated in the pathophysiology of MDD (Graeff 1997), and many experimental human studies suggest that depletion of tryptophan, the primary precursor to serotonin, is related to rates of relapse among those receiving pharmacological treatment for depression, as well as to lowered mood among healthy subjects (Benkelfat et al. 1994; Spillman et al. 2001; Van der Does 2001; Riedel et al. 2002).

14.6.1.1 The Degradation of Tryptophan by Activation of the Enzyme, Indoleamine 2,3-Dioxygenase

The degradation of tryptophan by activation of the enzyme, IDO is among the most studied of the mechanisms proposed to account for the pathological role of inflammatory immune factors in depression (Capuron et al. 2003; Dantzer et al. 2008).

IDO is activated by proinflammatory cytokines released during the acute-phase immune response, including IFN-α, IL-6, and TNF. In addition to reducing levels of serotonin via the breakdown of tryptophan, it has been proposed that IDO may also mediate depression through its neurotoxic metabolites (Dantzer et al. 2008). IDO activity diverts tryptophan metabolism from the production of serotonin to the synthesis of its primary metabolite kynurenine. Kynurenine is further metabolized into several neuroactive compounds that include 3-hydroxykynurenine (3-HK) and quinolinic acid (QUIN) or kynurenic acid (KA). These compounds, in turn, generate free radicals that cause neuronal damage due to oxidative stress (Wichers and Maes 2004). QUIN is an *N*-methyl-D-aspartate (NMDA) receptor agonist believed to inhibit neurogenesis, and KA is an NMDA receptor antagonist thought to be neuroprotective in astrocytes. These contradictory roles have led some researchers to speculate that a change in the production of neuroprotective toward more neurotoxic metabolites triggered by the immune inflammatory response is responsible for depressive symptoms (Myint and Kim 2003; Müller and Schwarz 2008).

The role of IDO in the physiology of depression is supported by a number of studies involving laboratory animals and humans. For example, the administration of pathogens such as LPS and BCG has been found to activate IDO in the brains of mice, following a time course that is compatible with the transition from sickness behavior to depression (Lestage et al. 2002; Moreau et al. 2008; O'Connor et al. 2009a). The causal role of IDO on depression-like behavior has been tested by blocking IDO or LPS-induced cytokines in laboratory animals. Pretreatment with the anti-inflammatory agent minocycline blocked the development of both sickness behavior and depression-like behavior in mice. Administration of the IDO competitive antagonist 1-methyl-D, L-tryptophan (1MT), by contrast, had no effect on LPS-induced proinflammatory cytokines and sickness behavior, but blocked the development of BCG-induced depressive-like behavior (O'Connor et al. 2009b). These results indicate that depression develops in an overlapping context of immune activation and sickness behavior, but nevertheless remains dissociated from sickness behavior, following a separate neurobiological trajectory. The results also demonstrate the important role that IDO activity may play in the pathogenesis of depression.

Further support for the role of IDO in depression is found in human studies. For example, depressive symptoms were significantly correlated with levels of QUIN and KA measured in the CSF of HCV patients who had received 12 weeks of treatment with IFN-γ (Raison et al. 2010). In patients undergoing treatment with IFN-α, decreases in peripheral blood tryptophan level were associated with increases in peripheral blood kynurenine level as well as increased symptoms of depression (Bonaccorso et al. 2002; Capuron et al. 2003).

14.6.1.2 Other Mechanisms Related to Serotonin Availability and Metabolism

Cytokines may decrease the amount of serotonin available for neurotransmission by up-regulating the expression and activity of the SERT. For example, using

animal models, Zhu et al. (2006) found that interleukin-1beta (IL-1β) and TNF-α stimulated serotonin uptake via activation of mitogen-activated protein kinase (MAPK) in a dose- and time-dependent manner. In another study, rats injected with synthetic double-stranded RNAs known to induce fatigue showed increases in the expression of IFN-α and SERT, while extracellular concentrations of serotonin in the medial prefrontal cortex decreased (Katafuchi et al. 2006).

14.6.1.3 Cytokines as Neurotransmitters

Cytokine receptors are expressed in neurons throughout the CNS and perform a wide range of neuromodulating functions (Schiepers et al. 2005; Hestad et al. 2009). The highest concentrations of cytokine receptors are found in the hypothalamus, hippocampus, and cortex—regions critical for antidepressant response and cognitive functioning (Loftis et al. 2010). Some have noted the possibility that cytokines function as neurotransmitters, exerting direct effects on the CNS (Bartfai and Schultzberg 1993; Schöning et al. 1999).

14.6.2 The Effects of Cytokines on the Hypothalamic-Pituitary-Adrenal Axis

Depression has a well-established association with hyperactivity of the HPA-axis (Pariante and Lightman 2008). Cytokines act as potent activators of the HPA-axis by inducing CRH and vasopressin (AVP). CRH and AVP are key regulators of the HPA-axis, and there is a significant amount of evidence linking increases in CRH and AVP levels to depression (see Owens and Nemeroff 1991; Holsboer 2000; Scott and Dinan 2002). For example, CRH has been found to be increased in the CSF of patients with major depression (Nemeroff et al. 1984), and administration of CRH to laboratory animals has been found to lead to behavioral changes that include depressive and anxiety-like behaviors, impaired sleep, anorexia, and reduced activity (Berridge and Dunn 1987). While the role of CRH has figured more prominently in associations between HPA hyperactivity and depression, AVP takes on a more significant role in the context of chronic inflammation (Dantzer et al. 2008).

In addition to direct stimulation of CRH and AVP, cytokines may also contribute to HPA-axis hyperactivity indirectly, through their effects on the glucocorticoid receptor. Cytokines have been linked to increased glucocorticoid receptor resistance through the effects of several signaling pathways, which include activation of the p38 MAPK and by stimulating an increase in the expression of a form of the glucocorticoid receptor (Pace et al. 2007). These changes, in turn, create a dysregulation of the CRH feedback system. The result is a feed-forward cascade that decreases the inhibitory effect of glucocorticoids on CRH and further intensifies cytokine production.

14.7 Mood Interventions and Alterations in Immune Status

Complementing evidence that depression is associated with states of immune compromise, a variety of mood-enhancing treatments and interventions—including antidepressant treatment, physical activity, ECT treatment, and psychosocial support—show the converse effect of enhancing immune response.

14.7.1 Antidepressants and Immune Function

Several lines of evidence suggest that antidepressants and mood stabilizers enhance immunologic defenses that include: (1) augmentation of NKCA (Irwin et al. 1992; Kook et al. 1995; Frank et al. 1999, 2004; Mizruchin et al. 1999), (2) suppression of proinflammatory processes by inhibiting proinflammatory cytokine production (Lanquillon et al. 2000; Maes 2001; Tuglu et al. 2003), and (3) antibiotic/antimicrobial activities (Lieb 2007). In addition, it is noteworthy that the time course of immune response following the onset of treatment with antidepressants and mood stabilizers often parallels changes in mood (Lieb 2007; Tanaka and Kinney 2011).

NKCA has been the most widely studied immune parameter in patients undergoing antidepressant therapy. The majority of studies in this area have found increased activity and/or circulating numbers of NK cells among those receiving treatment (Irwin et al. 1992; Kook et al. 1995; Frank et al. 1999, 2004; Mizruchin et al. 1999). Controlled clinical studies found a significant increase of NKCA in depressed patients following selective serotonin reuptake inhibitors (SSRIs) treatment (Kook et al. 1995; Mizruchin et al. 1999). Several studies showed an increase in NKCA that was concomitant with the amelioration of depressive symptoms (Irwin et al. 1992; Kook et al. 1995; Frank et al. 1999). In a double-blind placebo-controlled study, Frank et al. (2004) found that increase in NK function was found only for depressed patients who actually showed improved mood in response to the medication regardless of therapeutic modality. Although the exact mechanisms for NKCA recovery following antidepressant treatment remain elusive, experimental and observational studies suggest that SSRIs may up-regulate NKCA through their effects on serotonin (5-HT) receptors on immune cells (Evans et al. 2008).

In addition to stimulating changes in NKCA, antidepressants have been found to suppress proinflammatory processes by inhibiting proinflammatory cytokine production (Maes 2001). For example, decreased levels of the proinflammatory cytokine, TNF-α, have been observed in patients treated with tricyclic antidepressants and SSRIs (Lanquillon et al. 2000; Tuglu et al. 2003). Moreover, several controlled studies have reported that the increased production of the proinflammatory cytokines IL-6 and IFN-γ, which is found in depressed patients, is attenuated by antidepressant drugs and lithium (Kubera et al. 2001; Maes 1999; Knijff et al. 2007).

Several types of antidepressants and mood stabilizers also have *antibiotic* effects. In a recent review, Lieb (2007) cites a considerable number of in vivo and in vitro

studies demonstrating that antidepressant drugs and lithium have significant *antimicrobial* and *immuno-potentiating* effects. These effects include the ability to reverse resistance of bacteria to antibiotics (e.g., Kristiansen et al. 2010). It has been proposed that psychotropic medications derive their antimicrobial properties by acting as bacterial efflux pump inhibitors (Munoz-Bellido et al. 2000). Lieb (2007) also notes that several *antibiotics* have been found to have a positive effect on *mood*.

14.7.2 Other Interventions Known to Alleviate Depression or Distress and Their Relation to Immune Function

Other interventions known to alleviate depression or psychological distress have been associated with enhanced immune response. These interventions include electroconvulsive therapy (ECT), moderate physical activity, therapeutic ketamine administration, mindfulness training, and psychosocial support.

14.7.2.1 Electroconvulsive Therapy

ECT, used in the treatment of depression for several decades, often has a strong and rapid beneficial therapeutic effect on depressive symptoms (Husain et al. 2004; Kellner et al. 2006). The mechanisms by which ECT exerts its effects on mood are poorly understood. For example, there is no evidence of changes in monoamine metabolism or neuroplasticity following a single ECT session. However, a number of clinical studies investigating the immunological effects of ECT on depressed patients have shown evidence of alterations in *immune* function. For example, increased NKCA was found after a *single* ECT session (Fischler et al. 1992; Kronfol et al. 2002). Furthermore, a gradual and significant decline in levels of the proinflammatory cytokine, TNF-α has been observed over the course of repeated ECT sessions (Hestad et al. 2003). It is noteworthy that proinflammatory cytokines, such as TNF-α, which have been hypothesized to play a role in the pathophysiology of depression, were reduced to normal levels by the end of a course of ECT. These results suggest that one potential mechanism underlying ECT's efficacy in treating depression may involve an immunoregulatory process that normalizes excessive levels of proinflammatory cytokines and decreased levels of NKCA.

14.7.2.2 Physical Activity

A significant body of evidence shows beneficial effects of physical activity on both mood (Carek et al. 2011) and immune function (Haaland et al. 2008; Woods et al. 2009). Physical activity has been found to enhance mood and help protect against

the harmful effects of stress (Daley 2008). In patients with mild to moderate depression, the benefits of regular intensive exercise such as aerobic exercise or weight training are comparable to treatment with antidepressant medications, and exercise can also be used as an effective adjunct to antidepressant therapy (Dunn et al. 2005; Singh et al. 2005; Carek et al. 2011).

Less intensive exercise also offers mood-enhancing benefits of briefer duration. A single episode of exercise lasting as little as 30 minutes, for example, has been associated with short-term elevations in mood (Tate and Petruzzello 1995; Bartholomew et al. 2005; Reed and Ones 2006). A meta-analytic review of 158 studies concluded that aerobic exercise for brief durations of up to 35 minutes has mood-enhancing effects that appear to last for at least 30 minutes (Reed and Ones 2006). Moderate exercise results in significant enhancement of mood that declines more gradually over the course of several hours.

Exercise also has positive effects on cellular immune function (Haaland et al. 2008; Woods et al. 2009), and it is associated with decreased risk for infections, such as upper respiratory tract infections (Nieman et al. 1990; Moreira et al. 2009). The results of a meta-analysis of prospective controlled clinical studies of elderly people suggest that endurance, but not exhausting, exercise elevates T lymphocytes (e.g., CD8+ T cells), and that aerobic exercise may enhance immunologic memory in the context of vaccination (Haaland et al. 2008). Research by Nieman (2000), as well as the results of a comprehensive meta-analytic study (Shephard and Shek 1999), suggest that regular, moderately intense, exercise stimulates a significant and immediate increase in NK cells' circulating number and activity, which dissipates over the course of roughly an hour. Acute moderately intense exercise (e.g., walking for 30 min) also increases the circulating number of NK cells (Nieman et al. 2005).

Both mood and NKCA are rapidly enhanced in response to exercise, and both tend to dissipate within several hours after the conclusion of exercise, following a similar time course. This suggests that one mechanism by which exercise may ameliorate depressive symptoms over a relatively short period of time—a few hours or less—may involve changes in immune function (Tanaka and Kinney 2011). Other evidence supporting an immune-mediated effect on mood comes from studies showing that exercise can regulate immune response; exercise appears to lessen chronic inflammation status by reducing levels of CRP and IL-6, both of which are associated with increased risk for depression (Woods et al. 2009).

14.7.2.3 Ketamine

Ketamine, often used in clinical settings as an analgesic, is a high-affinity *N*-methyl-D-aspartic (NMDA) acid receptor antagonist with strong and rapid antidepressant effects (Berman et al. 2000; Zarate et al. 2006; Maeng and Zarate 2007). For example, in clinical studies conducted by Berman et al. (2000) and Zarate et al. (2006), depressed patients showed a significant improvement in mood within 1 hour of low-dose (0.5 mL/kg) ketamine administration. Low doses

of ketamine have also been found to help reduce depressive symptoms in cardiovascular patients after surgery (Bentley et al. 2005). Although the effects of ketamine on immune function remain largely unknown, a randomized, double-blind, placebo-controlled clinical study found that low-dose ketamine administration (0.5 mg/kg) attenuated the suppression of NKCA as well as the increases in proinflammatory cytokines that typically occur as a result of surgery (Bentley et al. 2005; Beilin et al. 2007). The results of these studies suggest the possibility that the immune-regulating effects of low-dose ketamine treatment may contribute to its antidepressant properties.

14.7.2.4 Psychosocial Interventions

Psychological interventions for the treatment of depression and emotional distress have also been investigated for their immunoregulatory potential. A number of studies have reported that *mindfulness training* and *cognitive behavior therapy* which are designed to reduce stress and have positive effects on mood, also have positive effects on immune function—including an enhancement of NKCA—in patients with medical conditions such as cancer, HIV, or chronic fatigue syndrome (Masuda et al. 2002; Robinson et al. 2003; Witek-Janusek et al. 2008). In another study, a structured psychological intervention that included health education, problem solving training, stress management skills, and emotional support resulted in increased numbers of circulating NK cells in patients with malignant melanoma (Fawzy et al. 1990). NKCA is also significantly elevated after experimental exposure to pleasurable psychological conditions, such as viewing a humorous video or watching a favorite actress in a film (Berk et al. 2001; Matsunaga et al. 2008). Underscoring the potential mediating role of immune factors on mood and affect, a study investigating the effects of a mindfulness-based stress reduction program on immune function and psychosocial well-being found that patients who reported improvement in well-being following the intervention had increased NKCA, whereas the NKCA of those who reported no improvement in well-being was not significantly different from baseline measures (Fang et al. 2010).

