IMMUNOTOXICOLOGY AND RISK ASSESSMENT

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Kluwer Academic Publishers
New York, Boston, Dordrecht, London, Moscow

eBook ISBN: 0-306-46810-7
Print ISBN: 0-306-45971-X

Created in the United States of America

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To Carol
wife and best friend

Preface

To adequately define the risk of adverse health effects caused by immune dysfunction, it is important that the reader understand the conceptual and practical aspects of classical immunology, immunotoxicology, and toxicology. Classical immunology explores immune mechanisms involved in defense against microbial agents and tumors. Also, it defines defects or dysfunction at the molecular level, highlights the relationship between defects and clinical disease, and provides most of the "state of the art" methodology used in animal or human immunotoxicology studies. In contrast, immunotoxicology (which has only existed as a discipline for 20 years) approaches science from a pharmacological perspective and is concerned with documentation of immunosuppression and developing sensitive tests to detect immunotoxicity in rodents. Finally, principles of toxicology are important in the interpretation of dose relationships and defining the risk by mathematical models and safety factors.

The aim of this book is to present basic immunological tenets and mechanisms at the cellular and molecular levels while preserving the toxicology focus on hazard identification, appropriate assays, dose response, and risk assessment. The approach adopted in this book is different from existing immunology or toxicology texts. For students of immunotoxicology, the chapters are arranged so that fundamental immunological concepts and mechanisms are presented sequentially as they would occur in the elicitation and termination of an immune response. Other specialized topics (e.g., allergy and autoimmune disease) and

mechanisms are logically arranged around the conceptual foundation of an immune response. For the toxicologist, pharmacologist, or governmental regulator, *in vivo* or *ex vivo* hazard identification methodology is an integral part of each chapter. Discussions of animal models to determine the biological significance of *ex vivo* or *in vitro* data and risk assessment paradigms are included as separate chapters. Information is presented so that the book can be read in easy installments.

The book is timely because governmental agencies in Europe and the United States have renewed their interest in the effects of xenobiotics on the immune system. Later this year, the U.S. Environmental Protection Agency will issue harmonized immunotoxicity testing guidelines for all chemicals regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Toxic Substances Control Act (TSCA). Centers within the U.S. Food and Drug Administration and the Organization for European Cooperation and Development (OECD) will soon issue additional testing guidelines.

Although immunological concepts and mechanisms may not be easy to grasp, motivated readers of this book will emerge well informed regarding contemporary immunology, xenobiotic-induced immunotoxicology, and new risk assessment uaradigms useful for analyzing immunotoxicity data.

It is the author's hope that with the understanding of the effect of xenobiotics on the immune system and proper risk assessment paradigms, we will be better able to interpret the data from human and animal studies. In turn, we will be able to make better, and more informed, decisions concerning the risk of adverse health effects produced by exposure to chemicals, pharmaceuticals, and other xenobiotics.

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Introduction to Immunotoxicology

INTRODUCTION

Immunotoxicology is a new subdiscipline or subspecialty area. It took shape in the early 1970s with the realization that chemicals such as dioxin could target the immune system in animals. In addition to the AIDS epidemic and the demonstrated lethality of immunosuppression, interest was heightened when it was shown that accidental exposure to polybrominated phenyls could adversely influence human health.

Immunotoxicology is a curious association of the young science of immunology, the emerging science of toxicology, and the established medical sciences of pharmacology and medical microbiology. Although diverse in nature, the goals of the four sciences are interconnected. Pharmacologists aim to define the agents that modify biological responses, while toxicologists study the toxic effects of agents on biological systems. The goal of the immunologist is to define the effect of exogenous and endogenous molecules on immune system function. Medical microbiologists focus on the effects of chemicals and other xenobiotics on the host defense against microbial agents (Gallagher *et al.,* 1993).

THE PARALLELOGRAM

Immunotoxicologists normally are cast into two roles. They must assess the potential human risk based on *in vivo* or *in vitro* data sets. In another role, they

may be asked to determine whether decrements in human immune function are associated with exposure to specific chemicals.

In both cases, the approach is similar. A parallelogram is constructed to model risk assessment (Fig. 1). The four corners of the parallelogram consist of *in vitro* and *in vivo* human studies and *in vitro* and *in vivo* animal studies. It is important to emphasize that data derived from a single comer of the parallelogram are usually biologically meaningless.

The parallelogram can be used in two ways. The relationship between suppression of rodent and human immune cell function *in vitro* and *in vivo* can be determined. The effects of xenobiotics on three of the four comers can be tested experimentally. These include studies on human and animal cells *in vitro* and *in vivo* effects in animals. Data from these studies may predict the outcome in the fourth corner *(in vivo* human effects).

The same approach can be used to determine possible relationships between immune function suppression and susceptibility to infection. In the second model, cells from humans are tested *in vitro* and cells from animals are tested *in vitro* and *in vivo* for increased susceptibility to infection. The susceptibility of humans to infections cannot be studied experimentally and must be extrapolated from other points on the parallelogram (Selgrade *et al.,* 1995).

IMMUNE SUPPRESSION, INFECTIONS, AND TUMORS

Biologically relevant immunosuppression is always accompanied by repeated infections with opportunistic bacteria or unusual tumors. The fact that opportunistic infections and tumors are unique makes them easily recognizable by physicians. Different opportunistic infections are associated with T, B, and neutrophil cell defects (Table 1).A Neutrophil defects are associated with recurrent streptococcus, *E. coli,* and pseudomonas infections. Defects in T cells are associated with increased infection with intracellular microbial agents such as herpes, pneumocystis, toxoplasma, and Coccidioides.

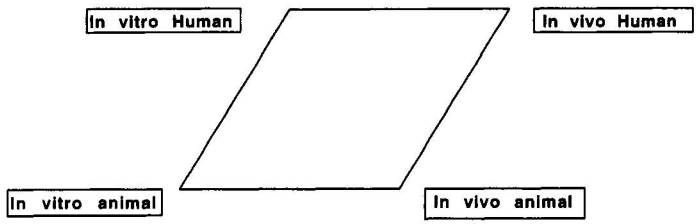

Figure 1. The parallelogram used to assess immunotoxicity.

Table 1. Opportunistic Infections Associated with T, B and Neutrophil Defects

Neutrophil defects	Opsonization defects	Cell-mediated defects
Staphylococci	Pneumococci	Cytomegalovirus
E. coli	Streptococci	Herpes simplex
Klebsiella	*Haemophilus*	Herpes zoster
Pseudomonas		*Listeria*
Bacteroides		*Legionella*
Aspergillus		Mycobacteria
		Pneumocystis
		Toxoplasma
		Candida albicans
		Cryptococcus
		Coccidioides

Modified from *Clinics in Immunology and Allergy,* Pinching, 1985, 5:469–91.

Tumors arising from immunosuppression are also unusual. Kaposi's sarcoma, an extremely rare skin tumor in normal subjects, is found with great frequency in immunosuppressed subjects with AIDS. Increased malignancies are also found after purposeful immunosuppression. Twenty-six percent of renal transplant patients surviving for 1 year develop cancer. At 10 years, 47% of patients developed cancers (Penn, 1985). Certain tumors are observed with great frequency. The incidence of cancers of the skin and lip were 21 times the expected rate in areas with high levels of sunshine. The incidence of non-Hodgkin's lymphoma increased 28- to 49-fold over an age-matched population (Penn, 1985).

EVALUATING IMMUNE SYSTEM STATUS

Immunotoxicity

Because xenobiotics have multiple effects on the immune system, it is difficult to arrive at a concise definition for immunotoxicity. However, the U.S. FDA considers an immunotoxic effect to be "any change in the structure or function of the immune system that is permanent or reversible." This definition has been embraced by most immunotoxicologists because it considers all possible outcomes of interactions between the immune system and xenobiotics.

The interaction between xenobiotics (e.g., chemicals or pharmaceuticals) and the immune system has four possible outcomes: (1) the xenobiotic may directly interact with the immune system to suppress or enhance the response to exogenous agents, (2) the xenobiotic may interact with host tissue and initiate hypersensitivity or autoimmune reactions, (3) the xenobiotic may not react di-

rectly with the immune system but initiate effects in other organs that influence the immune system, and (4) the xenobiotic may have no effect on the immune system (Munson *et al.,* 1991).

Evaluation of the Immune System

Choice of Experimental Animal. The choice for experimental animal is the species that absorbs, metabolizes, distributes, and excretes (ADME) the test chemical in a manner similar to humans. Often, the ADME of the test chemical is unknown before testing. Here, mice, rats, or dogs are used because there are methods available to detect effects on the immune response. Regulatory agencies require testing in both the rat and the mouse because validated testing methods are available.

Route and Duration. It seems obvious that xenobiotics should be tested in protocols in which the route of administration and duration are similar to the human situation. For example, animal studies on dioxin have used acute high-dose protocols inducing adverse effects whereas the human experience is chronic low-dose exposure with no effects on the immune system.

For target organ toxicity studies, the study duration may be much shorter. When antigen is used to stimulate the immune system in the presence of maximal test chemical levels, changes can be detected in 14- or 28-day studies. This is related to the rapid turnover of immunocompetent cells during the elicitation of an immune response (Munson *et al.,* 1991). Both the EPA and the FDA use either 14- or 28-day testing paradigms. Exposures of 90 days or longer are necessary to define induction of tolerance and compensatory mechanisms that may allow biotransformation of test material.