14.7.3 Positive Mood and Immune Function

Complementing the above findings on positive mood and immune function, there is considerable evidence suggesting that positive moods, such as joy, are associated with decreased vulnerability to viral infections, decreased physical pain, and *increased* longevity (Pressman and Cohen 2005; Cohen et al. 2006; Marsland et al. 2006). For example, a recent study that controlled for social and cognitive factors found that individuals with a positive emotional style were less likely to develop an upper respiratory infection when they were exposed to common cold viruses (Cohen et al. 2006).

14.8 The Infection-Defense Hypothesis of Depression

From the perspective of evolutionary biology, depression is a paradox. It is associated with serious disadvantages for survival and reproduction of both patients and their kin, and it is responsible for a wide range of social, emotional, and economic burdens as well as increased risk of mortality (Murray and Lopez 1996; Mykletun et al. 2007). Yet depression is common—indeed, it appears to be the most prevalent mental disorder—and it has significant heritability (Kessler et al. 2005; Sullivan et al. 2000). Here, and in a number of recent papers (Kinney and Tanaka 2009; Tanaka and Kinney 2011), we have described an *infection-defense* hypothesis of depression that has the potential to resolve this fundamental paradox, and to explain a number of symptoms, risk factors, and psychoimmunological features of depression that previous evolutionary theories have not explained.

14.8.1 Rationale and Support for the Infection-Defense Hypothesis

Depression often develops in a context of immune activation and sickness behavior (Raison et al. 2006; Dantzer et al. 2008). Dantzer (2001, 2009), extending the work of Hart (1988), has suggested that sickness behavior is an expression of a biologically-mediated *motivational state*, triggered by the innate immune system, that resets the organism's priorities to adaptively cope with the threat of bodily insult. Fever, malaise, anorexia, pain and fatigue all function to conserve metabolic resources and avoid further stressors that could exacerbate illness. The costs of shifting resources and priorities during sickness behavior appear to be offset by the critical advantages offered for fighting infection.

Only a portion of individuals who experience sickness behavior, however, go on to develop depression. There is evidence that secondary development of depression following sickness behavior is most likely to occur in those individuals who have an exaggerated vulnerability to infection (Dantzer 2009). This is consistent with evidence cited earlier that a wide range of medical and physiological or psychological conditions in humans—such as chronic stress and sleep deprivation, cancer, autoimmune diseases, and exposure to environmental toxins—are associated with increased vulnerability to infection as well as with increased risk of depression.

The *infection-defense* hypothesis of depression (Kinney and Tanaka 2009) proposes that immune vulnerability to infection elicits depressed mood, which in turn stimulates behaviors that help protect *vulnerable individuals and their kin* against infectious diseases. Moods potentially provide an elegant system for behavioral defense against infection, with the potential to affect a wide variety of behaviors. In addition, moods provide a way to orchestrate these behaviors in a

manner that is both timely and titrated. Akin to the adaptive functions of sickness behavior, the *infection-defense* hypothesis includes the concept that depressive symptoms conserve energy and prioritize resources for the immune system in fighting existing infections. Many symptoms of depression, such as anhedonia, psychomotor retardation, and fatigue help to conserve essential metabolic resources for fighting infection, while more costly antibiotic strategies such as fever that is associated with sickness behavior appear to be subverted (Maier and Watkins 1998).

The scope of the *infection-defense* hypothesis is much broader, however. We have proposed that depressive signs and symptoms offer advantages not only for fighting existing infections, but *also* for preventing individuals and their biological relatives from contracting *new infections* (Kinney and Tanaka 2009). Social withdrawal, low energy, and blunted affect, for example, may greatly reduce the spread of infections by reducing and discouraging social engagement with others.

The *infection-defense* hypothesis also proposes that there are bidirectional processes that mediate communication between the nervous and immune systems, and provide mechanisms for infections, immune processes, and mood to influence one another (Kinney and Tanaka 2009). The emerging role of neuroendocrine factors in the etiology of depression (Raison et al. 2006; Dantzer et al. 2008), along with evidence that antidepressant medications have antibiotic/antimicrobial effects and enhance immune function (e.g., Frank et al. 1999; Lieb 2007), strongly supports this tenet. The bidirectional nature of these processes is further underscored by significant evidence that a number of other mood-enhancing treatments and interventions—such as exercise (Haaland et al. 2008; Woods et al. 2009), ECT (Fischler et al. 1992; Kronfol et al. 2002; Hestad et al. 2003), and various psychosocial interventions (Robinson et al. 2003; Witek-Janusek et al. 2008; Fang et al. 2010)—enhance immune function, with time courses of immune response often paralleling those for mood (Tanaka and Kinney 2011).

14.8.1.1 Concluding Remarks on the Infection-Defense Hypothesis

Infectious diseases have been one of the strongest forces of natural selection throughout human evolution. For example, sickle cell anemia is a hereditary disease that is typically fatal, yet it is very prevalent in tropical regions. People who inherit only one copy of the sickle-cell gene, however, have increased resistance to malaria, allowing the gene to persist despite its severe costs (Kwiatkowski 2005). The *infection-defense* hypothesis provides an analogous evolutionary explanation for the persistence of depression-related genes and behaviors. Specifically, we suggest that, despite depression's costly burdens, activation of depressive moods and behaviors in response to infection or immune vulnerability is an attractive evolutionary strategy for fighting infection that provides a compensatory advantage for survival and helps to explain depression's symptoms, risk factors, and adaptive value.

14.9 Summary

A large body of converging evidence suggests that depression is often an inflammatory/immune-mediated response to infection, vulnerability to infection, and/or chronic activation of the innate immune system. This inflammatory response is stimulated by increased production of proinflammatory cytokines, which have wide-ranging effects on both neuroendocrine and neuronal systems, including an inhibitory influence on serotonergic transmission. This emergent model of depression helps to explain: (1) why depression is associated with immune alterations such as decreased NKCA, (2) why depression is associated with increased rates of infection and disease, and (3) why a wide range of environmental and physiological factors associated with increased vulnerability to infection are also associated with increased risk for depression.

In addition, the discovery of inflammatory-immune factors in the physiology of depression helps to explain an important psychiatric puzzle as to why genes associated with major depression have persisted, despite depression's association with increased morbidity and mortality. As we have outlined in the *infection-defense* hypothesis, signs and symptoms of depression such as anhedonia, social withdrawal, reduced energy and psychomotor retardation—and the genes that contribute to them—may be explained as adaptive responses to infection vulnerability, genes and responses that have been selected for because they serve to: (1) conserve metabolic resources for fighting infection, (2) reduce exposure to further infections or environmental stressors, and (3) reduce social contact to prevent the spread of infection to kin.

14.10 Recommendations

Associations among the environment, depression, and the immune system—and in particular, evidence that moods can serve as a behavioral defense against infection—carry important implications for understanding the causes, treatment, and prevention of depression. Challenging conventional wisdom about the treatment of depression, possible underlying infectious and immune factors deserve greater consideration in the treatment and prevention of depression. The following recommendations are noted:

1. As there is significant evidence that depressed patients have a heightened vulnerability to infection (Irwin and Miller 2007), depressed patients may be especially likely to have undiagnosed and untreated infections, disease, and/or other immune impairments. In fact, several studies have suggested that psychiatric patients *do* tend to have more physical illness than the rest of the population, and that this illness is frequently *unrecognized and untreated* (Rabinowitz et al. 1997). Thus, stronger efforts to diagnose possible underlying infections and immune disorders might aid in understanding the etiology in many cases of depression.

2. Environmental pathogens that trigger immune impairment and inflammation, such as toxigenic molds, pesticides, and other pollutants, also warrant greater attention for their potential role in the etiology of depression. For example, exposure to toxigenic mold is associated both with alterations in both NKCA and various neuropsychiatric symptoms, including depression (Anyanwu et al. 2003). Epidemiological, observational, and experimental research aimed at identifying environmental toxins that contribute to inflammation and depression are greatly needed and may potentially help to explain recent increases in rates of depression (Compton et al. 2006).
3. If depressed patients are especially likely to carry infectious diseases and/or to be immunologically vulnerable to them, then hygiene practices in clinical programs deserve greater attention. Shared waiting rooms or living spaces (in the cases of inpatient or residential programs) could inadvertently contribute to the spread of depression by bringing patients together in close proximity. Efforts to optimize hygiene by encouraging frequent hand-washing, disinfecting shared spaces, and avoiding unnecessary contact among patients and caretakers could potentially aid in preventing the spread of depression.
4. Appropriate steps to enhance immune function could potentially complement traditional antidepressant therapies. The reduction of immune-compromising factors—such as sleep disorders, chronic pain, dietary deficiencies, and insufficient exercise—deserves more investigation as a possible approach to treating and preventing depression. For example, moderate exercise, and nutritional supplementation to correct for deficiencies in omega-3 fatty acids and vitamin D, may be simple and economical ways to treat depression.
5. Pharmacologic and psychological techniques that have established antimicrobial or immune-enhancing properties also merit more study for their potential antidepressant effects. For example, preliminary results investigating the antidepressant effects of cyclooxygenase (COX)-2 inhibitors, which are anti-inflammatory drugs, have shown promising results (Müller 2010). Given the insufficiency of current psychopharmacological treatments that have been designed to target deficiencies in synaptic concentrations of serotonin and norepinephrine, our emerging understanding of immune factors in depression has the potential to lead to novel interventions that target neuroendocrine processes.

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Part IV
Infections and Cancer

Chapter 15
Environmental Toxicants and Susceptibility to Infection

Carsten Heilmann

Abstract The role of environmental toxicants for susceptibility to infections is of major importance. Even a modest influence by toxicants on the immune function may play a role for morbidity and mortality and affect individuals with conditions such as malaria, tuberculosis, AIDS, cancer, organ transplantation, and old age. A "window of vulnerability" for the sensitivity of the immune system to environmental toxicants is presumed to exist in the prenatal and the early postnatal periods. Many studies have shown that exposure to environmental toxicants, predominantly in the form of organochlorides, may induce an increased susceptibility to infection. In humans such exposure has particularly been shown to cause not only upper and lower airway infections, but also reactivation of latent viral infections. This chapter presents and discusses the evidence for the association between environmental toxicants and increased susceptibility to infection.

Key Points

- Although environmental toxicants generally have a modest influence on the immune system, the effects may be important on a population level.
- Evidence suggests the existence of a "window of susceptibility" possibly related to increased perinatal sensitivity of the thymus to organochlorides.
- Studies of wild animals show increased risk for infection in relation to exposure to environmental toxicants.
- Many studies in laboratory animals prove increased susceptibility to infection after exposure to environmental toxicants.
- Human field studies have suggested increased susceptibility to lower and upper airway infection following exposure to organochlorides.

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15.1 Introduction

Infections play a decisive role for human health and infectious diseases represent one of the major causes of increased mortality, in the third-world countries. In industrialized countries infectious diseases also play a role for mortality; however, they particularly influence morbidity. Infections not only have direct effects on individuals but are also important because of increased expenses due to loss of workdays and increased health expenditures. Even a modest influence by immunotoxicants on the immune function, either in the form of immunodeficiency or immune dysregulation, may theoretically play a role for morbidity and mortality. Immunotoxicants may exert their negative effect by increasing the risk of infectious diseases connected to chronic health problems and by increasing the duration and seriousness of otherwise less severe infections. As examples, modest effects on the immune function may play a negative role in individuals with conditions such as malaria, tuberculosis, AIDS, cancer, transplantation, and old age. Furthermore, the negative effect elicited by immunotoxicants may constitute a qualitatively modest but quantitatively important factor for the cause of common infectious diseases such as influenza, bronchitis, and otitis media. Finally, the cause of common latent infections may be changed; e.g., it is possible that immunotoxicants may cause reactivation of varicella zoster virus and herpes simplex virus infections (Arndt et al. 1999). As discussed by Selgrade (2007) and Luebke et al. (2004), adverse effects due to environmental toxicants may be difficult to point out on an individual level or by showing a relation to particular diseases but, nevertheless, such substances may have important consequences on the level of populations by increasing the impact of common infectious diseases. Polychlorinated biphenyls (PCBs) have been shown to influence negatively the immune response to diphtheria and tetanus toxoids given to infants as a part of the prophylactic vaccination program (Heilmann et al. 2006, 2010). The effect is not strong and in the majority of infants the diminished antibody level will not be of clinical importance. However, in a few children the antibody levels will however decline to subprotective levels and for this reason not only cause diminished protection but possibly also contribute to a reduced herd immunity. As discussed by Dietert (2008; Dietert et al. 2010) a "window of vulnerability" for the sensitivity of the immune system to environmental toxicants is presumed to exist in the prenatal and the early postnatal periods. In this phase of life the thymus plays a particularly important role for the development of T lymphocytes with an important role for the tissue type restricted immune defence and for the deletion of T lymphocytes with autoimmune reactivity. It is plausible that the activation of the aryl hydrocarbon receptor (Ahr) by exposure to dioxin and certain PCBs may affect the thymus function. In this context it is of interest that activation in mice of the Ahr by dioxin has been shown to influence the differentiation of proinflammatory interleukin (IL) 17-producing T helper lymphocytes and T regulatory cells (Quintana et al. 2008; Veldhoen et al. 2008). Exposure of mice to dioxin prenatally and during lactation causes increased sensitivity to influenza virus infection and results in an increased tendency for lung inflammation in outgrown animals (Hogaboam et al. 2008).

In humans it has been shown that exposure to PCB prenatally and through mother's milk negatively influence the antibody levels to tetanus and diphtheria toxoid later in life. Hence, the degree of perinatal PCB exposure was negatively correlated to antibody levels at age 5 and 7 years to a higher extent than the actual exposure levels at these time points (Heilmann et al. 2006, 2010). It is possible that the perinatal disturbance of the thymus function caused by immunotoxicants preferentially elicits increased risk of infection in small children, whereas the effect in older children and adults may be an increased predisposition for allergy and autoimmunity. Allergy in older children and adults may however also by itself cause increased likelihood of infection (Pelikan 2007).

A few studies have shown a connection between exposure to environmental toxicants and infection in wild animals. Hence, in sea otters death due to infection was particularly found in animals with a high level of PCB in the liver (Kannan et al. 2007). In artic gulls (glacous gull) infection with parasites was seen predominantly in gulls with a high exposure to selenium and mercury (Sagerup et al. 2009).

The connection between exposure to environmental toxicants and an increased tendency for infection has most clearly been shown in laboratory animals where confounders such as age, sex, nutrition, life style, genetic diversity, and the varying exposure to many different environmental toxicants are less of a problem. Some examples in relation to viral infections are given below; however, other reports of increased sensitivity to bacterial infections and parasitic infections exist. Luster et al. (1980) and Sugita-Konishi et al. (2003) have shown an increased sensitivity to *Listeria monocytogenes* in mice exposed to dioxin. Silbergeld et al. (2000) showed increased sensitivity to *Plasmodium yoelii* in mice exposed to mercury. Similarly, Inman and Chiu (2009) found that rats exposed to mercury had increased sensitivity to *Chlamydia* species.

However, as also underlined by others not only the situation in laboratory and wild animals should be considered. The data concerning humans are also of interest (Luebke et al. 2004).

In conclusion, various types of immune dysfunction may be caused by environmental toxicants. Both adaptive and innate immune dysfunctions seem to be affected. The impaired function of T-lymphocytes may be seen both as a dysfunction of the cellular immunity causing an increased morbidity to viral diseases as well as a diminished ability to produce specific antibodies to T-dependent protein antigens. A diminished ability to produce specific antibodies can be expected to increase primarily the risk for bacterial infections in the respiratory tract.

15.2 Lower Airway Infection

The association between exposure to environmental toxicants and infectious diseases has most often been shown in relation to lower airway infection.

In the years following 1968 patients appeared in the western parts of Japan who were suspected of having symptoms of intoxication with PCBs and possibly other

organochlorides (OCs). The source of pollution was traced back to contaminated rice bran oil. The characteristic disease induced by this contamination incidence was called Yusho (oil disease). Several years after the episode it became clear that PCB seemed to be toxic to the lung parenchyma causing contaminated individuals to develop signs of bronchitis with coughing, phlegm, and wheezing. It is unclear whether this tendency for bronchiolitis and small airway disease was caused by a direct toxic effect of PCB deposited in the lung parenchyma or if it was related to a temporary change in serum immunoglobulin (Ig) levels with increased IgG and diminished IgM and IgA (Shigematsu et al. 1978). In an investigation 14 years later some exposed individuals continued to have a tendency for coughing and phlegm and their lung function parameters were diminished (Nakanishi et al. 1985). Based on animal studies the same group of scientists has suggested that PCBs did not primarily cause the lung disease. The problem was more likely due to exposure to polychlorinated dibenzofurans (PCDFs) (Kunita et al. 1985). PCB contamination of food oil occurred in Taiwan in 1979 (the Yucheng incidence). Pregnant women ingested contaminated oil which caused many infants to be exposed prenatally and also postnatally through mother's milk (Guo et al. 2004). In connection with the Yucheng incidence, serum immunoglobulin changes similar to those observed during the Yusho episode were recorded (Lu and Wu 1985). By means of a retrospective interview-based analysis an increased occurrence of bronchitis and pneumonia was found among exposed infants as compared to an unexposed control group (Rogan et al. 1988). In adults a connection between bronchitis-like symptoms with coughing and wheezing and a high degree of occupational exposure to PCBs has been found (Smith et al. 1982).

Weisglas-Kuperus et al. (1995) examined if an increased occurrence of upper and lower respiratory air way infections could be found among healthy Dutch infants depending on the degree of exposure to PCBs and dioxin. However, no connection between high exposure and such infections was reported. More recently, the same group of scientist found a connection between high exposure to PCBs and a reduction in the occurrence of wheezing and shortness of breath in preschool children. In contrast, an association between exposure to PCBs (TCDD equivalents [TEQs]) and dioxin and coughing and phlegm was noted. In this publication, it was suggested that the increased occurrence of airway infections as a consequence of PCB TEQs and dioxin exposure might lead to a reduction in the occurrence of allergic symptoms (hygiene hypothesis) (Weisglas-Kuperus et al. 2000).

Studies of a population of Canadian Inuit infants have shown a connection between high prenatal exposure to PCBs and dichlorodiphenyldichloroethylene (DDE) through mothers' ingestion of fish and marine animals and the occurrence of, e.g., lower airway infections. Interestingly, no connection was found between postnatal exposure and the tendency for infection. Furthermore, PCBs seemed to be of greater importance than DDE (Dallaire et al. 2004). In a later study by the same group of scientists it was shown that Inuit school children, who were exposed to a relatively high level of PCBs prenatally, also had increased occurrence of lower airway infections (Dallaire et al. 2006).

In a study from Sweden the effect of prenatal exposure to PCBs on the frequency of airway infections was investigated in infants (Glynn et al. 2008). It was shown that the effect on the risk for infection depended on the kind of PCB congener in question. Hence, prenatal exposure to CB-28, CB-52, and CB-101 seemed to increase the risk of lower airway infections. In contrast, high prenatal levels of mono-ortho PCBs (CB-105, CB-118, CB-156, CB-167), di-ortho PCBs (CB-138, CB-153, CB-180), and high postnatal levels of mono-ortho PCBs, di-ortho PCBs, and DDE seemed to lower the risk of infections. It was hypothesized that the increased frequency of airway infections observed in infants with a high exposure to CB-28, CB-52, and CB-101 could possibly be related to the finding in this group of reduced concentrations of monocytes and lymphocytes.

Recently, researchers in Spain studied if exposure to PCBs and DDE was able to influence the occurrence of lower airway infections. Using a population-based investigation, they found that an association exists between high levels of maternal DDE in the first trimester and the occurrence of lower airway infections in their infants at the age of 6 and 14 months. Interestingly, no association between exposure to PCBs and the occurrence of infections was noted (Sunyer et al. 2010).

The differences found by various studies, in the occurrence of an increased risk for infection induced by exposure to OCs, are difficult to comprehend. However, the great variance in the levels of exposure to OCs may play a role. The exposure to OCs was much higher in the Canadian Inuit children than in the children of the Swedish and Spanish investigations. Another factor, which may be of importance, is whether OCs influence the lung function directly without influencing the immune function. In this context it is of interest that a negative association has been shown between the lung function of Dutch children between 7 and 12 years and the presence of dioxin in their mother's milk (ten Tusscher et al. 2001).

Although a negative effect of environmental toxicants on the lower airways has primarily been described in infants and young children such problems have also been encountered in adults. Occurrence of lower airway disease has most often been observed in relation to exposure to OCs; however an increased risk for lung infection has also been recorded in connection with welding. Pathological immunological findings have been observed in welders, but the most likely cause of lung disease is probably a direct physical or chemical effect (Antonini 2003; Tuschl et al. 1997).

15.3 Acute Otitis Media

Another type of infectious problem, which has often been discussed in relation to exposure to environmental toxicants, is acute otitis media (AOM). An association between exposure to OC and AOM was described for the first time in relation with the Yucheng episode 1978. Ingestion of PCBs and PCDFs through contaminated rice oil caused a variety of toxic symptoms in small children (Guo et al. 2004; Rogan et al. 1988). The occurrence of AOM in 11-year-old children who had

experienced interuterine and postnatal exposure to OCs was increased as compared to an unexposed control group. Furthermore, among exposed children the risk for AOM increased with increasing levels of PCBs and PCDFs (Chao et al. 1997).

In a study of 3.5-year-old Dutch children, an association between actual exposure to PCBs and dioxin and recurrent AOM was recorded. Interestingly, in this study a significant association between prenatal OC exposure and AOM was not found (Weisglas-Kuperus et al. 2000). Later the majority of the cohort was re-evaluated at school start, and at this time point an increased likelihood of otitis media was again found in children with a high postnatal exposure to PCBs (Weisglas-Kuperus et al. 2004).

In a study of Canadian Inuit infants from the Nunavik area, it was found that the prenatal exposure to OCs as judged by the presence of hexachlorobenzene (HCH), DDE, and dieldrin in early mothers' milk was associated with the occurrence of otitis media, in infants of 4–12 months (Dewailly et al. 2000). Later the same group in a new cohort of Inuit infants showed an association between exposure to OC and the occurrence of otitis media. In this study the association was particularly strong between high exposure to DDE and otitis media and it was predominantly observed among infants below 6 months (Dallaire et al. 2004). Later, this group further underlined the association between OC and otitis media. They showed that a high level of PCB-153 in umbilical cord blood was found in Inuit children who at pre-school age had an increased risk for AOM (Dallaire et al. 2006). Finally, in a large cohort of German children aged 7–9 years from a heavily industrialized area, an increased risk for otitis media was observed in children with a high exposure to OC (PCB, HCH, and DDE) (Karmaus et al. 2001).

Although the immunological background for the increased risk for AOM in children is not clear, the conclusion from the above studies indicates that following perinatal exposure to OCs there is an increased risk for AOM in children.

15.4 Viral Infections

Increased susceptibility to viral infections has particularly been described in relation to studies of laboratory animals. An increased morbidity to infection with encephalomyocarditis virus has been observed in mice following exposure to mercury chloride ($HgCl_2$), lead, cadmium, and nickel (Gainer 1977; Koller 1975). Furthermore, the degree of myocardial inflammation in mice infected with coxsackie B3-virus was increased if the animals had been exposed to heavy metals such as cadmium, methyl mercury, or nickel (Ilback et al. 1993, 1995, 1996).

Exposure to mercury chloride caused increased susceptibility to generalized herpes simplex virus type 2 infection in mice that were infected by peritoneal injection with virus (Christensen et al. 1996). This phenomenon seemed to be secondary to a reduction in the production of macrophage cytokines such as interferon-α/β and tumor necrosis factor essential for the early control of herpes replication (Ellermann-Eriksen et al. 1994).

A possible influence on resistance to infection following contact with dioxin has been investigated in mice by exposing the animals in uterus and postnatal to dioxin. Later as adults these mice were infected with influenza virus. Surprisingly, it was found that both the cellular and the humeral immunity was reduced in female mice, whereas this was not the case in male mice (Vorderstrasse et al. 2006).

In humans there are only few studies pointing to an increased susceptibility to viral infections following exposure to environmental toxicants. It is, however, noteworthy that the increased occurrence of lower airway infections and otitis media found after exposure to various OCs may be caused by an increased susceptibility to viral infections. Hence, viral disease is often the initial problem in children who later develop bacterial airway infections. The influence of environmental toxicants on the susceptibility to infectious diseases in children is probably most often subtle and does not cause a significant increase in hospital admissions. This could be the explanation why Fei et al. (2010) did not find an association between hospitalization for infectious diseases and exposure to perfluorooctanesulfonate and perfluorooctanoate, although these substances have been shown to cause immune suppression in both laboratory animals (Dong et al. 2009, 2011; Zheng et al. 2011) and in in vitro experiments (Corsini et al. 2011).

Direct evidence for an association between exposure to pesticides and viral disease has been found in an investigation of inhabitants from around Aberdeen, North Carolina. In this area, five waste sites for pesticides, volatile organic compounds, and heavy metals were situated. A group of inhabitants living close to the waste sites were compared with inhabitants who lived far from the waste sites. An increased relative risk for herpes zoster of 2.0 was found among younger people (18–40 years) living close to the waste sites. This study also included an investigation of a possible increase in other types of infections; however, no increased risk was found (Arndt et al. 1999). Later this group showed that individuals with a high concentration of DDE in the blood had reduced lymphocyte proliferative response possibly explaining the increased occurrence of herpes zoster (Vine et al. 2000).

The finding (Weisglas-Kuperus et al. 2000) of an increased occurrence of chicken pox among preschool children with a high exposure to PCB and dioxin is difficult to explain. However, these findings could be related to the symptoms being more aggressive in children with a high degree of exposure to OCs.

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Chapter 16
Childhood Acute Leukemia

Joseph Wiemels

Abstract Acute leukemia is the most common cancer in children but without definable causes in the majority of cases. Most cases are pre-B cell in origin, and this immunophenotype has increased in incidence over the past several decades in the Western world. Variable epidemiologic associations have been discovered between chemical exposures and childhood leukemia including parental smoking, pesticides, traffic fumes, and diet. However, much of the increase in leukemia rates is likely linked to changing patterns of infection during early childhood development. New evidence suggests that children who contract leukemia may harbor a congenital defect in immune responder status, as indicated by lower levels of constitutive neonatal IL-10 levels at birth. Medical record studies also demonstrate that childhood ALL patients received clinical care for infections within the first year of life despite having lower levels of exposure to infections.

Key Points

- Childhood leukemia is the most common cancer in children; lymphocytic subtypes dominate and have consistently risen in incidence over the past several decades.
- Childhood leukemias arise from two or more mutational events, and the first can often be traced to a fetal origin.
- Childhood ALL is inversely associated with surrogate markers of infection: exposure to daycare, normal vaccinations, and older siblings.
- Children who contract leukemia typically have more physician visits for infection during their first year of life and aberrantly low levels of IL-10 at birth, indicating an altered responder status to infections.

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- Infant leukemias are associated with *MLL* translocation and may have a chemical cause (anti-topoisomerase II activity).
- Childhood acute myeloid leukemias are more strongly associated with chemical exposures and not measures of immune development.
- Adult leukemias exhibit different subtypes from children and also more often exhibit a chemical-associated origin, e.g., occupational exposures, cigarette smoking, and solvents.

16.1 Introduction

Childhood acute leukemia is the most common cancer in children representing 31% of all cancers and about 3,250 new cases per year in the United States (Ries et al. 1999). Numerous breakthroughs in the past 50 years have increased survivability of the disease to greater than 80%, but long-term morbidities in survivors remain (Oeffinger et al. 2006). Clearly, identification of causes and prevention/early interventions is a goal, which can only be undertaken by understanding the causes of this disease. Known causes include ionizing radiation and congenital genetic syndromes such as Down's, neurofibromatosis, Fanconi's anemia, and Bloom's syndrome, all of which together explain less than 10% of cases. Incidence of the disease has increased close to 1% per year in the past 2 decades (Linabery and Ross 2008) with similar rates of increase decades earlier (Gurney et al. 1996; Kaatsch and Mergenthaler 2008), indicating that causal factors for the disease are likely to have become more prevalent in the population since that time. The most notable feature of this period of time is the rise of the "common acute lymphocytic leukemia" (cALL) peak in 2–6 years olds, a peak absent in some populations such as Africa and India, and in an ecological sense associated with countries that have higher socioeconomic status (Greaves and Alexander 1993). It is also notable that this peak may vary by ethnicity within the United States, with Hispanic:white:black ratio being 1.2:1.0:0.6 (Chow et al. 2010; Parker et al. 1998). Whether this is related to genetic or environmental reasons is unknown.