Sample Source. The ultimate goal of the immunotoxicologist is to compare effects of the test chemical in human and animal systems. This is often difficult because of differences in the sample sources. In humans, peripheral blood is used as the sample source. Lymphocytes in blood represent cells in transit and constitute only 2% of the total lymphocyte pool. Therefore, peripheral blood may not be representative of effects in lymphoid organs. In contrast, animal studies use splenic or lymph node cells from secondary lymphoid organs. Effects on lymphoid organs may predate effects observed in the peripheral blood.

Test Methods. The choice of test methods depends on whether a study's purpose is screening or mechanistic. In screening studies it is important to use

holistic assays that measure the function of either the antibody- or cell-mediated immune effector mechanisms. The sheep red blood cell plaque-forming assay is the method of choice for a screening assay of inductive immune responses. It requires the interaction of several cell types (e.g., T and B cells and macrophages), the production of cytokines, and the synthesis of antibodies. Moreover, the assay can be used to detect the effect of xenobiotics on isotypic switching from IgM to IgG antibodies. In contrast, natural killer cell function may be a screening assay for innate immunity to viruses and metastatic tumors. There are no good screening assays for cell-mediated immunity. Delayed hypersensitivity and cytotoxic T cell function have been used for screening, but they suffer from inherent assay variability. Mechanistic studies are, of course, hypothesis driven and specific *ex vivo* or *in vitro* tests can be used.

Interpretation of Results. Data from an immunotoxicity test cannot be interpreted without consideration of other changes occurring in the test animal. Decrements in immune function are only relevant if they occur at dose levels at which there is no other toxicity and there is a clear dose–response relationship. Changes in body weight, organ weights, food consumption, or hematology are often associated with exposure to chemicals.

It is necessary to take into consideration the normal range or control values of the assay in data interpretation. Significant differences between treated and control animals are biologically relevant when they exceed the expected variability of the assay, the normal control range, or historical controls.

THE IMPORTANCE OF ADME MEASUREMENTS

Toxicokinetic studies are conducted to determine the absorption, distribution, metabolism, and elimination of xenobiotics (Buchanan *et al.,* 1997). These measurements are useful in

1. Dose selection
2. Understanding the relationship between exposure, time-dependent target organ dosimetry, and adverse effects
3. Elucidating the effects of sex, species, and age on the ADME

Minimal toxicokinetic studies can be performed without the use of radiolabeled chemicals. If animals are of the same species and strain as employed in toxicology studies and routes of exposure are similar in both studies, one sex from one species can be used. There should be enough animals to guarantee a minimum of three data samples per time point (Buchanan *et al.,* 1997). Three

dose levels should be sufficient to demonstrate proportionality, namely, 0.1 LD_{50}, 0.01 LD_{50}, and 0.001 LD_{50}. Samples (8–12) should be collected from early in the exposure to the time point when the limit of detection in serum is reached. Time points should allow sufficient time to both tissue and plasma levels versus time profiles.

2

Innate Immunity

INTRODUCTION

There are two different immune systems in mammals. Acquired immunity is a consequence of microbial insult. Usually, the acquired response requires that cells in the immune system undergo proliferation and differentiation to produce effector cells and immunologically active protein molecules. In contrast, innate immunity is facilitated by preexisting anatomic and physiological barriers, antimicrobial peptides, inducible enzymes, and pluripotential molecules. Some molecules involved in innate immunity are normally present in the blood. Others are secreted by monocytes, neutrophils, or intestinal Paneth cells (Kagan *et al.,* 1994). There is no clear demarcation between innate and acquired immunity. Acquired immunity should be considered a continuum of innate immunity.

ANATOMIC AND PHYSIOLOGICAL BARRIERS

Barriers can be active or passive in nature. Bacteria grow only in a very narrow temperature range, and elevated body temperature restricts or prevents growth, In mammals, infection-induced fever is a natural attempt to prevent bacterial growth until the acquired immune system becomes activated. Avian species have a slightly different innate temperature-mediated defense system. Chickens have a normally elevated body temperature that prevents infection with organisms such as anthrax that are deadly to most other species.

Skin Barriers

The skin is an effective barrier to microbes. It consists of an impenetrable dead layer (the stratum corneum) and the living epidermis. Below the epidermis is the dermis containing blood vessels and connective tissue. Interspersed in the dermis are hair follicles and sebaceous glands.

The stratum corneum is constantly being sloughed off and the epidermis is renewed every 30 days. The skin thus forms a protective barrier, many chemicals and compounds binding irreversibly to the stratum corneum and being sloughed off with the dead skin. Similarly, the renewal of the epidermis every 30 days makes prolonged infections unlikely.

The sebaceous glands also contribute to the innate skin immunity. These glands produce sebum, which lowers the skin pH to 3–5. The low pH prevents most bacteria from colonizing the skin. However, this pH range provides a suitable environment for fungal growth that often results in skin infections.

Respiratory Tract Barriers

Several mechanisms prevent respiratory infections by bacteria and viruses. Cells in mucous membranes express adhesion molecules that bind influenza, *Bordetella pertussis,* and *Neisseria gonorrhoeae.* Respiratory cells also produce mucus, which traps and localizes the invading bacteria. Using the mucociliary escalator, ciliated cells move the mucus-trapped bacteria upward and out of the respiratory tract.

Stomach Barriers

The pH of stomach fluids forms a passive barrier to infection of the intestinal tract. In adults, the pH of the stomach is between 2 and 3. Bacteria cannot survive in the acid environment and intestinal infections are uncommon in adults. Newborns, however, have a less acid pH and bacteria can survive and pass into the intestine. Intestinal infections are common in neonates.

COLLECTIONS

Collectins are a family of mammalian lectin containing proteins that include mannose-binding lectin (MBL), bovine conglutinin, lung surfactants A and D, and bovine collectin 43 (Hoppe and Reid, 1994). Structurally, collectins are multimeric proteins (e.g., 9–18 polypeptides) having “flower bouquet” or “X-like” structures. Each polypeptide consists of an N-terminal segment, a col-

lagen sequence, and a C-terminal lectin. They are structurally and functionally related to the Clq component of the classical complement pathway (Malhotra *et al.,* 1994). Collectins bind to sugar residues on bacteria via the lectin domains and to phagocytic cells using the collagen sequence (Lu, 1997).

Mannose-Binding Lectin

Because MBL (also termed *mannose-binding protein)* has multiple functions, it is the most studied of the collectins. MBL has a "flower bouquet" structure and the circulating serum form is hexameric. Normal serum level in infants is 1780 ng/ml (Turner, 1996).

In the presence of calcium, MBL naturally and selectively binds to sugars such as N-acetylglucosamine, mannose, and N-acetylmannosamine. Binding to sugars is usually weak with a constant of $K_d \sim 10^{-3}$ M, but the molecule's hexameric nature increases the functional avidity of MBL. MBL is active in host defense, and high concentrations of glucosamine and mannosamine sugars are expressed on bacteria, fungi, HIV, and influenza (Haurum *et al.,* 1993).

MBL can act as an opsonin resulting in the ingestion and killing of MBL coated bacteria. Enhanced phagocytosis may be facilitated by two different receptors for MBL. One receptor is a unique 126-kDa Clq receptor on the surface of macrophages and neutrophils (Tenner *et al.,* 1995). Although the data are controversial, a second collectin receptor has also been described. Composed of two identical 60-kDa chains, this receptor has homology with calreticulin, an intracellular calcium-binding protein (Malhotra *et al.,* 1990).

MBL provides a mechanism for activation of the complement system that is independent of antibody. In peripheral blood, MBL circulates in a complex with MBL-associated serine protease (MASP) (Kawakami *et al.,* 1982). In the mouse, this MASP 100-kDa protein has 30–40% homology with Clr and Cls. Moreover, structurally, MASP more closely resembles activated C ls (Matsushita and Fujita, 1992). When the MBL/MASP complex binds to a bacterium, MASP is activated and cleaves both C4 and C2. This results in the creation of a C3 convertase consisting of C4b2a complexes on the bacterial cell wall. Binding of C3b fragments to the cell surface provides multiple opsonins that interact with complement receptors on macrophages and neutrophils. Because the complement pathway is activated solely by lectins, this mechanism has been called the *lectin complement activation pathway* (Turner, 1996).

There are different forms of both MBL and MASP. In the rat, there are two forms of MBL (types A and C). The oligomeric type A variety has a mass of 650–750 kDa and activates complement. The lower-molecular-mass (200 kDa) type C form does not fix complement. In humans, there may be two forms of MASP. It is not clear whether MASP1 and MASP2 have the same structure and function as the mouse protein (Turner, 1996).