The disease is comprised of several subtypes that vary in phenotype and age incidence patterns (Fig. 16.1). The broadest categories are the lymphoid and myeloid split, with about 80% of leukemias being lymphocytic. Infant leukemias (<1 year of age) predominantly exhibit 11q23 *MLL* gene rearrangements and may have lymphocytic (pro-B), myeloid, or undifferentiated features (Fig. 16.2). Leukemia among young children (2–10 years) is dominated by a pre-B lymphocytic phenotype, and the cALL subtype, which is defined as 2–6 years of age, CD10+, CD19+ pre-B cell leukemia. Teenagers tend to trend towards adult-like leukemias, with an increasing frequency of myeloid types and a disappearance of cALL with increasing age. An appreciation of these phenotypes is critical as risk factors and immune dysfunction in particular are likely to affect their relative incidence differentially. Furthermore, within these subgroups are further groups defined by specific genetic features such as particular translocations, the presence

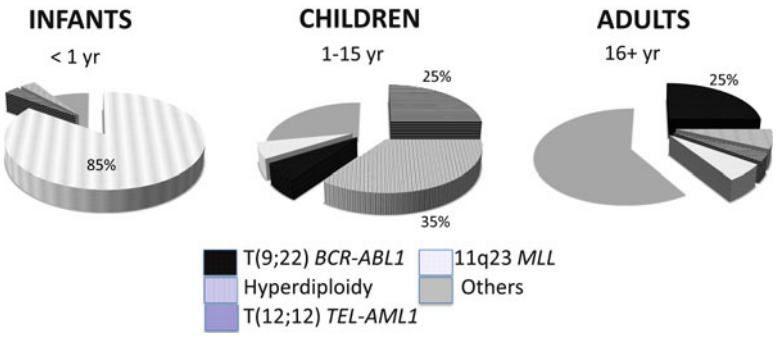

Fig. 16.1 Cytogenetic subtypes of childhood acute lymphoblastic leukemia by age group

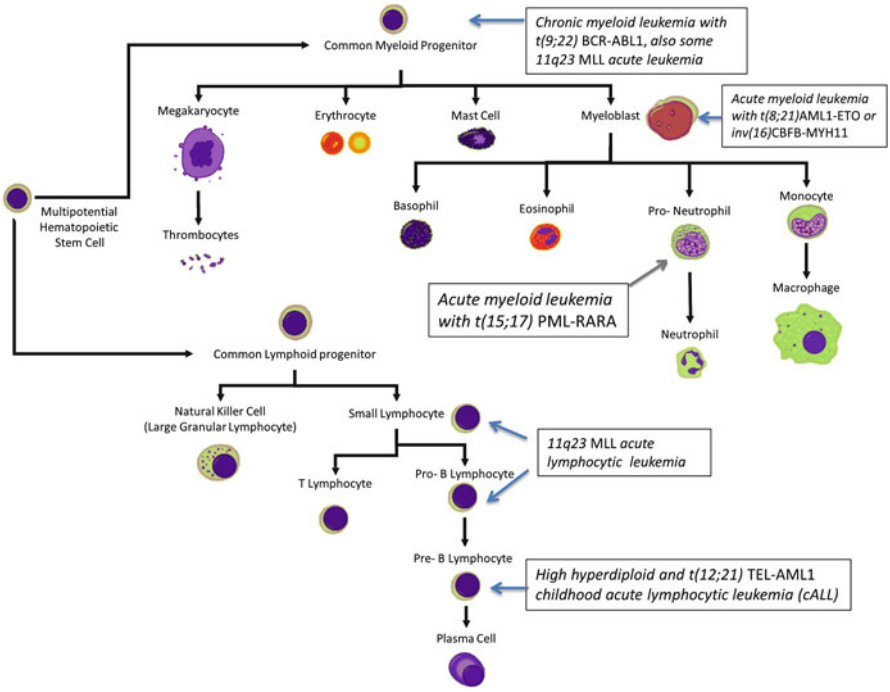

Fig. 16.2 Hematopoiesis, or the development of blood cells from a common stem cell precursor. The cell of origin for various types of leukemia described in the text are shown. Nearly every other cell type is also capable of exhibiting leukemia or, in the case of more differentiated B-cells, lymphoma and myeloma

of extra chromosomes, and gene mutations; the formation of each of these mutations appear to occur within specific time periods during the child's life with the mechanistic formation likely having distinct causes.

The genetic and epigenetic aberrations frequent in the childhood leukemias are often important prognostic indicators, and identification of several of these are

critical to modern disease classification protocols (Pui et al. 2011). The uniform characterization of childhood leukemias under modern clinical protocols also provides relevant subgroup information for etiologic studies and will increasingly become a critical component of leukemia epidemiology studies.

16.2 Natural History of Childhood Leukemia: Timing of Initiating Events

Childhood leukemia like all cancers is a product of two or more molecular changes in stem-like cells that have growth and repopulating ability. The majority of leukemias exhibit a pre-B cell phenotype, meaning that they exhibit cell surface markers of normal pre-B cells, and appear to be clonal outgrowths of normal pre-B cells "frozen" at a particular differentiation stage (Fig. 16.2). Less common are leukemias with myeloid or T-cell lineage. Being blood cells, leukemias have an inherent capacity for mobilization in the bloodstream and extravasation. Precursor blood cells also have an enormous capacity for "blast-like" growth with their normal function to produce 10^{11} cells per organism per day. These attributes are among the six "hallmarks of cancer" (Hanahan and Weinberg 2000), and the fact that hematopoietic precursors harbor these "cancer-like" attributes may be a reason by which leukemias seem to have far fewer genetic aberrations compared to solid tumors, which need to evolve these capacities through genetic mutations. The genetic simplicity of leukemia combined with the young age of the childhood leukemias has allowed researchers to delineate the timing of the formation of genetic aberrations in the lifetime of the child.

Most of the common genetic mutations in leukemia have been assessed for "backtracking to birth," meaning that they have been assessed for their formation during the fetal period (Wiemels 2008). This research was made possible by the availability of neonatal heel-prick blood spots, or "PKU cards" for children with leukemia. Several common translocations assessed on Guthrie cards, including t(12;21) *TEL-AML1*, t(8;21) *AML1-ETO*, inv(16) *CBFB-MYH11*, have indicated a clear presence of the mutations on neonatal blood spots at birth in children who contract leukemia later (McHale et al. 2003a, b; Wiemels et al. 1999b, 2002b). Several other mutations, including t(1;19), *FLT3*, and *RAS*, are clearly postnatal (Chang et al. 2010; Wiemels et al. 2002a, 2010). *MLL* translocations (11q23) appear to occur within temporal proximity to diagnosis, meaning that infants (<1 year old) have prenatal translocations, and young children have postnatal translocations (Gale et al. 1997; Maia et al. 2004).

16.3 Causes of Mutations in Leukemia

Apart from the MLL translocations, most mutations associated with leukemia are insufficient to cause disease by themselves. This is the case for *TEL-AML1* and *AML1-ETO*, the most common translocations for ALL and AML, respectively. Studies using cord bloods from normal born children indicate that these translocations may occur at a rate of 1% or more in the normal population (Mori et al. 2002; Zuna et al. 2011).

This remarkable result suggests that a significant proportion of the population carries preleukemic clones, and the vast majority of these clones are self-limiting and do not result in disease. What causes these frequent events? Cell kinetics in fetal and child hematopoiesis is remarkably high and takes place over several organs—the aorta-gonad-mesonephros region, followed by the liver, spleen, and bone marrow. The high degree of cellular proliferation creates a situation whereby perturbation via environmental insults including chemicals may induce mutations. The epidemiology of mutation-specific subtypes of leukemia is only just beginning, but there are signs of progress. Children with t(12;21) *TEL-AML1* translocations were more than fourfold more likely to be born from mothers who were exposed to paints during their pregnancy when compared to controls; this increased risk was not noted for other cytogenetic subtypes of leukemia (Scelo et al. 2008). To move forward, all leukemia epidemiology studies must consider genetic and cytogenetic subsets and combine cases of rare subgroups from multiple studies worldwide to gain statistical power.

While causes of specific rearrangements have only just started to be investigated, the general causes of childhood leukemia have been under investigation for decades as reflected by dozens of studies. The only consistent and confirmed environmental cause is ionizing radiation from sources such as diagnostic imaging during pregnancy or atomic bomb exposure during childhood and young adulthood (Little 2008). Other suspected contributors of leukemogenesis include diet of the mother and child, parental smoking, pesticides and household chemicals, traffic fumes, and immunologic modifiers, which we will cover in the next section. Smoking and pesticides have been recently and comprehensively reviewed and will not be further covered here (Chang 2009; Metayer and Buffler 2008).

As we are currently in the genome-wide association study age, the contribution of genetic modifiers is of current interest. To date, variation in several genes implicated in B-cell development (*ARID5B*, *IKZF1*, *CEBPA*) and cell cycle regulation/DNA repair (*CDKN2A/B*) are confirmed genetic risk factors for childhood ALL (Papaemmanuil et al. 2009; Prasad et al. 2010; Sherborne et al. 2010; Trevino et al. 2009), but dozens of other genetic risk factors are certain to emerge in the coming years. Besides these confirmed risk factors, candidate gene studies have implicated dozens of other genetic risk factors in candidate gene studies assessing pathways such as DNA repair, folic acid metabolism, and carcinogen detoxification. A recent meta-analysis of the multitude of studies has been published (Vijayakrishnan and Houlston 2010), but results of candidate gene studies must be taken with caution since publication bias and influence of underpowered studies may lead to false conclusions.

16.4 "Common" Childhood Leukemia: A Consequence of Aberrant Immune Modulation?

As noted above, most subtypes of childhood leukemia are initiated in the womb, but only a fraction of those "initiated" preleukemic clones will progress to leukemia. By far, the most critical epidemiologic modifiers identified to date for the majority of childhood leukemias pertain to immunological development. The idea that exposure

to infections and immune development might influence the etiology of leukemia in children grew out of two observations and led to two related hypotheses. Kinlen noted that leukemia incidence was often increased when children and families were moved and mixed in a new setting, such as the movement of children out of central London during World War II, and the creation of "new towns" in Britain in the decades following that war (Kinlen 1988, 1995). Kinlen proposed a "population mixing" hypothesis on the origins of leukemia and posited a specific viral infection as potentially causative. In examining data from the perspective of individuals rather than the population as a whole, Greaves noticed that children who received lower levels of immune stimulation during childhood developed a higher risk for leukemia and posited that a normal course of infections were protective (Greaves 1988, 2006). A specific viral cause is not involved in this hypothesis; rather, the lack of immune stimulation in children who are relatively isolated followed by an aberrant over-response to common infections later in childhood is thought to induce leukemia in children who harbor preleukemic mutations. Several common childhood leukemia aberrations are prenatal in origin and occur at a much higher frequency than the disease (Mori et al. 2002; Wiemels et al. 1999b), and those children who have a preleukemic mutation combined with aberrant immune development will be at the greatest risk. Given the current lack of discovery of any specific leukemia virus and the generally sporadic patterning of leukemia incidence among populations, one could argue that prevailing evidence favors the Greaves hypothesis although there are many similarities between that and Kinlen's hypothesis.

Since it is a rare disease, nearly all childhood leukemia studies gather information on cases and controls after children are diagnosed with leukemia. Immunological development is quite difficult to assess in these retrospective studies. Data is typically questionnaire-based and variables include those that are easily remembered without bias by families enrolled in such studies. These include childcare history, vaccinations, infections, and allergies/asthma. Childcare history, or daycare, is considered a proxy measure for exposure to infection—the more contacts a child has in daycare setting, the better chance for exposure to new infections. Studies of daycare and leukemia sometimes demonstrated no difference in frequency, or more often a *reduced* level of daycare in children who contract leukemia compared to controls. A recent meta-analysis by Urayama and colleagues compiled evidence from 15 studies and yielded a combined OR = 0.76, 95% confidence interval (CI): 0.67, 0.87, indicating a reduced level of prior childhood contacts in leukemia patients via daycare setting in the majority of studies, which is interpreted as a reduced level of immune stimulation/modulation in those children (Urayama et al. 2010). Greaves' "delayed infection" hypothesis states that a dysregulated immune response may occur when there is a lack of "priming" by infections during early childhood, resulting in an abnormal immune response to infections later in life and an increased risk of childhood leukemia, particularly childhood ALL (Greaves 2006). Other support for Greaves' hypothesis has come from studies showing an inverse relationship between childhood ALL and higher birth order (Dockerty et al. 2001; Westergaard et al. 1997) along with the daycare attendance studies, which both indicate that an increased opportunity for early childhood infections protects against leukemia.

Additional support is provided by studies of normal childhood vaccinations. While vaccinations are largely ubiquitous making it difficult to assess their role in a population (very few unexposed persons) studies of *Haemophilus influenzae*, a vaccination phased in over the past 2 decades, indicate that vaccination reduces risk to leukemia (Groves et al. 2000; Ma et al. 2005). This is not likely to be a specific effect; rather, another example of an immunomodulatory stimulation resulting in an immune system with less hyperresponsiveness.

Greaves' hypothesis is similar to the "hygiene hypothesis" proposed by Strachan to explain the rising prevalence of allergy in the western population (Strachan 1989). Strachan hypothesized that early childhood infections may be protective against allergy, but that declining family size and improved sanitation may have reduced exposure to infectious agents during early childhood thus resulting in the rising prevalence of allergy (Strachan 1989). The hygiene hypothesis has been supported by epidemiologic studies that reported an inverse association between allergy and higher birth order (Bernsen et al. 2003; Lewis and Britton 1998; Westergaard et al. 2005) or early daycare attendance (Haby et al. 2000; Kramer et al. 1999), similar to the associations seen with childhood leukemia. Allergies and asthma have generally been associated in an inverse relationship with leukemia as detailed in a recent meta-analysis on the subject (Linabery et al. 2010). On the face of it, this contradicts the data above on allergies; if allergies and leukemia share the same risk factors related to immune development, shouldn't their incidence patterns be similar? The case-control studies that assess allergy do so by assessing parental recall of allergies. Case parents may be more likely to ruminate about factors that may have affected their child's risk to leukemia leading to false positive associations. In addition, control families tend to misreport allergies that may have occurred after a "reference date" (diagnostic date for the corresponding leukemic children), therefore, overreporting exposures, infections, and medical conditions (Schuz et al. 2003). Interestingly, the two studies that utilized medical record abstraction rather than patient interview found that allergy was a *risk* factor for leukemia (Chang et al. 2011a; Spector et al. 2004), which is more plausible given the similar risk factors for these two diseases. This illustrates a situation whereby systematic bias may affect a series of case-control studies and produce an aberrant conclusion.

Leukemia is a cancer of the immune system, and while immune stimulation by a normal course of infections and vaccinations appears to be protective, there are other data suggesting that children who contract leukemia may be born with an aberrant immunity that makes them respond to infections more vigorously. The United Kingdom Childhood Cancer Study (UKCCS) reported that children diagnosed with ALL had significantly more clinically diagnosed infectious episodes in the first year of life compared to controls (Roman et al. 2007); this result was replicated in an independent study (Cardwell et al. 2008). The number of infectious episodes in children with ALL increased with increasing indices of infectious exposure (birth order, regular social activity outside the home, and social deprivation at birth), a phenomenon not observed among healthy control children (Simpson et al. 2007). The UKCCS also clearly demonstrated fewer social contacts for children contracting leukemia, indicating that overall

exposure to infections were likely lower than controls. This suggests that there may be two separate phenomena influencing leukemia risk: (1) a lower repertoire of infections during early immune development which will increase risk (i.e., daycare); (2) an altered congenital responder status to infection results in a functionally aberrant clinical presentation of occasional infections in these same children (i.e., greater propensity to need clinical care when contracting infection). In support of this, we recently discovered that a key cytokine is severely deficient among children who grow up to contract leukemia, a greater than tenfold difference when comparing the highest tertile to the lowest (Chang et al. 2011b). IL-10 is a key regulator for modulating the intensity and duration of immune responses to infections (Chang et al. 2011b). It has been reported that an increased risk for repeated respiratory infections during infancy and childhood associated with an elevated production of IL-5 by T cells at birth could be attenuated by IL-10 production (Zhang et al. 2009). It is possible that children with a dysregulated immune function at birth are at higher risk for developing leukemia due to constitutively lower expression of IL-10, a cytokine that is critical to prevent an overactive inflammatory response to pathogenic infections. Biological stress from postnatal infection in combination with a dysregulated immune response may confer a growth advantage for a preleukemic clone leading to its rapid expansion and an increased opportunity for the occurrence of a second mutation required for the development of childhood leukemia (Greaves 2006). The mechanism behind this constitutive lower IL-10 expression at birth will be a critical research question in discovering the causes of childhood leukemia.