DEFENSINS

Defensins are small (29–35amino acids) antimicrobial and cytotoxic proteins produced by neutrophils, lung macrophages, and intestinal cells (Kagan *et al.,* 1994). cDNA for seven myeloid defensins have been described: rabbit NP-1, NP-2, NP-3a, NP-4, NP-5 and human HNP-1, HNP-2 (Nicolas and Mor, 1995). Defensins are structurally rigid, triple-stranded antiparallel ß sheaths with six invariant cysteines that form three intramolecular bonds. Bacterial cells are destroyed by the creation of voltage-dependent channels in the lipid bilayer. These molecules have been shown to kill most gram-positive and gram-negative bacteria and fungi. Human neutrophil peptide (HNP)-1 can directly inactivate several viruses such as herpes simplex virus (HSV) types 1 and 2, cytomegalovirus, vesicular stomatitis virus, and influenza virus A/WSN (Daher *et al.,* 1986). Defensins are also able to kill malignant cells normally resistant to cytolysis by natural killer cells or TNF-α (Nicolas and Mor, 1995). Some members of the defensin family are also able to inhibit ACTH-induced steroidogenesis (Masera *et al.,* 1995)

Intestinal tract defensins are designated cryptidins. Six isoforms have been described. Cryptidin structure resembles the hematopoietic defensins but differs in the length of the amino terminus. Intestinal Paneth cells are the only cells in the adult intestine that secrete cryptidins (Ouellette and Selsted, 1996). Paneth cells are epithelial granulocytes at the base of Lieberkühn crypts distributed throughout the small intestine and the proximal colon of mammals (Ouellette and Selsted, 1996). Like the hematopoietic defensins, cryptidins have broad-spectrum antimicrobial effects. In addition, cryptidin release contributes to the maintenance of the mucosal barrier by preventing infections.

Defensins are also found in the respiratory tract. The tracheal antimicrobial peptide (TAP) is found along the entire length of the conducting airways. It is primarily expressed in ciliated epithelial cells of the pseudostratified epithelium. Like other defensins, TAP is a protein with six cysteines involved in the formation of disulfide bonds. TAP has broad-spectrum antimicrobial and antifungal activities (Lawyer *et al.,* 1996)

PROLINE- AND ARGININE-RICH ANTIMICROBIAL PEPTIDES

Neutrophils produce Pro- and Arg-rich antibacterial proteins including Bac-5 and Bac-7 (Frank *et al.,* 1990). The proline and arginine content of these proteins is 47 and 25%, respectively. These peptides are active against gram-negative bacteria and act by decreasing the ATP content and transport of amino acids and nucleotides (Nicolas and Mor, 1995).

The most studied of these neutrophil peptides is PR-39 originally isolated from pig intestine. PR-39 increases neutrophil phagocytosis of gram negative organisms such as *Salmonella choleraesus.* These peptides do not lyse the cells via pore formation. Rather, they potentiate the activity of magainin A, a pore-forming peptide (Shi *et al.,* 1996).

NITRIC OXIDE

Nitric oxide is a short-lived free radical produced from L-arginine and released by a number of cells during response to infections. It may be rapidly converted to nitrite, a stable metabolite. NO has nonspecific toxic effects on a wide range of bacteria, but may also damage host tissue (Drapier, 1995).

NATURAL KILLER (NK) CELLS

NK cells represent a third lymphocyte lineage, being neither T cells nor B cells. Following cell contact, they release perforin and granzymes. Perforins act much like defensins and create transmembrane pores in the target cell lipid layer (Liu *et al.,* 1995). Granzymes trigger an endonuclease that degrades DNA and induces apoptosis (Symth and Trapiani, 1995).

IMMUNODEFICIENCY AND INNATE IMMUNITY

Innate immunity plays a role in defense of the neonate while the immune system is developing. Children with age-related opsonic defects often have reduced levels of innate defense proteins such as MBL (Super *et al.,* 1989).

Genetic defects also cause MBL immunodeficiencies. Mutations in codons 52, 54, or 57 of exon 1 alter the tertiary structure of the MBL collagen sequence. These mutations are single amino acid substitutions in which glutamic acid (codon 57) or aspartic acid (codon 54) is substituted for glycine. Homozygous mutations result in low serum MBL levels consisting of low-molecular-mass (100 kDa) three-chain subunits (Lipscombe *et al.,* 1995). Because of the lack of structural stability, these chains do not form oligomers and cannot activate complement. When co-occurring with other immunological abnormalities (e.g., C4 deficiency), low MBL levels increase the risk of infections.

ROLE OF INNATE IMMUNITY IN DISEASE

Innate immunity may restrict viral infections. Defects in innate immunity result in increased viral load, whereas a functional immune system terminates the infection. In adult Caucasians, but not Asians, there is a strong correlation between nonfunctional MBL and persistent hepatitis B infections (Thomas *et al.,* 1996). In contrast, Japanese encephalitis virus (JEV) infections are terminated by increased nitric oxide. The latter inhibits viral RNA synthesis, viral protein expression, and viral release (Lin *et al.,* 1997).

Bacterial infections of the lung are also terminated by innate immunity. *Klebsiella pneumoniae* infections are controlled by nitric oxide, which upregulates macrophage microbicidal activity (Tsai *et al.,* 1997). Inhibition of NO production results in a 10- to 46-fold increase in klebsiella colony-forming units in the lung and blood.

Innate defense mechanisms are defective in cystic fibrosis (CF) patients. Human ß-1 defensin is responsible for the control of opportunistic lung pathogens. This defensin is often inactivated by the high salt concentration in airway surface fluids from CF patients. Production of TAP may also fail in CF patients (Ko *et al.,* 1997). This results in defective killing of pseudomonas strains and increased pulmonary infections in CF patients (Stolzenberg *et al.,* 1997).

Host resistance to *Listeria monocytogenes* is also mediated by the innate immune system. In the absence of IL-10, peritoneal macrophages produce a proinflammatory cytokine cascade (e.g., IL-12, IFN-γ, IL-1α,and IL-6) upregulating a Th1 response. The peritoneal response results in earlier clearance of the organism and decreased tissue damage that is characteristic of Listeria infections (Albright *et al.,* 1997).

Innate immunity is also responsible for resistance when acquired immunity is downregulated. Infection with *Trypanosoma musculi* downregulates the acquired immune system yet the infection does not spread within the host. NK cells and activated peritoneal macrophages control the infection. The NK cells kill the parasite or cells infected with the parasite. Macrophages secrete additional cytotoxic molecules (Albright *et al.,* 1997).

HAZARD IDENTIFICATION

The function of collectins is usually measured in opsonization assays. Traditionally, MBL activity is determined in a yeast assay (Turner *et al.,* 1985). Antimicrobial factors in the alveolar lining fluids (e.g., surfactants A and D) are also determined by opsonization. In the assays, the opsonized bacteria are usually *Staphylococcus aureus* (Coonrod and Yoneda, 1983).

In the rat, only surfactant A acts as an opsonin for phagocytosis (Pikaar *et al.,* 1995). Flow cytometry has been used to detect the opsonization of *Escherichia coli* by rat alveolar macrophages. The data are expressed as percent microorganism killed.

NK cells can kill several different tumor cells. The lysis of K562 or YAK target cells by human, mouse, or rat NK cells can be measured in chromium release assays.

REGULATORY POSITION ON INNATE IMMUNITY

Regulatory agencies have expressed an interest in the effects of xenobiotics on innate immunity. The harmonized FIFRA/TSCA guidelines list an NK cell function assay as a measure of innate immunity. However, measurement of NK cell activity is a optional and is not mandated by the EPA. Testing paradigms for biochemical pesticides under FIFRA subpart M require that the effect of chemicals on NK cell function be determined in a 30-day study.

3

Cells of the Immune System

INTRODUCTION

The immune system can be classified in several ways. Classical immunologists consider it to be a system mediated by soluble antibodies (humoral response) and immunocompetent cells (cellular response). Anatomists often classify the immune system on the basis of lymphoid organs, i.e., primary lymphoid organs (e.g., thymus and bone marrow) and secondary lymphoid organs (e.g., spleen and lymph nodes). Pathologists often regard the immune system as subsets based solely on anatomic locations, including the gut-associated lymphoid tissue (GALT), the mucosal-associated lymphoid tissue (MALT), the lymph-associated lymphoid tissue (LALT), and the skin-associated immune system (SAS). The unifying feature of these classifications and subdivisions is that the lymphocyte is the effector cell for all immunological reactions.

IMMUNE EFFECTOR CELLS

Lymphocytes

Two pools of lymphocytes are found in the body. The largest pool resides in the secondary lymphoid organs such as the spleen and lymph nodes. Lymphocytes in these organs are usually undergoing stimulation and differentiation. Resting lymphocytes in the peripheral blood represent the smallest pool. At any point in time, only 2% of the total lymphocyte pool circulates in the peripheral

blood (Anonymous, 1995a). The number of lymphocytes in the peripheral blood varies with the species. In humans, lymphocytes comprise 20–40%of the total white blood cell population. In rats, over 70% of the total white blood cells are lymphocytes.