16.5 Infant Leukemias and *MLL* Translocation: Chemically Induced Leukemias?

The infant leukemias are quite unique among all childhood leukemias in age distribution and the singular association with one particular genetic mutation—translocations at the *MLL* gene in chromosome 11q23. Mutations in this gene occur in more than 85% of infant leukemias and close to 100% of prenatal and congenital (close to birth) leukemias. These leukemias can harbor lymphoid or myeloid and occasionally undefined lineage characteristics but share a common transcription profile (Armstrong et al. 2002). *MLL* has been termed a "promiscuous" gene as its translocation to nearly 100 different partners has been associated with leukemia (Burmeister et al. 2009). As noted above, *MLL* translocations in infants are typically prenatal events. One may ask whether *MLL* translocation is simply more common than other translocations in the fetus, resulting in its more frequent presence in infants. Several observations counter this, however. First, infant leukemias with *MLL* appear to have more mature immunoglobulin gene rearrangement pattern than the common childhood ALLs of older children, indicating that the leukemia cells actually were initiated later, possibly in the

third trimester of pregnancy (Fasching et al. 2001; Steenbergen et al. 1994; Wasserman et al. 1992). Second, *MLL* translocations are highly transforming and the human disease has an extremely short latency, indicating that secondary mutations in this leukemia subtype may not be the rate-limiting event for full disease, and the leukemia incidence rate may mirror the translocation frequency (Kim-Rouille et al. 1999). *TEL-AML1* translocations, for instance, are present at a 10^{-2} (or 1%) frequency among neonates and the vast majority do not progress to leukemia (Mori et al. 2002; Zuna et al. 2011), compared to 10^{-5} frequency for infant leukemia with *MLL* translocations.

MLL translocations are common in another set of leukemia patients—iatrogenic leukemias induced by cancer chemotherapy of other cancer sites. Interestingly, *MLL* translocations were observed in adult therapy-related leukemias after the introduction of cancer chemotherapy drugs which target topoisomerase II, including the epidophyllotoxins, during the 1980s (Larson et al. 1992). This observation resulted in a theory that *MLL* translocations in idiopathic leukemias may be a product of dietary, medicinal, or environmental chemicals which target topoisomerase II (Ross et al. 1994). The intense research interest that followed described many possible mechanisms by which drugs and other conditions might enhance breakage of *MLL* gene. Specific topoisomerase II sequences in the *MLL* gene, chromosomal scaffold attachment sites, apoptotic nucleases, and unique structures derived from transcriptional torsional forces may contribute to *MLL* breakage (Aplan et al. 1996; Broeker et al. 1996; Sim and Liu 2001; Strick et al. 2000; Strissel et al. 1998, 2000; Vaughan et al. 2002). The mechanism is still not fully clear and the exact chemical constituents that might contribute to the formation of *MLL* translocations in infants has not been fully elucidated. An intriguing report from Brazil and another international study implicated a type of insecticide and a therapeutic analgesic as potential agents (Alexander et al. 2001; Pombo-de-Oliveira and Koifman 2006). While the epidemiology of idiopathic infant leukemias in the United States has not yet found consistent culprits (Ognjanovic et al. 2011; Slater et al. 2011), the strong epidemiologic associations with specific clinical therapies in iatrogenic leukemias continue to focus interest on topoisomerases in the causes of infant ALLs. Efforts at examination of *MLL* breakpoints at the molecular scale have identified a translocation hotspot in therapy-related leukemias (Aplan et al. 1996; Broeker et al. 1996); interestingly about half of infant breakpoints have the same molecular phenotype as therapy-related breakpoints suggesting that diverse mechanisms of formation (more than simply topoisomerase II inhibition) may play a role in infants (Cimino et al. 1997; Felix et al. 1998; Jung et al. 2010).

MLL leukemias are not alone in harboring a chemical etiology. Certain myeloid subtypes are also suggestive. The *AML1* gene is involved in many translocations and has also been linked to anti-topoisomerase II agents (Roulston et al. 1998; Rowley and Olney 2002). Classical clastogenic chemotherapy and environmental alkylating agents are associated with leukemias that have less precise breakpoints but commonly exhibit broken chromosomes and aneuploidy (Larson et al. 1996).

16.6 Lessons from Leukemia Clusters

Perhaps no other cancer type has captured both public and scientific attention in the same way that childhood leukemias have in the topic of cancer clusters. The most famous clusters include the Seascale cluster in the Lake District, UK; the Woburn cluster in Massachusetts; and the Fallon cluster in Nevada. Typically, chemico-physical causes are blamed with local culprits being ionizing radiation from a new nuclear plant (Seascale), trichloroethylene (Woburn) and tungsten alloy dust or petroleum fuels (Fallon). Only one of these reached a legal standard of proof (Woburn) at this writing, but scientific proof has been elusive. Studies of radiation exposure in the Seascale cluster were negative; however, it has been noted that the children who got leukemia were preferentially migrants to the area and experienced the most population mixing, providing credence to the population-mixing hypothesis or infectious causes of this cluster (Dickinson and Parker 1999; Greaves 1997). In Fallon, the unique exposure environment includes arsenic and ^{210}polonium in the water, a tungsten/cobalt refinery, and a Navy flight and aircraft carrier base with its attendant exposures including jet fuel occupational exposures. The leukemias from the Fallon cluster share a unique profile—nearly all cases were from children who lived near agriculture fields and open irrigation ditches forming an annulus around the town (Francis et al. 2011). This suggests some possible infectious cause transmitted through a water-borne means. The US armed forces exhibited a similar peak incidence of leukemia at the same time suggesting transmission of an infectious agent throughout the armed services branches (Francis et al. 2011). The timing was coincident with an adenovirus epidemic throughout the military (Metzgar et al. 2007; Russell et al. 2006); however, no concrete link between adenovirus and leukemia is currently available.

16.7 Adult Leukemias

Any discussion about the causes of leukemias should include a consideration of adults, who contract about 90% of all leukemias diagnosed or about 40,000 individuals per year in the United States. Adult leukemias are different diseases from their childhood counterparts. First of all, some subtypes exist in adults and not children and vice versa. The most common leukemia in adults is chronic lymphocytic leukemia (CLL), which is not seen in children. Adults also display a wider range of leukemia subtypes with lower incidence of B and T-cell ALL but much higher chronic myeloid leukemia (CML), AML, and myelodysplastic and myeloproliferative syndromes. These latter four subtypes are myeloid in nature, meaning that they are derived from the precursors to cells critical in innate immunity rather than adaptive immunity. These cells, particularly neutrophils and their precursors, produce large amounts of enzymes that can both produce cytotoxic mediators as part of their normal function (e.g., myeloperoxidase) and activate environmental chemicals that reach the bone marrow, producing genotoxic intermediates. A prime example of this

phenomenon is exposure to benzene, which is metabolized in the liver to produce various phenols and quinone intermediates (which themselves are not leukemogenic when ingested). The zonal localization of Phase I and II enzymes in the liver facilitates the formation of these benzene metabolites, which enter the hepatic vein and general circulation; whereas phenol and hydroquinones themselves are detoxified by conjugation in the liver prior to entering general circulation (Medinsky et al. 1995). Benzene metabolites are activated to nucleophilic compounds by myeloperoxidase in the bone marrow causing DNA damage and also may be subsequently reduced by quinone oxidoreductases (Smith 1996; Wiemels et al. 1999a). Benzene is a confirmed cause of both leukemia and lymphoma (Hayes et al. 2000; Steinmaus et al. 2008) and has been linked to the formation of chromosome rearrangements typical of leukemia even in normal healthy, exposed persons as well as hematopoietic defects (Lan et al. 2004; Zhang et al. 1998, 2011b). Other causes of leukemia in adults are chemicals associated with the rubber industry (butadiene, etc.), radiation, smoking, and chemotherapies for cancer (Bowen 2006).

A second difference between adult and childhood cancers is the molecular phenotype of some of the same disease classifications. The *TEL-AML1* and hyperdiploid subtypes, which together account for half of childhood leukemias, are rare if not absent among adult ALLs, the latter of which are dominated by more complex chromosome aberrations as well as a different dominant repertoire of translocations including *BCR-ABL1* (the "Philadelphia chromosome") (Liu et al. 2009). Unlike the childhood leukemias, there is some *specific* viral involvement of some types of adult leukemias. HTLV1 causes T-cell leukemia, rarely, in individuals infected in endemic areas (Goncalves et al. 2010).

16.8 Environmental Causes: Synthesis of the Evidence

Like most cancers, leukemia has been linked to certain environmental chemicals and ionizing radiation. Chemicals with specific capacity to target the bone marrow via selective accumulation and metabolic activation are the prime candidates. The myeloid series harbors higher levels of enzymes with metabolic activation and is more consistently linked to chemical exposures such as cigarette smoking, occupational solvent exposures, and alkylator chemotherapies in therapy-related leukemias. The 11q23 *MLL* translocation is linked to chemicals with topoisomerase-II inhibition activity and results in leukemias of both myeloid and lymphoid subtypes.

As leukemia is a neoplasm of the immune system, it is highly plausible that immune modulators will also modulate risk, particularly for the adaptive immune leukemias derived from pre-B and T-cells. Infections, vaccinations, population mixing, and birth order are linked to lymphocytic leukemias, in particular the pre-B cell childhood ALLs. The discovery of causes of childhood leukemia clusters has been vexing to the research community, but evidence best implicates patterns of infections, possibly specific infections, spreading through naïve communities as an ultimate cause. Prior to an infection being the ultimate stimulus, a normal series of

early infections appears to be protective for leukemia. Recent evidence from epidemiologic and laboratory suggests that newborns who grow up to get leukemia may be born with low IL-10 levels and a propensity to react to normal infections in a hyperresponsive manner during their first year of life.

16.9 Future of Childhood Leukemia Research

Childhood leukemia like all cancers is currently being subjected to intensive analysis by the "new genetics," meaning genome-wide association studies (GWAS), high dimension expression and copy number variation, mutation analysis, and array-based intensive DNA methylation analyses. The power of GWAS studies is clear but limited due to the modest effect sizes of a handful of significant SNPs. The small effect sizes of the handful of GWAS genes discovered so far limit the potential for the genes to be useful in prevention or clinical modalities; however, the identities of such genes illuminate cellular pathways that may otherwise not be known to be involved in these diseases. Just as environmental epidemiology studies will become more powerful with precise disease classifications, GWAS studies will become more powerful when larger numbers of diseases in precise classifications are studied, such as those described by recurrent chromosomal abnormalities such as *TEL-AML1*, high hyperdiploidy, and *PML-RARA*. These GWAS studies will require consortium studies involving pooling of samples to obtain required sample sizes.

Next generation sequencing analyses will certainly discover new genes and pathways in leukemia. The transformative potential of this technology is evident in the discovery of *IDH1/2* and *TET2* mutations in adult acute myeloid leukemia and brain cancer and the discovery of the critical nature of DNA methylation pathways in these two diseases (Abdel-Wahab et al. 2009; Langemeijer et al. 2009). Mutations in these genes are not present in childhood AML or ALL, which, besides having several common translocations, mutations, and deletions, have not been fully vetted for genetic and epigenetic architecture. Coordinated analyses involving gene expression, gene copy number change, and epigenetic modifications such as the TARGET initiative will help to complete knowledge of the range of modifications in leukemia cells (Zhang et al. 2011a). These initiatives must ultimately be linked up to population-based epidemiologically derived data on infections, exposures, diet, and other environmental factors for true clarity to be reached on the causes of childhood leukemias.

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Part V
Prevention of Chronic Diseases

Chapter 17
Reducing the Prevalence of Immune-Based Chronic Disease

Rodney R. Dietert, Jamie C. DeWitt, and Robert W. Luebke

Abstract As described in prior chapters of this book, chronic diseases connected to immune and inflammatory dysfunction are often categorized based on the affected tissues and, as a result, can be overlooked as being immune-mediated diseases. These diseases are numerous in nature, costly to individuals, families and nations, and pervasive. They represent an emerging global health threat where effective strategies capable of reducing disease prevalence have been slow to emerge. This concluding chapter includes information on four additional immune/inflammatory-based diseases or conditions: psoriasis, autoimmune thyroiditis, frailty, and an isolation-withdrawal state. It also provides an overview of plans for reducing the risk of immune-based chronic disease and emphasizes the importance of acting early in life and using safety data that are directly relevant to priority chronic diseases. The risk-reduction strategy combines four key components: widespread outcome-based safety testing that precedes human exposure, whole-life-considered therapeutic interventions, strategic research, and effective public health policies.

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Key Points

- Chronic diseases, including those that are immune-based, represent a global health threat.
- Reducing the prevalence of chronic diseases is necessary to address the current healthcare, economic, and quality-of-life crises that are occurring around the globe.
- Early-in-life public health interventions are likely to be most effective at preventing chronic diseases.
- Widespread outcome-based safety testing that is directly relevant to risk of chronic diseases and precedes the exposure of populations should drive interventions. Immunotoxicity assessment is a necessary component of effective safety testing.
- Therapeutic strategies should be based on lifetime health risks including knowledge of patterns of interlinked chronic diseases and probable comorbidities. An individual diagnosed with a first chronic disease/condition rarely goes through life without additional diagnoses (as shown in the prior chapters and the figures in this chapter).

17.1 Introduction

Organ failure and death from acute organ toxicity are no longer the most likely adverse health outcomes from exposure to chemicals or drugs. Instead, chronic disease is the most significant environmentally-influenced health burden of today. Chronic diseases, also called noncommunicable diseases (NCDs), are recognized as a serious problem in developed countries but the threat posed by their burden is not restricted to developed nations. Warning signs suggest that this is presently or soon to be the dominant global health problem (Greenberg et al. 2011). In fact, it is estimated that 60% of all deaths globally are attributed to NCDs (e.g., cardiovascular disease [CDV], asthma, Alzheimer's disease, diabetes), and that percentage is expected to rise to 70% in fewer than 20 years (WHO 2005).