Using light microscopy, all lymphocytes have the same morphology and staining characteristics. However, functional studies reveal that there are many lymphocyte subpopulations. Lymphocytes are divided into T and B cells depending on whether the cells are induced to maturation in the thymus (T cells) or the bone marrow or organs analogous to the bursa of Fabricius (B cells) in chickens. From a disease perspective, T cells are involved in inflammatory responses to intracellular bacteria, immunoregulation, and the lysis of tumor cells. Conversely, B cells produce protein antibodies directed toward bacteria and other extracellular pathogens. With the advent of flow cytometric analyses and fluorescence-labeled monoclonal antibodies reacting with cluster of differentiation (CD) markers on lymphocytes, it is possible to further dissect subpopulations of T and B cells. Pan T and B cell markers have been identified. In humans and rodents, all T cells express CD3 markers and all B cells express CD19–CD21 surface proteins. Using antibodies with broad-spectrum CD reactivity, subpopulations of T cells can be identified. T helper/amplifier and T cytotoxic/suppressor cells are identified by the presence of CD4 and CD8 markers, respectively. Based on experimental data, it has become clear that the CD4 and CD8 populations are heterogeneous with respect to immunological function. The CD4 population contains helper cells for antibody production (Th) and amplifier cells (Ta). Subpopulations of CD8 cells include two killer cell subsets (Tc1 and Tc2) involved in tumor lysis and cells with possible suppressive activity (Ts). The CD4 helper/amplifier populations can be further dissected by defining the presence or absence of leukocyte common marker isoforms and the CD30 marker. T helper cells that assist B cells in the production of antibodies express CD4, CD45RA+, CD30+ markers. In contrast, CD4 cells that amplify the response (amplifier T cells) express CD4, CD45RO+, CD30–. An alternate nomenclature has been used to divide helper and amplifier cells. Thus, CD4, CD45RA+, CD30+ T helper cells were given the designation of Th2 cells. These cells are resting cells with a life span in the peripheral blood of 5–7 weeks. Th2 cells produce a pattern of cytokines that accelerates antibody production (especially IgE) and enhances eosinophil production (Mossman and Sad, 1996). Elevated numbers of Th2 cells are often found in disease states such as asthma, allergic responses, and helminth infections. Although it is known that Th2 cells play a critical role in defense against helminths, it is unclear whether elevated Th2 cells in allergic diseases are a cause or a consequence of the disease (Table 1).

The CD4, CD45RO+, CD30– amplifier population was given the designation Th1 cells. These cells are long lived in comparison to Th2 cells. The extended life span of Th1 cells may be the basis for immunological memory. Th1

Table 1. Association of Th2 Cells with Specific Disease States

Helminths	
Trichuris muris	Th2 response in resistant mice cures
Nippostrongylus brasiliensis	Th2 response associated with rejection
Heligimosomoides polygyrus	Th2 response associated with expulsion
Brugia malayi	Th2 response correlates with protection
Schistosoma mansoni	Th2 associated with granulomas
Bacterium	
Borrelia burgdorjeri	Th2 cells confer resistance
Allergy	
Immediate allergy	Th2 detected in late asthma and allergen-specific T-cell clones

Modified from *Immunology Today,* Mossman and Sad, 1996, vol. 17:138–45, with permission of Elsevier Science.

cells activate cytotoxic CD8 T cells and mediate inflammatory responses and delayed hypersensitivity reactions (Mossman and Sad, 1996) Thus, the Th1 amplifier cells are elevated in tuberculosis, tuberculoid leprosy, and *Listeria monocytogenes* and *Candida albicans* infections (Table 2).

Presently there are no reagents separating the T cytotoxic and T suppressor subsets. In reality, the designation of T suppressor cells may be a misnomer as many T cell subsets can suppress reactivity by the secretion of various cytokines. Subsets of CD8 cells are identified by the expression of the CD28 marker. Cytotoxic T cells involved in tumor lysis are CD8+, CD28–. A second population of CD8 cells expressing the CD28 protein is weakly cytotoxic and secretes a potent antiviral protein.

There are several B cell subpopulations. Antibody-producing B cells have receptors for complement components C3d (CD21) and C3b (CD35) and recep-

Table 2. Association of Th1 Cells with Specific Disease States

Protozoans	
Leishmania major	Th1 cures and confers resistance
L. donovani	Th1 cures local leishmaniasis
L. brasiliensis	Th1 localized disease
Fungus	
Candida albicans	Th1 response correlates with resistance
Bacteria	
Mycobacterium tuberculosis	Th1 correlates with resistance
M. leprae	Th1 found at site of delayed hypersensitivity reactions

Modified from *Immunology Today,* Mossman and Sad, 1996, 17:138–45,with permission of Elsevier Science.

tors for antibodies (CD32). Interaction of ligands with the receptors stimulates the B cells to differentiate into plasma cells that produce protein antibodies. B cells constitute approximately 20% of the circulating lymphocyte population and 40% of the splenic lymphocytes.

A second population of B cells, called B-1 cells, localize in the peritoneum and pleural cavities. They are distinguished from the normal B cell pool by several characteristics including the expression of both T and B cell markers. They appear early in ontogeny and are capable of self-renewal without bone marrow promotion. Generally, B-1 cells produce IgM, IgA, and IgG antibodies with low affinity and broad specificity for bacterial antigens such as phosphorylcholine, lipopolysaccharide endotoxins, and α 1–3 dextrans. These molecules are major components of the gram-negative bacterial cell wall.

Because of the localization in the gut and the production of antibacterial antibodies, B-1 cells are important in mucosal immunity. In addition, they produce antibodies reactive with thyroglobulin and single-stranded DNA. Clearance of denatured self antigens in autoimmune diseases such as Sjögren's syndrome or systemic lupus erythematosus (SLE) may be the major function of antibodies produced by B-1 cells.

There is a third population of lymphocytes. These cells are neither T nor B cells. Rather, they represent a unique lineage with specific effector functions. Because cells of this lineage lyse tumor cells without prior sensitization, they are called *natural killer* cells. NK cells are more difficult to enumerate by flow cytometry because they express markers found on both T and B cells. However, antibodies directed toward the CD16 and CD38 markers have been used to identify the NK cell population. The subset of NK cells that actually lyse cells from solid tumors express both the CD16 and the NKH1 marker (Storkus and Dawson, 1991).

Lymphocyte Development and Maturation. During ontogenesis in mammals, hematopoiesis occurs first in the yolk sac and the fetal liver. When the bone marrow develops, hematopoiesis shifts to the marrow. The maturation of stem cells in the marrow requires a stable microenvironment of reticular cells (adventitial and fibroblastic), adipocytes, endothelial cells, macrophages, and T cells (Mayani *et al.,* 1992; Weiss and Geduldig, 1991). The maturation of these cells requires a complex interaction of cytokines and lineage-specific growth factors (Fletcher and Williams, 1992; Kincade, 1992). Rodents are the exception to the rule. Hematopoiesis also occurs in the red pulp of the spleen and the thymus. In the adult mammal, over 95% of hematopoiesis occurs in the bone marrow (Mayani *et al.,* 1992). However, the distribution of the stem cells within the marrow differs according to species. All extramedullary space in the rat marrow is occupied with hematopoietic stem cells. In contrast, similar tissue in the dog, human,

and rabbit is localized in the epiphysis of long bones, the skull, and the central skeleton.

T Cell Development. The thymus is the master organ of the immune system. It receives lymphocyte precursors from the bone marrow and stimulates growth, maturation, and differentiation of select CD4 and CD8 lymphocyte subsets. Immature lymphocyte precursors from the bone marrow enter the cortex of the thymus where they take on the appearance of large lymphoblasts. Approximately 5% of the resting cells do not express CD4 and CD8 markers (CD4−, CD8−). Another population of double-negative cells (80%) is rapidly dividing and rearranging TCR genes to develop a complete repertoire of antigenic recognition (Fig. 1). IL-7 produced by stromal cells initiates an irreversible transition process. Although the exact intrathymic effects are unclear, most hormones of the thymus also modulate the maturation of T cells. These hormones are thymulin, thymopoietin, thymic humoral factor (THF), and thymosins (Pawlowski and Staerz, 1994).

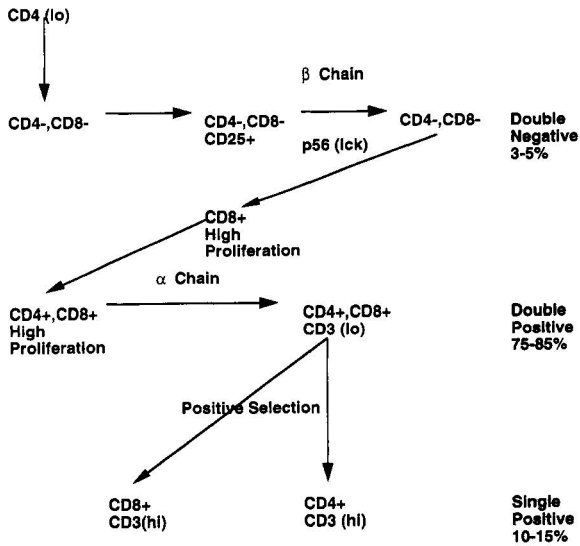

Figure 1. The maturational pathway of T cells in the thymus followed by most T cells expressing the αβ T-cell receptor (TCR). Transition from the CD25+ double-negative cells to single-labeled cells requires the synthesis of β chain protein and activation of the protein tyrosine kinase p56*lck*. Modified from *Immunology Today,* Anderson and Perlmutter, 1995, 16:99−105, with permission of Elsevier Science.

Normal transition of the double-negative cells requires the expression of a CD25 molecule, the production of a TCR ß chain protein, and the expression and activation of p56 *lck* that allows intracellular signaling. However, the p56 *lck* signaling process is unique to thymocyte development. Neither p59 *lck* nor p59fyn, which are closely related to p56 *lck* in structure and function, increase activity during the T cell transition process. Moreover, generalized inhibition of the common FTK-mediated growth pathway does not inhibit p56 *lck* activity. Most evidence supports the hypothesis that' p56 *lck* serves to control the recombination of the variable portions of the MHC 11 V_α and $V_ß$ regions of the TCR.

Under the influence of thymic hormones produced by epithelial cells, the double-negative cells begin to mature over a period of 2 or 3 weeks. Initially, proliferating cells express low levels of CD8. Most cells transition into double-positive (CD4+, CD8+) cells. Such cells constitute over 80% of the total cells in the adult thymus. As the double-positive cells move from the cortex to the thymic medulla, TCR α is also expressed on the cell surface. At this point, the CD3 marker is upregulated and expressed on the lymphocytes (Fig 1). About 50% of the cells are CD3+,TCR α/β and half are CD3–,TCR α/β .

To develop the complete repertoire of antigenic specificities, TCR α and ß chains undergo random rearrangements. To ensure that cells leaving the thymus are capable of MHC-restricted expansion but unable to react with host tissue, a small proportion of double-staining cells have low binding affinity for peptide/self MHC complexes expressed on stromal cells, fibroblasts, or thymic epithelial cells. These dual-staining cells are stimulated to further differentiate (positive selection). The purpose of positive selection is twofold. First, selection is required for additional T cell maturation. Second, interactions with MHC bias the T cell repertoire to self-restricted expansion. The remaining unselected population containing the autoreactive cells or T cells with high-affinity TCR for MHC has a short life span of 3–4days and undergoes negative selection by programmed cell death or apoptosis (Pawlowski and Staerz, 1994).

At 12 days of the maturation process, CD4+, CD8– TCR α/β represent 12–15% of the total thymocytes. These cells ultimately evolve into helper cells with class II MHC-restricted activity. Because these cells have been processed by the thymus, they are commonly called *T helper* cells. By day 19, CD4–,CD8+TCR α/β cells appear in the thymus. Although these cells comprise only 3% of the total thymocytes, they are released into the peripheral blood in high numbers to become class I MHC-restricted T CD8 cytotoxic cells.

There is a distinct, second lineage of α/β cells. In the thymus, a small population (5%) carries a CD4–, CD8– TCR α/β phenotype. Although these cells are often autoreactive, they can exit the thymus without undergoing positive or negative selection. Because these cells are processed differently than the conventional thymocytes, it has been speculated that these T cells have an alter-

native function in defense and are most efficient in recognizing self peptides presented by MHC proteins (Anderson and Perlmutter, 1995).

B Cell Development. In chickens, a gut-associated lymphoid organ called the bursa of Fabricius is the sole source of B cells and antibody-producing plasma cells. Extirpation of the bursa causes a severe defect in the plasma cells resulting in reduced levels of immunoglobulins in the serum. However, bursectomized chickens have normal T cell immunity. Located in the hindgut (cloaca), the bursa is anatomically similar to the thymus. However, the bursa is packed with plasma cells rather than small T lymphocytes. Like the thymus, the bursa undergoes atrophy when the chicken undergoes puberty (4–6 weeks of age). Many immunologists have suggested that mammalian intestinal Peyer's patches (PPs) and other gut-associated lymphoid tissue are analogous in function to the bursa of Fabricius.

In mammals, the role of the gut-associated lymphoid tissue in B cell development is unclear and may be species dependent. In rabbits, there is no evidence that PPs, appendix, or sacculus rotundus play a role in B cell differentiation (Cooper and Lawton, 1972). Rabbit gut lymphoid tissue contains only the precursors of IgA-producing B cells that migrate to other areas of the gut following antigen stimulation (Craig and Cebra, 1971). In this species, there is a consensus that the bone marrow is the sole source of B cells (Cooper and Lawton, 1972). In other species, PPs are the primary source of B cell differentiation. In the sheep, physiological and anatomic data show that PPs function as a primary lymphoid organ (Reynolds *et al.,* 1991) and provide the necessary microenvironment for B cell proliferation and antigen-driven mutation of immunoglobulin genes (Reynaud *et al.,* 1988) .

Lymphocyte Recirculation

Lymphocytes are responsible for surveillance of tissue and the immune response to blood-borne microbial agents. To achieve these goals, evolutionary pressures created a bicameral lymph system. Naive and immature lymphocytes circulate only in the blood whereas memory or activated lymphocytes can travel in the blood, leave the circulation, enter the tissues, and return to the blood via the lymph system.

The tissue surveillance mechanism takes advantage of the natural pressure of blood flowing through the system. Extravasation of lymphocytes occurs by movement through plump, cuboidal cells called *high endothelial venule cells* (HEVs). These cells appear very different from conventional endothelial cells, which have a flat morphology (Fig. 2).

Similar to Velcro adhesive, the HEVs slow and stop, rolling activated lymphocytes. Slowing the rolling lymphocyte entails interactions between L-selec-

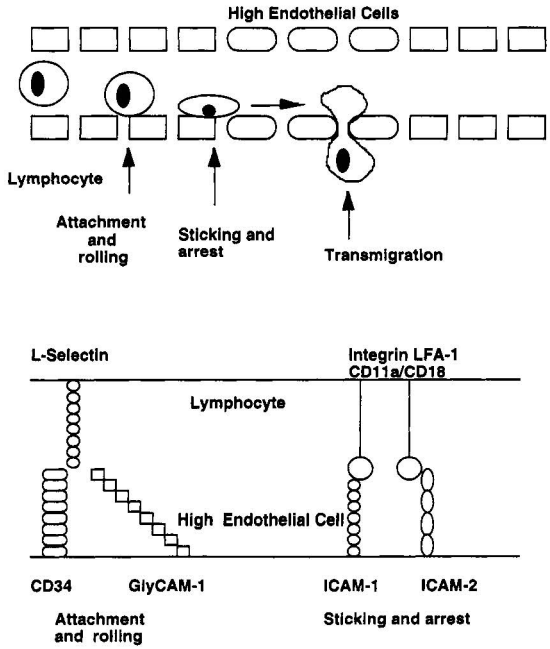

Figure 2. Lymphocyte emigration through the high endothelial venule cells into the tissue. The initial interaction with HEVs in the lymph node is mediated by lymphocyte L-selectin, which interacts with mucin counterreceptors CD34 and glycosylation-dependent adhesion molecule 1 (GlyCAM- 1). Increased adhesiveness is mediated by activation of G-protein-coupled receptors. Lymphocyte arrest is mediated by the integrin leukocyte function-associated molecule 1 (LFA-1; also known as $\alpha_L\beta_2$ or CD11a/CD18). The mechanisms involved in transmigration of lymphocytes are unclear, but a coating of glycocalyx on the HEV surface may play a critical role. Modified from *Immunology Today,* Girard and Springer, 1995b, 16~M9–456, with permission of Elsevier Science.

tins on the lymphocyte, CD34, and a glycosylation-dependent adhesion molecule (GlyCAM-1) on the HEV. A second signal mediated via G-protein coupled to receptors activates the lymphocyte. It is unclear whether these receptors are ligands for cytokines or other local activation factors.

Arrest of lymphocyte movement requires two separate interactions between the lymphocyte and HEV. Pan lymphocyte integrin molecule LFA-1 ($\alpha_L\beta_2$ or CD1 1a/CD18) interacts with the HEV intercellular adhesion molecules 1 and 2 (ICAM-1 and 2). A second lymphocyte integrin called a4 ß7 reacts with MadCAM-1 on HEVs (Hama, 1994). The latter interactions arrest movement of all memory cells that express high levels of a4 ß7.

Passage through the HEVs and into tissue requires remodeling of the HEV junction and the basement membrane. HEVs secrete hevin, a major acidic protein that is rich in cysteine. Hevin reduces the adhesiveness between HEVs. Other molecules such as the hyaluronan receptor (CD44) or the vascular adhesion protein (VAP-1) may also play a role in the basement membrane remodeling (Jalkanen *et al.,* 1986; Salmi and Jalkanen, 1992; Girard and Springer, 1995b).

The transendothelial passage of lymphocytes is highly efficient and occurs within minutes. Of the lymphocytes circulating in the venules containing HEVs, 25% bind to the latter. Rolling, attachment, and arrest of lymphocytes take only a few seconds. Transepithelial migration occurs in less than 10 min (Smith and Ford, 1983). Approximately 1.4×10^4 lymphocytes can extravasate from a single lymph node every second (Cahill *et al.,* 1976). Along with the cells, the liquid portion of blood is forced into the tissue. Cells, liquids, and any microbial agents traverse the tissue, enter open ended lymphatic vessels, and are transferred to progressively larger vessels. Finally, the cells enter a lymph node.

After passing through the lymph nodes, the cells progress through the lymph system and into the thoracic duct. The duct empties into the subclavian vein near the heart. In this classic pathway of recirculation, approximately 25×10^9 cells per day reach the blood via the thoracic duct. This number represents only 5–10% of the total lymphocytes (Pabst and Binns, 1989).

Other Immunocompetent White Blood Cells

Polymorphonuclear Leukocytes (PMNs). Phagocytosis and intracellular destruction of pathogenic organisms are the major functions of this white cell subset. The number of PMNs in the peripheral blood is species dependent. In human peripheral blood, PMNs constitute 50–60% of the total white blood cells, whereas rat blood contains 11–12% PMNs.