The impact of NCDs on global death does not reflect the massive societal and economic impact that is linked to the persistent nature of these diseases. The duration from initial diagnosis of a chronic disease until death is often decades; because the diagnosis of a chronic disease is associated with a heightened risk of additional chronic diseases as the individual ages (Dietert et al. 2010), the individual faces ever-increasing healthcare needs and often experiences a declining quality of life.

The extent of the NCD problem has led to increased attention and commitments from international organizations such as the United Nations and the World Health Organization (Mamudu et al. 2011; Partridge et al. 2011; World Health Organization 2005). In fact, there is some evidence to suggest that the prevalence of common chronic diseases and conditions is already challenging the limits of some healthcare systems (Gulley et al. 2011) and may be greater than the estimates obtained using certain medical databases (Chini et al. 2011). For example, one research group showed that hospital information system (HIS) estimates of common chronic

diseases were underestimates when compared with the numbers obtained using a pharmacy database approach (Chini et al. 2011).

Recognition of the role of environmental factors on the prevalence of chronic diseases is also drawing additional attention. Recently, the Public Health and Environment Division of the WHO called for more effective national and international public health interventions to manage chemicals and limit their public health impact (Prüss-Ustün et al. 2011). Additionally, strategies are under consideration to incorporate health economics into clinical translational research. This was recently applied to nutrition interventions (Wong et al. 2011). Across the various chapters of this book, multiple categories of environmental factors (environmental chemicals, drugs, dietary components, type of microbial environment, birth delivery mode, etc.) were implicated in the increased risk of one or more chronic diseases. Integrated strategies across all categories of environmental risk factors will be important for increased prevention of chronic diseases.

Most chronic diseases are recognized to have their origins in early life (Hanson and Gluckman 2011). Therefore, opportunities for public health-driven early-life interventions are likely to be the most effective from both a biological and an economic standpoint. Such interventions could be aimed at risk reduction and/or tailored to better manage existing conditions to reduce the risk of additional chronic diseases in later life (Dietert et al. 2010). Protection against chronic disease should be viewed over the entire template of a lifetime. Critical environmental exposures may be temporally removed from the onset of disease. For example, if the risk of chronic disease is impacted most in early development (Hanson and Gluckman 2011), the process of aging allows the adverse effects of problematic prenatal and childhood environmental exposures to become obvious. This is particularly true for immune dysfunction-driven diseases that are initiated by developmental immunotoxicity.

By definition, juvenile-onset chronic conditions such as Kawasaki disease, childhood asthma, juvenile idiopathic arthritis, and type 1 diabetes have early-life origins. But even inflammatory diseases of aging can have early-life origins. For example, atherosclerosis, which is normally an adult-onset diagnosis, is now viewed as most often initiated during childhood (Kelishadi 2010). Even frailty, which is defined as an end-stage systemic weakness condition often immediately preceding death, represents a pro-inflammatory condition that appears to have early developmental linkages (Kuh et al. 2006a, b; Chang et al. 2012; Yao et al. 2011).

The prior chapters in this book describe the connection between environmental conditions, immunotoxicity and immune insult, immune dysfunction, and chronic disease. They also highlight the extent to which chronic diseases represent a present-day global health threat. Prevalence data suggest that chronic diseases are increasing and that prior preventative strategies have served to lessen the overall rate of the increase rather than actually reducing the health burden of chronic diseases.

In this chapter, we introduce information on additional immune-based chronic diseases and conditions: psoriasis, autoimmune thyroiditis, frailty, and an isolation-withdrawal condition state; the emphasis is placed on comorbidities. We then utilize the combined disease-specific information presented in this book to:

(1) discuss opportunities to better protect populations against the increasing threat of immune-based chronic disease and (2) reduce the prevalence of these diseases. If the prior chapters have defined the extent of the problem, the current chapter attempts to point toward potential solutions.

17.2 Four Additional Examples of Chronic Diseases/ Conditions: Psoriasis, Autoimmune Thyroiditis, Frailty, and Isolation-Withdrawal

The previous chapters in this book provide insights into several prominent chronic diseases associated with immune dysfunction. The chapters take a new approach by considering the cause–effect relationships among environmental risk factors, immunotoxicity and immune insult, subsequent immune dysfunction, and disease characteristics and impact. However, it is important to realize that despite this novel framework for discussing chronic diseases, the landscape of immune-based chronic diseases is not restricted solely to those with designated chapters in this book. In fact, given that approximately 100 autoimmune conditions are reported to exist (AARD 2010), there are certainly more immunotoxicity–immune dysfunction–chronic disease linkages remaining to be considered than were included here. Hence, this book is more of a starting point for consideration of significant associations and potential cause–effect relationships rather than an epilog.

17.2.1 Psoriasis

Psoriasis (PS) is a dermatological disorder that is also characterized as an immune-mediated inflammatory disease (Davidovici et al. 2010). Improper T helper (Th) 17 regulation and elevation of Th1 and Th22 cell numbers appear to be important in mediating PS (Lowes et al. 2008; Kagami et al. 2010; Tokura et al. 2010). The misregulated inflammation that is a hallmark of psoriasis also appears to link this disease with other inflammatory conditions such as CVD (e.g., atherosclerosis) (Ghazizadeh et al. 2010; Boehncke et al. 2011; Mehta et al. 2011). A recent study in children identified exposure to environmental tobacco smoke at home, stressful life events, and a high body mass index as risk factors for childhood-onset psoriasis (Ozden et al. 2011). Tobacco smoke also has been implicated as a risk factor that appears to link elevated Th17 function, overexpression of interleukin 22 (IL-22), and risk of psoriasis in adults (Torii et al. 2011). Alcohol also was reported to be a risk factor in a case-controlled study of adults in Europe (Jankovic et al. 2009).

PS represents a cutaneous disease that has been linked with systemic comorbidities (Davidovici et al. 2010). Yang et al. (2011) recently examined 1,685 adult PS patients in Taiwan for 29 possible comorbidities. Among patients with severe

PS, these investigators reported elevated risk for congestive heart failure, ischemic heart disease, renal failure, diabetes, liver diseases, and peptic ulcer. The PS–CVD linkage is supported by the suggestion that the underlying systemic inflammatory dysfunction of PS can lead directly to CVD and an increased risk of CVD-associated mortality in these patients (Boehncke et al. 2011). Armstrong et al. (2011) reported that a common mechanistic basis between PS and atherosclerosis may explain the tight comorbid connection between PS and CVD. In addition, De Simone et al. (2011) suggested that endothelial dysfunction is common in PS and that PS patients suffer from subclinical atherosclerosis. Not surprisingly another inflammation-driven disease, chronic obstructive pulmonary disease (COPD) also has been reported to be a comorbid condition of PS in a case-controlled study from Israel (Dreiher et al. 2008) and a population-based study from Asia (Chiang and Lin 2012).

An additional study used the Taiwan National Health Insurance claims database to examine 51,800 PS cases matched in a 1:4 ratio with controls drawn from a sample cohort of 997,771 enrollees (Tsai et al. 2011). The investigators reported an overlapping array of comorbidities for PS that include elevated risks for heart disease, hypertension, hyperglyceridemia, diabetes, hepatitis B and C infection, fatty liver disease, cancers of the digestive organs, peritoneum, lips, oral and pharynx, depression, asthma, allergic rhinitis, and the autoimmune conditions rheumatoid arthritis, systemic lupus erythematosus, vitiligo, pemphigus, and alopecia areata (Tsai et al. 2011). A third study from Taiwan found that PS conveyed an overall elevated risk of cancer that was greater in males than in females and was independent of treatment (Chen et al. 2011). The same investigators reported that skin, urinary, bladder, and GI tract-associated cancers were particularly elevated in the psoriasis patients. Figure 17.1 illustrates major comorbidities that have been associated with PS.

17.2.2 Autoimmune Thyroiditis

Autoimmune thyroiditis (AT) is a chronic autoimmune-inflammatory disease in which the immune system attacks the thyroid gland often resulting in thyroid dysfunction in patients. AT can be divided into two separate conditions, Grave's disease in which hyperthyroidism is common, and Hashimoto's thyroiditis which usually features hypothyroidism. Environmental risk factors have been suggested for these diseases (Burek and Talor 2009). For example, living next to petrochemical complexes has been reported as a risk factor for Hashimoto's disease (de Freitas et al. 2010). Additional purported risk factors include long-term excess iodine intake (Rose et al. 2002), selenium deficiency (Duntas 2006, 2010), and certain environmental pollutants such as polybrominated biphenyls (Bahn et al. 1980), pesticides (Duntas 2011), and polyaromatic hydrocarbons (Lindsay et al. 1992). As with many autoimmune diseases, certain infections (e.g., *Toxoplasma gondii*) may serve as environmental triggers of disease onset (Wasserman et al. 2009).

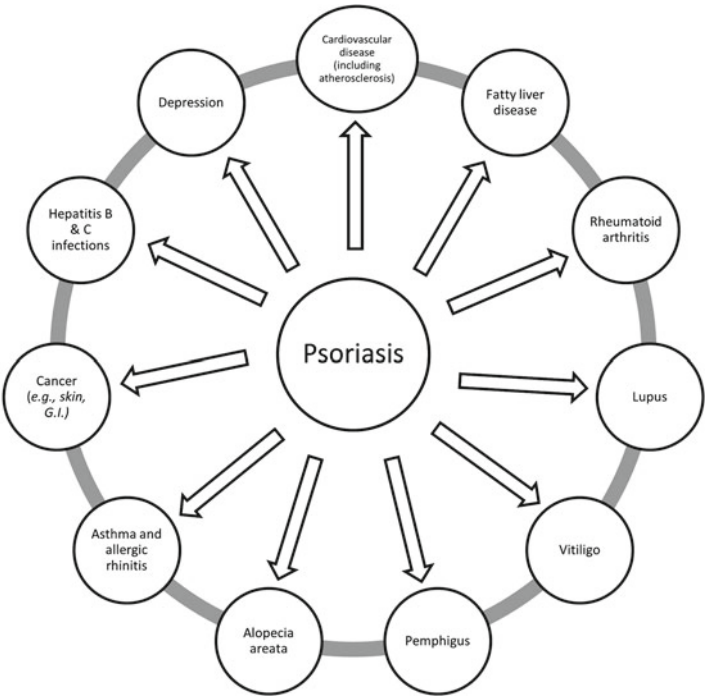

Fig. 17.1 Comorbid diseases and conditions that have been reported for the immune-based chronic disease, psoriasis, are shown

AT has been associated with numerous disease comorbidities. Hemminki et al. (2010) recently reviewed the epidemiology and comorbidities of Grave's disease. Not surprisingly, many of these are other autoimmune conditions. This is consistent with the findings of Boelaert et al. (2010) who reported that 9.67% of UK patients with Grave's disease and 14.3% of patients with Hashimoto's thyroiditis had a second autoimmune disease. Significant comorbidities were found for rheumatoid arthritis, systemic lupus erythematosus, vitiligo, celiac disease, Addison's disease, and pernicious anemia. The relationship between AT and autoimmune rheumatic diseases also has been described by other investigators (Lazúrová et al. 2009). Among patients with AT, the elevated risk for additional diseases is not restricted to autoimmune conditions; the association of AT with various cancers has also been reported including a strong relationship between Hashimoto's thyroiditis and papillary thyroid cancer (Kim et al. 2011), and a recently reported association of benign thyroid diseases such as Hashimoto's thyroiditis with an elevated risk of breast cancer (Muller et al. 2011). Both hearing loss (Berker et al. 2011) and depression (Eller et al. 2010) have been reported among patients with AT. Examples of comorbidities that have been associated with AT are shown in Fig. 17.2.

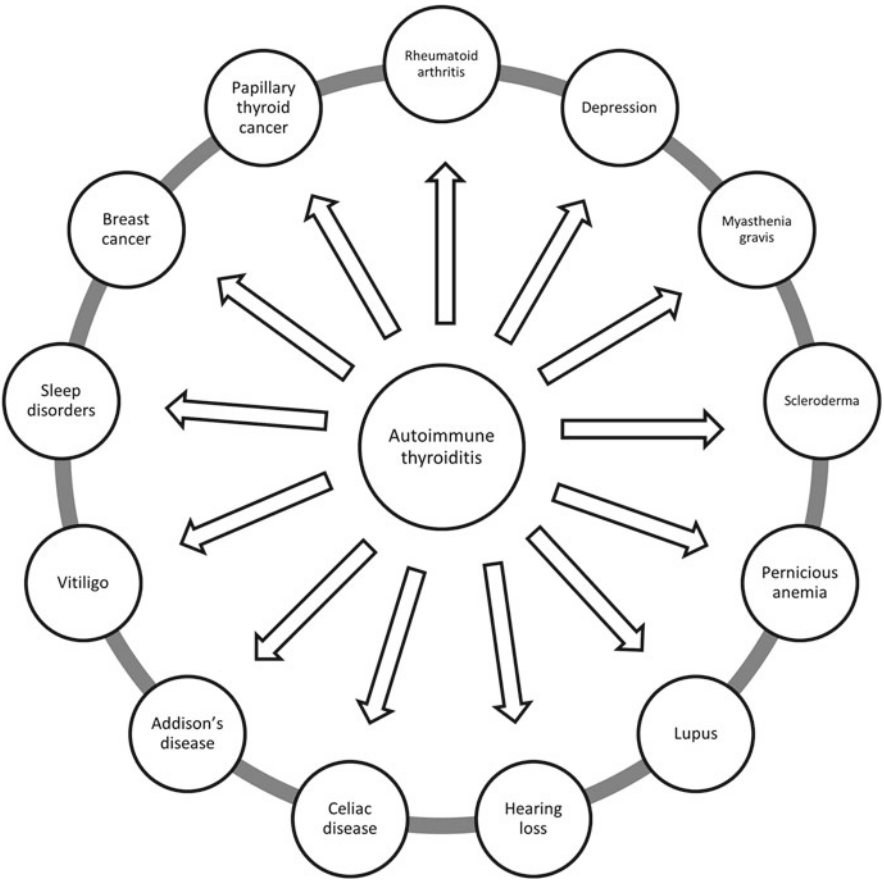

Fig. 17.2 The reported comorbidities for autoimmune thyroiditis are illustrated

17.2.3 Frailty

Frailty has been defined as a largely geriatric syndrome featuring impaired resistance to stressors linked to a decline in physiological reserves (Afilalo 2011). It is among the best predictor for the risk of death (Song et al. 2007, 2010). It is increasingly recognized as a distinct clinical entity with a specific physiological state (Fried et al. 2001; Lang et al. 2009; Sanders et al. 2011). Frailty often presents as a phenotype that includes muscle weakness, fatigue, and inflammatory pathway activation (Ho et al. 2011). Comorbid relationships reported for frailty are shown in Fig. 17.3 and include: diabetes, obesity (women), CVD, insulin resistance, depression (women), atherosclerosis, sarcopenia, COPD, and chronic kidney disease (Roebenoff 2000; Morley et al. 2005; Blaum et al. 2005; Afilalo et al. 2009; Hubbard et al. 2010; Chang et al. 2012; Abdel-Rahman et al. 2010). Unlike the situation with PS and AT, frailty is usually an end-stage condition.