The PMN is produced by and matures in the bone marrow. In humans, there is a 9-day transit time from the marrow into the blood. In a 4-day mitotic pool, precursors differentiate from myeloblasts to promyelocytes. Maturation occurs in a 5-day storage pool where cells differentiate into PMNs. Once the cells enter the blood, they may circulate (circulating pool) or marginate (marginating pool) in the vessel walls and capillary beds. The half-life in blood and that in tissue are 1 and 5 days, respectively.

Monocytes. Monocytes are the largest of the white blood cells, and are approximately three times the size of red blood cells. In the bone marrow, monoblast and promonocyte precursors have a transit time of 6 days to peripheral blood. The half-life in blood is approximately 3 days in humans and 18 hr in mice. Monocytes may circulate in blood or migrate randomly into tissue where

they assume the morphological and functional characteristics of tissue macrophages. Tissue macrophages such as Kupffer cells, alveolar macrophages, or dendritic cells have a half-life of several months.

Monocytes and macrophages provide critical functions to the immune system. Phagocytosis and intracellular bacterial killing are important functions. Of greater importance, monocytes present peptides and polysaccharides to immunocompetent lymphocytes in a way that stimulates the immune system to produce antibodies or cytotoxic T cells. In addition, reactive oxygen metabolites, cytolytic proteinases, and tissue necrosis factor (TNF) produced by monocytes have tumoricidal activity.

Eosinophils. Eosinophils are motile phagocytic cells that contain red granules (eosin staining) and refractive crystals (Charcot–Leyden crystals). Normally, only 2–5% of the white blood cells are eosinophils. Increased numbers of eosinophils are often found in allergic reactions, chronic inflammatory reactions and helminth infections. Eosinophils exert both beneficial and detrimental effects.

Large hypodense eosinophils terminate allergic reactions by secreting a carboxypeptidase that inactivates histamine. Unfortunately, major basic protein (MBP) secreted simultaneously causes extensive tissue damage. MBP is, however, important in the defense against helminths. The extruded MBP is extremely toxic to the parasites.

Basophils. Basophils comprise less than 1% of the total circulating white blood cells, but they are the major effector cell in immediate allergic reactions in the skin. Basophils express high numbers of receptors for the IgE allergic antibody. Allergen-induced cross-linking of the cell-bound IgE initiates an energy-dependent process that culminates in the release of heparin, histamine, eosinophil chemotactic factor, and trypase (Goust, 1993; Tharp, 1990).

STRUCTURE OF SECONDARY LYMPHOID ORGANS

The Lymph Node

Lymphocytes within lymph nodes are the major defense against bacterial pathogens in the skin and tissue. Lymph nodes are the first organized structure encountered by both microbes and lymphocytes. After emptying into the subcapsular sinus, the blood percolates through the node. This allows interactions between microbes and different lymphocyte subsets at different sites within the node (Fig. 3).

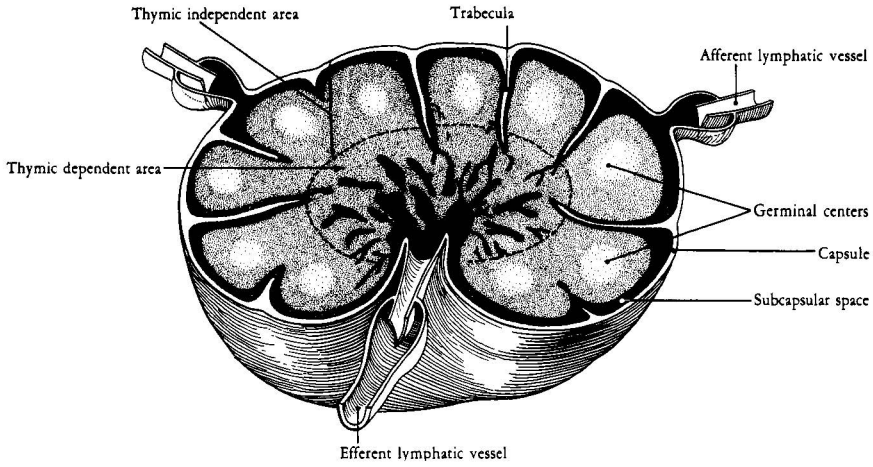

Figure 3. The thymic dependent and independent areas of a lymph node. Reprinted from *Immunology,* Bellanti, 1971, with permission of W. B. Saunders Co.

There are three distinct areas of immunocompetent cells in the lymph node (Fig. 3). The outer cortex contains primary follicles of IgM+, IgD+ virgin B cells interspersed in a framework of follicular dendritic cells. Bacterial stimulation of the follicle causes selection and differentiation of plasma cells and memory B cells (Kroese *et al.,* 1990; Szakal *et al.,* 1989). In addition, antigenic stimulation produces secondary follicles with a germinal center and surrounding mantle. The mantle layer houses the memory B cells (Szakal *et al.,* 1989).

In the lymph node, the paracortex is considered to be the thymic dependent area consisting primarily of CD4 helper cells, a few cytotoxic CD8 cells, macrophages, granulocytes, and neutrophils. Stimulation and activation of the immune system occurs in the paracortex. In the case of viral infections, the interfollicular area of the paracortex becomes enlarged.

The Spleen

The spleen has the primary responsibility for mounting an immune response to blood-borne pathogens. Quantitatively, it is the most important organ in terms of lymphocyte migration and filtration. Located in the upper left quadrant of the abdomen, the spleen weighs approximately 150 g in humans. Almost 25% of the total lymphocyte population is found in the spleen. In addition, about 50% of the lymphocytes in the blood (2.5×10^{11} lymphocytes) migrate through the spleen each day (Pabst, 1988).

The spleen has a red and white pulp (Laman *et al.,* 1992). Morphologically, the splenic artery branches until it terminates into splenic arterioles in the red pulp of the spleen (Fig. 4). The red pulp contains red cells and only small numbers of lymphocytes. A high volume of blood passes through the vascular sinuses within the red pulp. The vascular sinusoids are lined with phagocytic macrophages that function to degrade aging or defective red blood cells and ingest blood-borne pathogens. Bacterial infections cause a massive increase in the red pulp via production of immature granulocytes. Approximately 5% of the total blood flow passes through the sinusoids each minute, collecting in venules progressing in size to the splenic vein. The splenic vein carries blood from the spleen to the portal circulation.

The spleen is normally involved in hematopoiesis in select species. In rats and mice, the splenic red pulp is also involved in extramedullary hema-

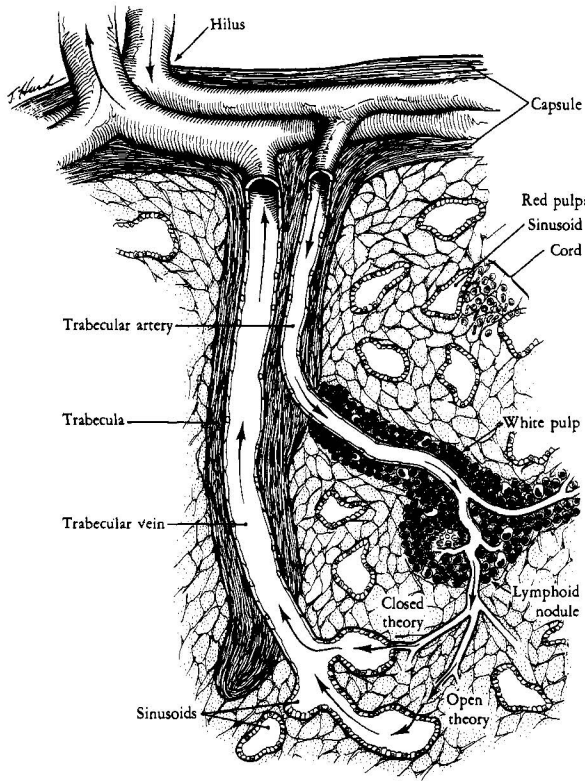

Figure 4. Schematic structure of the spleen. Reprinted from *Immunology,* Bellanti, 1971, with permission of W. B. Saunders Co.

topoiesis of granulocytes. Normoblasts and megakaryocytes are found in the spleen.

Most of the immunocompetent cells are found in the white pulp. These lymphocyte clusters are located in the periarteriolar sheath (PALS). Roughly two-thirds of the PALS are CD4 cells while the remainder are CD8 lymphocytes. Germinal centers also exist in the white pulp. Some B cells in the zone are organized into secondary follicles. A rim of lymphocytes and macrophages in the PALS form a marginal zone. Unlike other mammals, the marginal zone predominates in the rat spleen.

The microenvironment of the marginal zone is unique. Specialized macrophages called *marginal metallophilic macrophages* are found in the zone. Moreover, the morphology of B cells is different relative to other areas of the spleen. There are no centrocytes or centroblasts in the germinal centers and the B-cell phenotype (IgM+, IgD−)is unique. Immunocompetent B cells, in the marginal zone, represent mixed populations. Virgin, nonactivated B cells and B memory cells are found in the zone. For reasons that are unclear, these B cells respond only to carbohydrate immunogens (e.g., pneumococcal polysaccharides) that have repeating subunits.