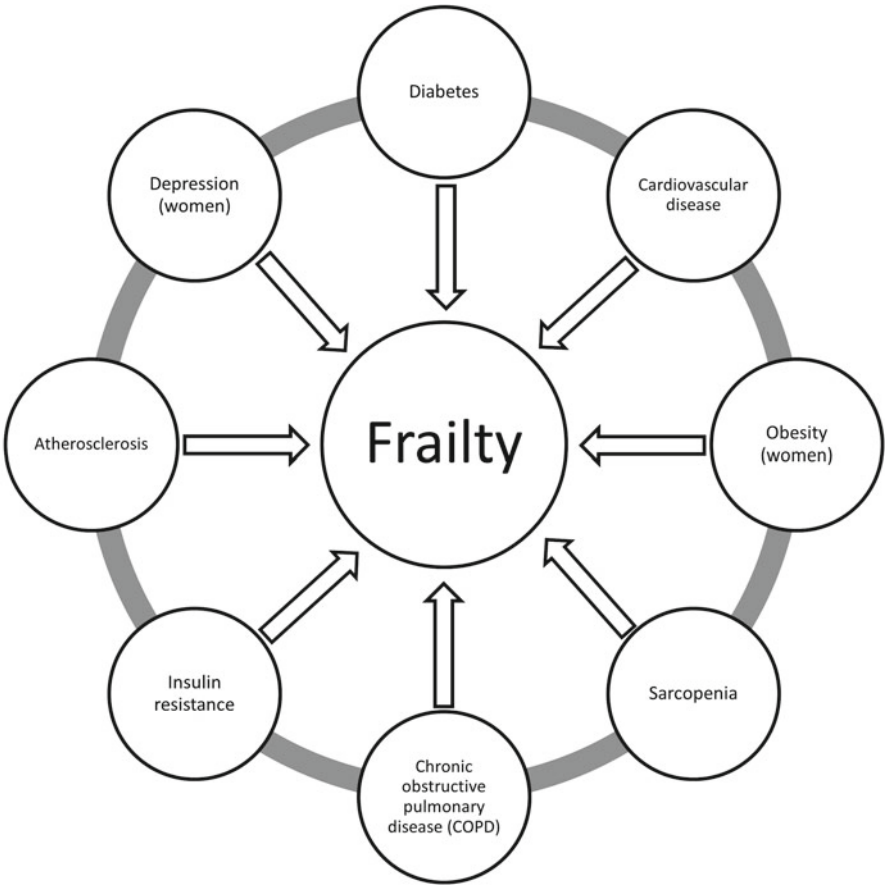

Fig. 17.3 Diseases and conditions connected to inflammatory dysfunction and frailty are illustrated. Cohorts diagnosed with the primary conditions shown in the outer circle are reported to have an increased risk of later-life frailty

In other words, the comorbid chronic diseases associated with frailty are often diagnosed before the onset of the frailty phenotype.

Chronic low-grade inflammation has been identified as a major contributor to the process of aging (Candore et al. 2010) and a major component of frailty (Hubbard and Woodhouse 2010; Chang et al. 2012). Among the elevated inflammatory biomarkers associated with frailty are C-reactive protein (Walston et al. 2002), IL-6 (Leng et al. 2007), TNF-α, and oxidative stress (Serviddio et al. 2009). Figure 17.4 illustrates a potential pathway to frailty involving immunotoxicity, inflammatory dysfunction, and comorbid chronic inflammation-associated diseases. In this example, environmental tobacco smoke is shown as an environmental toxicant that induces misregulated inflammation in children. While the route to frailty would usually pass through one or more inflammatory chronic

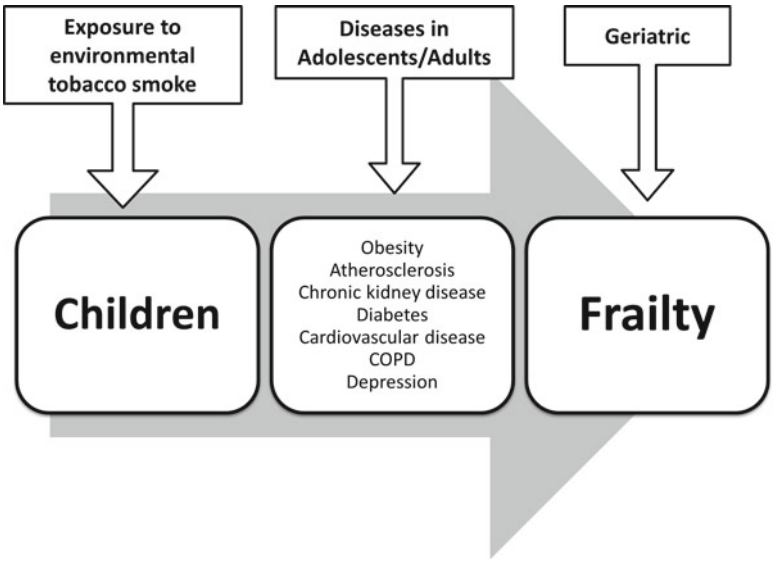

Fig. 17.4 A putative route to frailty is illustrated in which early-life exposure to tobacco smoke results in childhood immune/inflammatory dysfunction and the increased likelihood of one or more chronic diseases followed by geriatric frailty

diseases, it is also possible that frailty is the primary disease that appears during the aging process. This figure illustrates the need to identify immunotoxic hazards among chemicals and drugs and protect against these types of dysfunction-driven pathways to chronic disease.

17.2.4 Isolation-Withdrawal as an Outcome of Inflammatory Dysfunction

Among the potentially underappreciated impacts of immune/inflammatory dysfunction are the roles that it can play leading to a group of conditions that tend to be debilitating and can isolate individuals from their families and communities as well as society in general: depression, behavioral disorders, sleep problems, and sensory loss. Depression has been discussed in detail in a prior chapter of this book. These four outcomes of inflammatory dysfunction greatly affect quality of life and the ability of an individual to function in society. Not surprisingly, they are comorbidities, individually or in combination, with a myriad of inflammatory and/or autoimmune diseases. Figure 17.5 illustrates the broad spectrum of inflammatory-driven conditions that have comorbidities with one or more of these highly-debilitating health problems.

In keeping with this focus, it has been suggested that improper immune activation-inflammation may be linked to suicide risk during major depressive

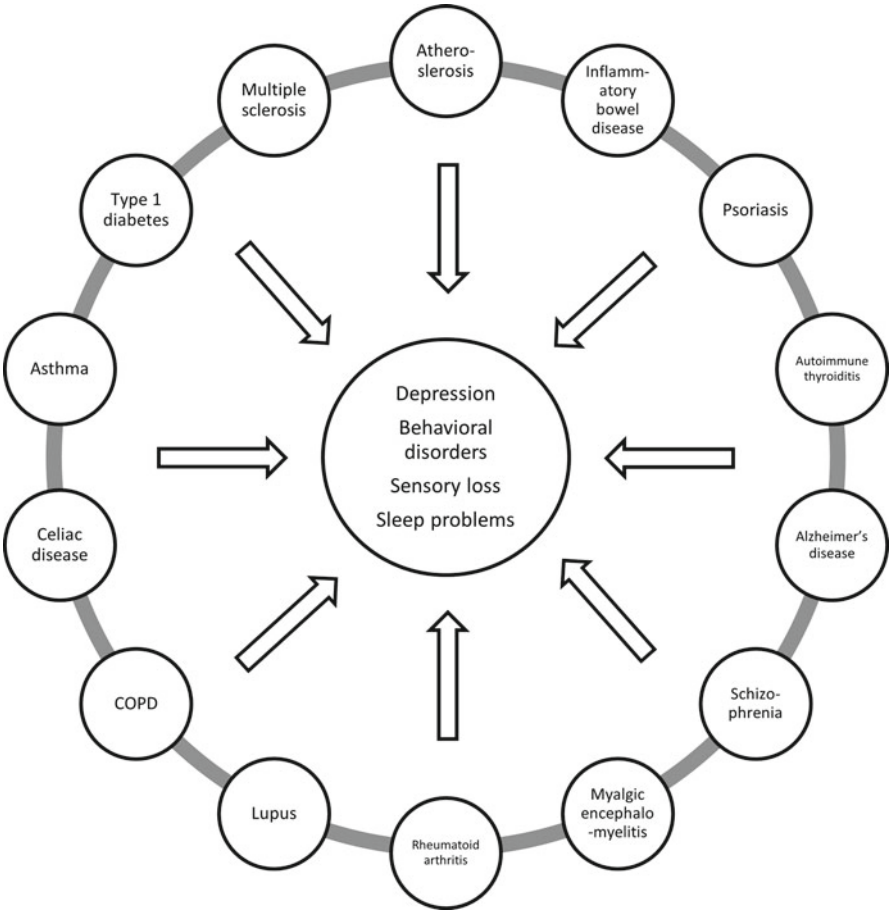

Fig. 17.5 A cadre of inflammation-driven conditions that can lead to isolation/withdrawal are shown in the center circle. Chronic diseases reported to be comorbid for the isolation conditions are shown around the periphery of the circle

episodes (Sublette et al. 2011). The possibility exists that inflammation-driven pathophysiology is connected to certain suicide attempts. Some investigators reported specific inflammatory cytokine differences among individuals with depression who had previously attempted suicide compared with either nonsuicidal depressed patients or healthy controls (Janelidze et al. 2011), although cause–effect relationships are yet to be established. Figure 17.6 illustrates the reported associations of inflammatory dysfunction conditions with an elevated risk of suicide (Smith et al. 2006; Gradus et al. 2010; Kuo et al. 2010; Kurd et al. 2010; Alberdi-Sudupe et al. 2011; Goodwin 2011; Ludvigsson et al. 2011; Prati et al. 2011).

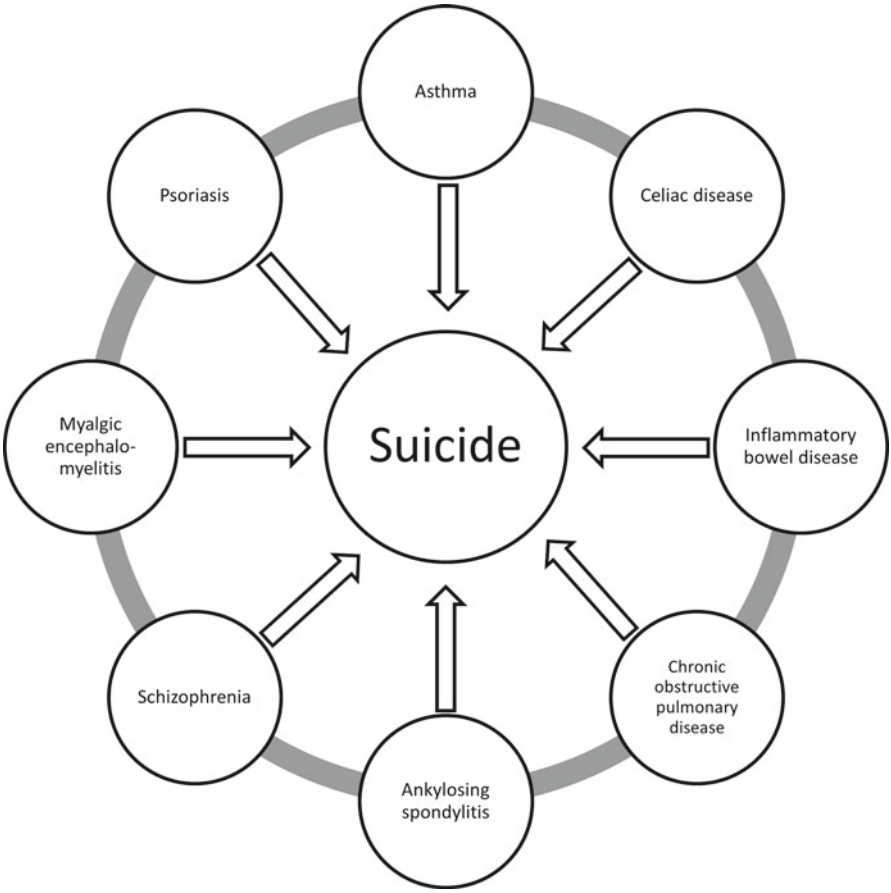

Fig. 17.6 Inflammation-driven diseases and conditions that are reported to elevate the risk of suicide and suicide attempts are illustrated

17.3 Utilizing Strategic Prevention and Intervention to Break Chronic Disease Patterns

The examples of comorbidities for PS, AT, frailty and isolation, along with those for the other diseases and conditions in previous chapters, support the utility of recognizing and addressing lifelong patterns of immune-based disease (Dietert et al. 2010). Risk of chronic disease appears to operate much like a web that emerges once an individual is diagnosed with the initial chronic disease. Environmentally-induced immune dysfunction, underlying genotype, and infectious agent triggers help to cement the progression to initial disease onset. Dietert et al. (2010) linked this initial chronic disease, which can often appear during childhood as an

"entryway disease," to a specific pattern of chronic disease risks. Once the underlying immune dysfunction and subsequent entryway disease or conditions appear, the health trajectory becomes rather set in terms of likely additional health risks across an individual's lifetime. At that point, timely and specific intervention, which may involve therapeutic approaches to correct the underlying immune dysfunction, are needed to interfere with the chronic disease pattern and reduce the risk for additional interlinked diseases. The problem is that to date, approaches for preventing specific chronic diseases have been limited to a few well-studied examples (e.g., antismoking efforts to reduce the prevalence of lung cancer).

17.4 Outcome-Based Safety Testing for Risk of Chronic Disease

The programs developed for the safety testing of chemicals and drugs emerged during an era when risk of life-threatening infections and mortality from acute toxicity of organs were the primary concerns. Current hazard identification programs and associated risk assessments have been remarkably successful in protecting populations against organ failure and almost immediate death following chemical exposure or use of a medication. Toward these outcomes, regulated safety testing has a proven, highly successful record. But are those the most significant health concerns of today or of prior decades?

If safety testing in its origins was designed to identify the risk of organ failure and death from acute organ toxicity, that is no longer the most useful prime directive for today. The entire focus needs to shift to match the present-day challenges of environmentally-influenced chronic diseases. Data from several studies (WHO 2005; Klijs et al. 2011; Prüss-Ustün et al. 2011) indicate that our foremost health challenge is reducing the risk of acquiring chronic diseases and relieving the intensive medical care and premature death associated with them. Not surprisingly, an organ-specific safety testing network that has evolved only slightly from one designed to detect acute toxicity and death is not optimally-positioned to ensure protection against today's most significant chronic diseases. But in fact, that is precisely what a safety testing system should do today; it should protect first and foremost against our highest priority chronic diseases.

As presented throughout this book, immune and inflammatory dysfunctions are major contributors to chronic diseases. Hence, a potential calibration for the relevance of current safety testing of chemicals and drugs could be to pose the following question: what routinely required safety testing data give us risk estimates for asthma and allergic diseases, type 1 diabetes, IBD, rheumatoid arthritis, PS, AT, multiple sclerosis, lupus, depression, COPD, atherosclerosis, autism, and later-life frailty? At present the most likely answer is: none. This suggests that there is a disconnection between the extensive safety data that are presently collected and the data that are needed to reduce the risk and, ultimately, the prevalence of immune-based chronic disease.

17.5 Strategic Approaches to Reduce the Prevalence of Immune-Based Chronic Diseases

17.5.1 Safety Testing Relevant for Chronic Diseases

According to the U.S. Centers for Disease Control and Prevention (CDC), chronic diseases are the most common, costly and preventable health problems faced in the United States. Previous studies have estimated that about half of adults in the United States have at least one chronic disease (Wu and Green 2000), 75% of healthcare expenditures were devoted to chronic conditions (Anderson 2004), and 7 out of 10 of deaths in the United States were due to chronic disease (Kung et al. 2008). For example, arthritis was identified as the leading cause of disability affecting approximately one in five US adults (CDC 2006) and recent asthma prevalence in the United States was estimated at 8.2% (Akinbami and Moorman 2011). If, in fact, chronic diseases are among our most costly, pervasive and largely preventable health threats, then the first and foremost goal of safety testing should be to deliver the necessary information for chronic disease prevention.

Additional research into environmental risk factors is needed; however, research occurs post-use of the potential exposure-related causes of chronic disease. It is usually initiated after factors are suspected of elevating the prevalence of disease and usually lags decades behind emerging epidemics of disease as exemplified by research related to the environmental causes of asthma and autism (Dietert 2011; Dietert et al. 2011). For this reason, chronic disease-relevant safety testing conducted before large populations are exposed to a risk factor is the most effective approach for both protection of populations and overall societal and economic costs.

In the case of immunotoxicity, the diseases discussed in this book represent a useful landscape of chronic diseases that may be preventable. For this reason, extensive and relevant preemptive immunotoxicity testing is needed as the frontline defense against immune-based chronic disease. Required immunotoxicity testing for agents regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) has emphasized detection of both chemical- and drug-induced immunosuppression as well as chemicals that are potential sensitizers, although the data collected are of questionable relevance for detection of the majority of immune-based chronic diseases presented in this book. How might future chronic disease-relevant testing differ from the historic battery of immune tests that has directed our immunotoxicity evaluations to date (Luster et al. 1992)?

The most significant difference in the approach to testing is the need to place a greater emphasis on cell-mediated, innate immune, and inflammation-associated functions that drive much of chronic disease. Predictive biomarkers for the highest priority chronic diseases need to be developed, including biomarkers for respiratory and food allergies, autoimmune diseases, and inflammatory diseases. In fact, Dietert (2008) argued that the most effective testing protocol should be driven by the biomarkers of dysfunction directly related to the highest priority diseases. Biomarkers for diseases discussed in this book including asthma, rheumatoid

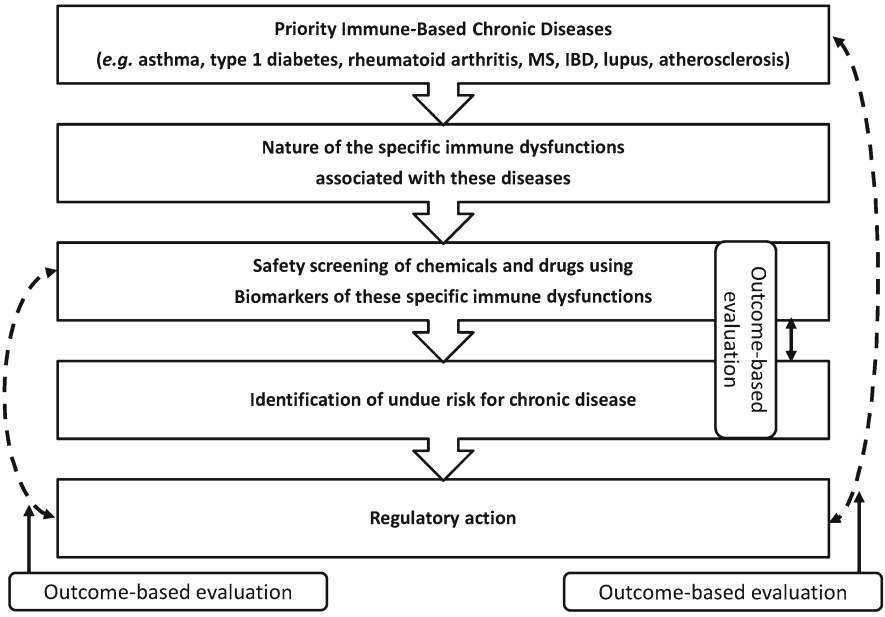

Fig. 17.7 A flow chart for outcome-based immunotoxicity testing designed to reduce the prevalence of chronic disease is shown. Widespread and disease-relevant immunotoxicity testing is necessary to prevent problematic exposures that cause immune dysfunction and contribute to the ongoing epidemic of chronic disease

arthritis, lupus, type 1 diabetes, psoriasis, autism, atherosclerosis, autoimmune thyroiditis, and inflammatory bowel disease may be the best candidates for development. Figure 17.7 illustrates a general flow chart based on a priority disease-driven model.

Various commonalities and nexus points of allergy, autoimmunity, and inappropriate inflammation appear to exist. For example, alterations in innate immune receptors such as the toll-like receptors (TLRs) are likely to be important in the early processes of many of these diseases (Leifer and Dietert 2011). Figure 17.8 illustrates some of the TLR alterations that have been associated with elevated risk of specific immune-based chronic diseases. Options exist for whether testing would focus on end mediators of the allergic, autoimmune and inflammatory diseases or, instead, measure more upstream common nexus points of environmentally-induced alterations that have the capability of predicting the immune dysfunction associated with these chronic diseases. These are shown in Fig. 17.9. Regardless of this downstream vs. upstream decision, immunotoxicity testing to identify the risk of allergy, autoimmunity and inflammation is likely to place a greater emphasis on the functional status of dendritic cells, macrophages, and T-cell populations. Challenge systems designed to mimic the triggering of the improper immune-inflammatory responses also may be needed to place upstream markers in context to minimize

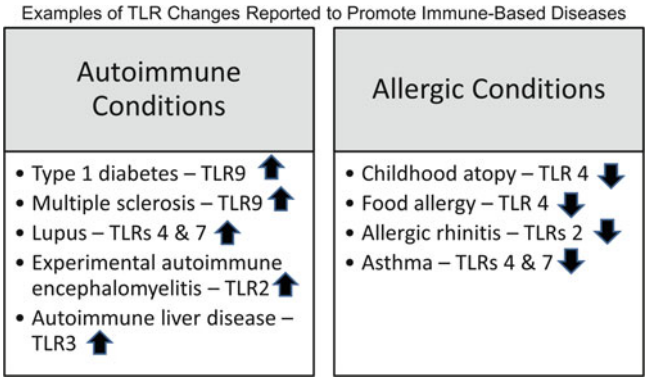

Fig. 17.8 Reported associations of altered expression levels of toll-like receptor (TLR) genes with specific autoimmune and allergic diseases are indicated. These represent promising biomarkers of these important categories of chronic disease

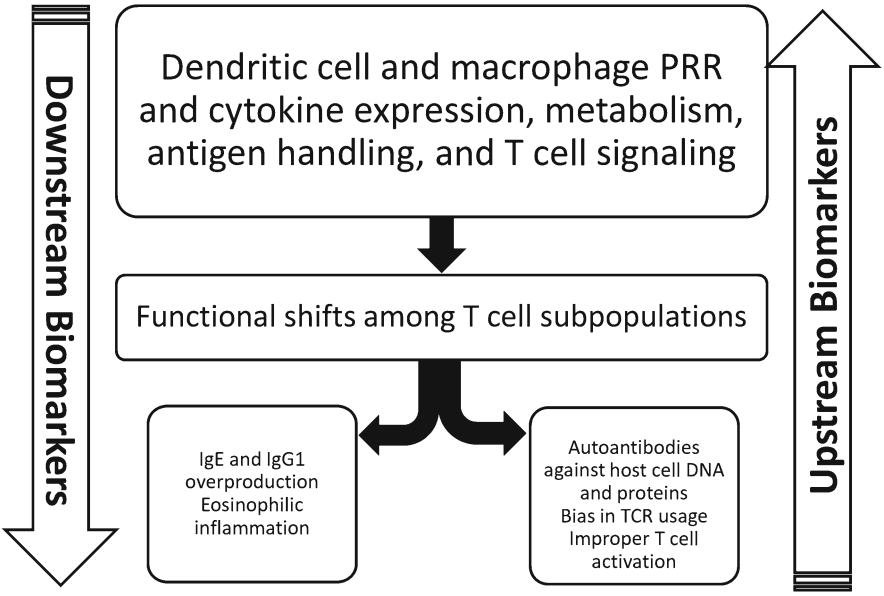

Fig. 17.9 The diagram focuses on immunological factors connected to autoimmune, allergy and inflammatory disease and the decision opportunities that exist to focus on downstream mediators of tissue pathology and disease (e.g., IgE) or upstream innate immune and T-cell promoters of broader dysfunction (e.g., TLR expression on dendritic cells)

false-positive signals, perhaps as a second tier of testing. Protection of the public's health would be better served if disease-relevant immunotoxicity testing was equal to the task of identifying global threat levels associated with chronic immune-based diseases.

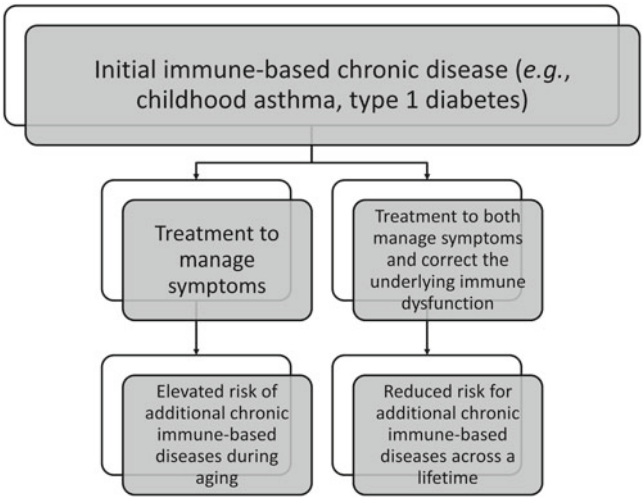

Fig. 17.10 A decision tree for therapeutic treatment of immune-based chronic disease is shown. Therapeutic intervention that only treats the presenting disease symptoms and masks the underlying immune and/or inflammatory dysfunction may fail to address the elevated risk of one or more serious chronic diseases in later life. In contrast, alternative interventions that address both the immediate symptoms of the presenting disease as well as the underlying immune dysfunction are more likely to reduce the risk of later-life disease. This, in turn, would help to reduce the overall prevalence of chronic disease and the disease burden carried by individuals as they age

17.5.2 Therapeutic Interventions: Need for Immune-Corrective Approaches

As discussed by Dietert et al. (2010), one of the opportunities to reduce the prevalence of chronic diseases is to pursue therapeutic options that break patterns in which individuals develop multiple chronic diseases across a lifetime. Because chronic diseases exist in interrelated patterns that are connected by underlying immune dysfunction, there are opportunities to treat patients in such a way as to reduce the risk of specific later-life diseases. For example, a diagnosis of asthma need not inherently chart a course for additional allergic diseases and later-life lung cancer; psoriasis does not have to lead to skin cancer, COPD, and/or atherosclerosis. Hashimoto's thyroiditis should not be a prelude to sleep disorders, lupus, and/or breast cancer; patients with IBD should not have to face the prospects of colorectal cancer, rheumatoid arthritis, or major depression. These subsequent elevated disease risks could be reduced if treatments of the initial conditions included not only a reduction of symptoms but also approaches to correct the underlying immune and/or inflammatory dysfunctions. Figure 17.10 illustrates a comparison of these therapeutic options and likely outcomes.

In some cases, therapeutic options may already exist for later-life risk reduction if the attending physicians are aware of these comorbid disease risks and the benefits

to be gained via more comprehensive and holistic therapies. In other cases, new therapeutic approaches may need to be developed and implemented. However, intervening effectively to break these patterns of chronic disease has the potential to: (1) change the entire life course of health for individuals, (2) to reduce the overall prevalence of chronic disease, (3) to enhance quality of life, and (4) to reduce the cost of healthcare.

17.6 Metrics for Measuring Chronic Disease Prevention

Charting the effectiveness of public health-driven risk reduction on the prevalence of chronic disease is an important component of outcome-based safety testing and more lifetime-driven therapeutic strategies. Ironically, most of these have been designed and implemented to measure the effectiveness of public health programs urging people to alter their lifestyle to avoid hazards (e.g., cigarette smoking, indoor air pollution factors, high-fat foods) already "released" into the environment. For example, Miron-Shatz and Ratzan (2011) suggested the use of a digital or electronic health scorecard as a tool for calibration of lifestyle choices relative to risk reduction for chronic diseases. Of course, the effectiveness of this tool is limited by the extent to which environmental risk factors have been adequately identified for chronic diseases. Individuals, families, and communities cannot avoid hazards if they remain unknown. Therefore, outcome-driven safety testing, including effective immune assessment of risk, should identify the risk of chronic disease before exposure occurs. Such testing needs to incorporate some type of metrics that can measure its impact on prevalence of chronic diseases. In fact, the historic weakness in regulated safety testing today is the almost complete disconnection between the data collected and outcomes relative to impact on disease. This needs to be addressed if future safety testing is to be both efficient and effective.

17.7 Conclusions

Chronic diseases represent the most serious health threat we face today and immune/inflammatory dysfunction is at the heart of many chronic diseases. As described in the chapters of this book, environmental factors play a major role in causing chronic disease. Additionally, once one chronic disease has been diagnosed, often in childhood, others are likely to follow as the individual ages.

Yet, there is considerable irony in the current public health programs designed to reduce the prevalence of chronic disease. Most of the chronic disease-producing environmental risk factors that have been identified to date have been discovered via research conducted decades after chronic disease epidemics. Such a strategy is not only highly inefficient, it is also costly both economically and in terms of human capital. Disease-relevant safety testing combined with the avoidance of harmful exposures could have reduced the incidence of many of these diseases.

This chapter describes: (1) the extent to which chronic diseases are interconnected as patterns, (2) how terminal conditions such as frailty and CVD are related to chronic inflammation, (3) the strategies to make safety testing both outcome-based and relevant to protection against chronic disease, and (4) the strategies to make therapeutic interventions in the management of chronic disease more effective in the prevention of additional chronic conditions during aging.

Immune-based chronic disease is a huge target for health risk reduction. It is one where a combination of widespread and redirected safety testing, more holistic medical approaches and intensified integrative research can reverse the present trajectory of increasing prevalence of disease and the burdens that have been associated with that.

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