PRIMARY IMMUNODEFICIENCIES

DiGeorge Syndrome

Abnormal fetal development of the third and fourth pharyngeal pouches causes defective development of the thymus, thyroid, and parathyroids. Because of the inhibition of thymic maturation, there are low numbers of T cells in peripheral blood. Most such children die shortly after birth, death being caused by cardiac abnormalities. Abnormal parathyroid development is reflected in altered serum calcium and serum electrolyte levels. Ramifications of the altered calcium levels include muscular twitching and tetany (Webster, 1985).

Wiskott–Aldrich Syndrome (WAS)

This syndrome is usually an X-linked defect causing eczema and thrombocytopenia. The major clinical sign is hemorrhage related to poor platelet maturation. Frequently, such patients die in the early years of life from viral infections or B-cell lymphomas.

WAS patients have poor primary antibody responses to polysaccharide antigens. T-cell immunity is also impaired and most patients cannot mount delayed hypersensitivity skin reactions to tuberculin, candida, or streptokinase/streptodornase (SK/SB). A unique gene mutation has been found in WAS patients.

The WASP (Wiskott–Aldrichsyndrome protein) gene has no significant homology to any known gene but has characteristics of a transcription factor (Fischer and Wood, 1995).

PHENOTYPIC, GENETIC ABNORMALITI ES

Adenosine Deaminase Deficiency

Severe combined immunodeficiency (SCID) is an autosomal recessive disorder manifested as the absence or inhibition of lymphocyte enzymes (Table 3). About one-third of SCID patients have a defect in the enzyme adenosine deaminase. This enzyme normally converts adenosine and deoxyadenosine to inosine and 2'-deoxyinosine. Lack of a functional enzyme causes the accumulation of deoxyadenosine, adenosine triphosphate (ATP), and S-adenosyl homocysteine. In turn, these products inhibit ribonucleotide reductase necessary for DNA synthesis in T and B cells.

Purine Nucleoside Phosphorylase Deficiency

In children, there is a defect in production of the enzyme purine nucleoside phosphorylase (PNP), leading to accumulation of deoxyguanosine and guanosine triphosphate in the serum. Deoxyguanosine has a toxic effect on precursor and mature T cells. This is reflected in a lack of functional T cells in the peripheral blood. In contrast, B cells are not affected by the presence of deoxyguanosine (Stiehm, 1993) and B-cell immune function is usually within normal limits.

Ataxia Telangiectasia (AT)

Early symptoms of the disease are ataxia when the child begins to walk and telangiectasia on the conjunctiva at 3 years of age. AT is an autosomal recessive

Table 3. Phenotypic Genetic Syndromes with Immune Abnormalities

Adenosine deaminase deficiency
Ataxia telangiectasia
Bloom's syndrome
Down's syndrome
Schimke's immuno-osseous dysplasia
Shwachman syndrome
Short-limbed dwarfism and cartilage hair hypoplasia

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disorder of DNA repair. In most patients, there are deficiencies in the B-cell production of specific antibody isotypes (e.g., IgA), decreased delayed hypersensitivity reactions, and marked defects in (CD8) T cytotoxic cell function.

Bloom's Syndrome

These patients, usually young children, have facial erythema and telangiectasia related to sun sensitivity. A defective DNA repair mechanism is the underlying cause of the disease. The nature and severity of the immune defect varies from patient to patient. Some patients have only low antibody levels, reflecting reduced CD4 cells. Others have decreased numbers of CD8 lymphocytes and a consequent defect in cellular immunity. Increased risk of lymphoid malignancy is associated with the defective repair mechanism (Fischer and Wood, 1995).

Down's Syndrome

Individuals with Down's syndrome have multiple immunological abnormalities including abnormal distribution of lymphocyte subsets, cellular immune function deficits and autoimmune phenomena. However, the immune defects in these patients are not severe enough to increase the incidence of infections. It was hypothesized that these changes are part of the early age-related senescence of the immune system in Down's patients (Fischer and Arnaiz-Villna, 1995).

Schmike's Immuno-osseous Dysplasia

This disease results from an autosomal recessive gene defect producing skeletal dysplasia, dwarfism, progressive nephropathy, and changes in skin pigmentation. Associated with the syndrome is a low number of CD3 T lymphocytes with α/β TCR receptors (Stiehm, 1993).

GENETIC SYNDROMES WITH IMMUNE DEFECTS

In addition to the phenotypic abnormalities, there are other genetic defects that have inherent immune defects (Table 4). Progeria and familial microencephaly have associated T-cell defects. Folic acid deficiency results in depressed cell-mediated immunity and other B-cell defects (Stiehm, 1993).

CHEMICALS AFFECTING LYMPHOID TISSUE

Splenic and lymph node lymphocytes are targets for fungal toxins. Ochratoxins from *Aspergillus* and *Penicillium* are cytotoxic. However, the cytotoxic

Table 4. Assorted Genetic Syndromes with Associated Immune Defects

Congenital folic acid malabsorption	T- and B-cell defect
Ectodermal dysplasia with aplastic anemia	T-cell defect
Familial microencephaly with normal IQ	T- and B-cell defect
Progeria	T-cell defect

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response is dependent on dose, route of administration, and the test species. The trichlorothecene family are potent inhibitors of protein synthesis in lymphocytes. *Fusarium* produces two potent trichlorothecenes: T-2 toxin and deoxynivalenol.

Synthetic 2-acetyl-4(5)-tetrahydroxybutyl-imidazole (THI) depletes both T and B cells in blood, spleen, lymph nodes, and thymus. Although the mechanism of action is not fully delineated, THI may function as a vitamin B_6 antagonist. THI competes with pyridoxal 5'-phosphate at the cofactor binding sites on PLP-dependent enzymes.

HAZARD IDENTIFICATION HEMATOLOGY

Total white blood cell (WBC) and differential counts from peripheral blood samples are usually included in immunotoxicity studies. Normal baseline values for WBCs differ in relation to such factors as age, sex, the strain of the rat or mouse, disease status, animal husbandry, stress, and diurnal variation. In addition, hormones may influence the number of white cells in the blood during a 24-hr period. WBCs, particularly lymphocytes, fluctuate with the corticosteroid levels in the blood.

To assess the biological relevance of changes in total WBCs, lymphocytes, monocytes, and neutrophils, both the percentage and the absolute numbers of WBC subsets are determined. While the former endpoint supplies meaningful information, the latter is more biologically relevant. Bone marrow toxicity is reflected by the appearance of immature red cells (reticulocytes), neutrophils (band cells), or lymphocytes in the blood. Statistically significant, dose-related changes of 10–20% from baseline values are considered relevant.

Because the baseline values vary widely, it is often difficult to ascertain treatment-related effects. Hence, WBC counts are regarded as an insensitive correlate of immunotoxicity. In 30 animal studies, the concordance between leukocyte counts and biologically relevant *in vivo* immune defects was only 43% (Luster *et al.,* 1993).

Lymphocyte Phenotyping

Lymphocyte subsets are enumerated by fluorescent staining and flow cytometry. As previously described, lymphocytes express subset-specific markers. Monoclonal antibodies directed toward mouse, human, and monkey lymphocyte markers are highly specific for surface markers and can be used to enumerate subsets. In the rat, lymphocyte markers are found on a variety of lymphoid and nonlymphoid cells. Therefore, it is difficult to identify lymphocyte subsets by staining with a single antibody. Moreover, there is no specific CD3 marker for rat T cells. When phenotyping rat lymphocytes, dual labeling is the only method that accurately distinguishes between T-cell subsets and NK cells. The combinations of antibodies used to identify rat lymphocyte subsets are shown in Table 5.

The distribution of lymphocyte subsets is different in the blood and spleen. Because the immune system is poised to defend the host, over 60% of circulating lymphocytes are T cells and 30% are B cells. NK cells represent the smallest proportion of the total lymphocytes (10–15%). The bulk of the T cells in the circulation are CD4 helper cells (60–70%). Cytotoxic/suppressor CD8 cells represent 20–25% of the T cells. In the spleen, percentages of T and B cells are equal — approximately 40% each. But the predominant T-cell population is the CD4 helper subset.

The distribution of B cells secreting IgM, IgA, or IgG and the maturation status of B cells can also be determined by flow cytometry. Using anti-immunoglobulin isotype-specific antisera, it is possible to determine the various isotype-specific B-cell subsets producing antibodies. During the B-cell maturation process, different markers are found on the cell surface. In the early B cell stage, CD10, CD19, CD11b, and HLA-DR are expressed on the cell's surface. Only CD19, CD20, CD21, CD24, CD11b, and HLA-DR are found on mature B cells in the peripheral blood.

Data from flow cytometry studies should be expressed both as percentages of cells and as the absolute number per cubic millimeter of blood. Whereas percentages give some information, the latter endpoint is the more biologically and clinically relevant. The absolute number is a mathematical calculation that

Table 5. Dual Staining of Rat Lymphocytes and Identification by Flow Cytometry

Total T cells	CDS (OX19)
T helper/amplifier	CD5, CD4 (W3/25)
T suppressor/cytotoxic	CDS, CD8 (0x8)
Natural killer cells	CD5-, CD8
B cells	κ chains (OX12)

considers the total WBC count, the percentage of total lymphocytes, and the percentage of each subset. The formula for determination is as follows:

$$\text{Absolute lymphocytes/mm}^3 = (\text{total WBCs/mm}^3)\ \frac{(\%\ \text{of total lymphocytes})}{100}\ \frac{(\%\ \text{of each lymphocyte subset})}{100}$$

If one assumes that the cytometry studies have been performed under optimal conditions, statistical analyses of the data should be considered. Because lymphocyte subsets are part of a network and, hence, do not function as separate entities, multivariate statistical analysis is the method of choice.

In the older literature, the helper:suppressor (T4:T8) ratio was often used as an index of immunosuppression. This is particularly true in AIDS patients who have ratios below 1.0 (normal ratios range between 1.7 and 2.2). It is unclear if a decreased T4:T8 ratio is a generalized phenomenon in immunosuppressed patients. In immunosuppressed renal transplant patients, there is great variation in the ratio which has no relationship to episodes of rejection. In these patients, a falling T4:T8 ratio correlated only with acute viral infections.

Depending on the matrix, immunohistochemistry can be used to identify monocytes, T and B cells in the spleen, lymph nodes, and thymus. Fresh frozen tissue (human, murine, or rat) combined with antibody-mediated, immunoperoxidase-amplified stains have been used extensively and excellent results have been obtained. Attempts were made to identify murine or rat T and B cells in formalin-fixed, paraffin-embedded tissue. The approach has not been successful. Epitopes are altered or deleted from immunocompetent cells following formalin treatment (Schuurman *et al.,* 1994).

In the mouse model, changes in the splenic lymphocyte subsets are a sensitive indicator of immunotoxicity. Based on the data from 24 separate studies, the concordance between changes in the number of immunocompetent cells and biologically relevant *in vivo* effects is 83% (Luster *et al.,* 1993).

Because of differences in sample sources, correlations between human and animal data are difficult. Most animal studies use splenic lymphocytes from a secondary lymphoid organ in *ex vivo* assays. In humans, peripheral blood is the only available sample source. It is unclear whether human peripheral blood reflects effects on other parts of the immune system. Human peripheral blood contains only 2% of the total lymphocyte population (Westermann and Pabst, 1990).

There is some evidence suggesting that the blood does not mirror disturbances in the lymphoid tissue. For example, patients with Hodgkin's disease show no alteration in peripheral lymphocyte subsets whereas subsets in the spleen are abnormally skewed (Gupta and Tan, 1980). Data also show that HIV

destroys cells within the lymph nodes long before changes occur in the peripheral blood.

Organ Weights

Lymphoid organ weights (e.g., thymus, spleen, lymph nodes) have often been used as an index of immunotoxicity. In practice, the weights vary enormously and large standard deviations from the mean values are common. Differences between organ weights from treated and control animals are rarely statistically significant. The variations in thymic and lymph node weights reflect difficulties in excision. Because of their anatomic position, lymph nodes are difficult to dissect. In contrast, the thymus is easily accessible but embedded in a fatty tissue matrix. With the thymic and fatty tissue closely resembling each other, it is difficult to separate the organ and the fat. Thymic weights in normal young animals with no histological abnormalities may vary by 20%.

In standard toxicology studies the spleen is considered the main target organ for immunotoxicants. Splenic weight or splenic/body weight ratios are often used as an index of immunotoxicity. The splenic/body weight ratio concordance with *in vivo* biological effects is only 61% (Luster *et al.,* 1992a). Therefore, splenic weights are a relatively insensitive correlate of immunotoxicity .

The low concordance may reflect the fact that factors unrelated to immunotoxicity can increase or decrease splenic weights. Increased production or degradation of erythrocytes will increase splenic weights. Conversely, euthanasia with barbiturates causes contraction of the spleen reducing the total weight. The relationship between splenic weight changes and *in vivo* effects is often unclear.

Splenic Cellularity and Viability

Splenic cellularity and viability are used as concurrent screens for immunotoxicity. By definition, cellularity is the number of nucleated cells present in an immunocompetent organ. Determination of splenic cellularity is rather insensitive. Cellularity must change by 30% before the effect is considered biologically relevant. The necessity for large weight changes for biological relevance is reflected by a 51% concordance between splenic cellularity and biological effects (Luster *et al.,* 1992a).

A decrease in the viability may suggest an immunotoxic effect within the nucleated cell population. The most common vital stain used to determine viability is trypan blue. Living cells do not take up the dye, but dead or dying cells readily internalize the dye. While trypan blue staining is simple to perform, care must be undertaken to remove extracellular protein from the test sample. Protein in the media leads to false estimates of viability because the dye binds readily to the protein, preventing it from entering dead cells.

Histopathology

For many years, histopathology has been the bellwether for hazard identification in standard toxicology studies. Recently, both the sensitivity and the accuracy of the histopathology endpoint have been questioned in studies conducted for less than 28 days. Before a histological event can be visualized by light microscopy, approximately 60% of the normal cell population within an organ must be altered (Irons, 1985). Thus, subtle changes may not be detected by light microscopy.

Other data suggest that conventional histopathology may underestimate immunotoxicity in short-term studies. Fourteen laboratories in nine countries participated in a study designed to detected immunotoxic effects of azathioprine and cyclosporin A. It was concluded that basic histopathology was not able to detect effects on the immune system (Anonymous, 1996). Enhanced pathology that examined additional organs and used structural assessment and semiquantitative grading of changes in the principal lymphoid organs did, however, reveal histological changes. In another study reported by the RIVM of the Netherlands, 50% of known immunotoxic chemicals *(N* = 20) were misidentified when immunological endpoints were restricted to total and differential WBC counts and histopathology of the spleen (Anonymous, 1995a). Finally, 30% of immunotoxic compounds *(N* = 53) in a National Institute of Environmental Health and Safety (NIEHS) validation study elicited no changes in the pathology of secondary lymphoid organs (Luster *et al.,* 1992b).

Despite the limitations, it is important to examine the immunocompetent organs from the high-dose treatment group and the controls in standard toxicology studies. The thymus should be examined to detect possible effects on the developing immune system. The spleen is a logical choice as it is the repository forrecirculating lymphocytes. Depending on the route of administration, two different nodes should be examined. Mesenteric nodes are examined when exposure is via the oral route, whereas bronchial nodes are examined following inhalation exposure.

In young animals, the thymus is large, well defined, and can be examined by light microscopy. Immunotoxicity is indicated by a number of different changes. Changes in the ratio of cortex to medulla, altered cellularity, the incidence of degenerative or apoptotic lymphocytes, or the number of tingible body macrophages are commonly associated with immunotoxicity. In short-term 28-day studies, changes in thymic weights may be more relevant than changes in splenic weight (Anonymous, 1994).

In the spleen, the T and B cells are segregated in defined areas. The splenic T cells are localized in the PALS. B cells are located in splenic B-cell follicles. Changes in the red/white pulp ratio, extramedullary hematopoiesis, and the appearance of histiocytes in the spleen suggest immunotoxicity. Decreased cellularity or altered appearance of the PALS, marginal zones, or follicles are of particular interest (Anonymous, 1994).

Changes in the lymph nodes are also used as indices of immunotoxicity. T cells are segregated in the paracortical region in lymph nodes. B cells are predominant in lymph node germinal centers. Changes in architecture and/or cellularity are indicative of immunotoxicity. Other relevant microscopic changes include the number and appearance of primary follicles and germinal centers in the cortex or alterations and cellularity of the paracortex. In the medulla, changes in the number or appearance of plasma cells or sinus histiocytes suggest immunotoxicity (Anonymous, 1994).

In the normal histological evaluation of the femoral bone marrow, the frequency of different cell types and cell structure is determined. The frequency of cell types can be ascertained by two methods. Generally, a bone marrow smear is used to define cellularity and expressed as the myeloid/erythroid (M/E) ratio. In the mouse, the normal ratio is 1.49:1.0 with a standard deviation of 0.47. The ratio in the rat varies with age. In rats less than 1 month old, the ratio is 0.62:1.0. The ratio in older rats varies from 1.75:1.0 to 1.93:1.0. The disadvantage of the bone marrow smear is that it is possible to have reduced numbers of total cells with a normal M/E ratio.

Bone marrow cross sections are used for histopathological analyses. The histology may be compromised by the fact that the bone is decalcified before analyses. Measurement of other endpoints such as cellularity, presence of abnormal cells, or evidence of hyperplasia or hypoplasia can also be associated with immunotoxicity (Anonymous, 1994).

EFFECTS OF AGING, STRESS, AND HORMONES

The histopathology of immunocompetent organs can be modified by many exogenous and endogenous factors. Clearly, the age of the animal is reflected in lymphoid histology. At sexual maturity, the thymus begins to involute with major effects on the cortex and minor effects on the medulla (Schuurman *et al.,* 1991). Because of the decrements in cortex function, the host has a lower production of virgin T cells necessary for primary immunological responses. In addition, the parenchyma is replaced by adipose and connective tissue. Lymph nodes may be smaller in older adult animals but are capable of antigenic stimulation and expansion of germinal centers (Schuurman *et al.,* 1994).

Psychological stress induces the secretion of corticosteroids via the hypothalamus–pituitary–adrenal axis. In some species, the major target organ is the thymus, Within a few days, the lymphocytes within the cortex disappear and the lobules decrease in size. In contrast to the normal thymus, the medulla has a higher density of lymphocytes (Van Baarlen *et al.,* 1988). Similar histology is observed in acute debilitating diseases and severe systemic toxicity.

An altered balance between estrogens and androgens can also affect the histopathology of the thymus. Depending on the stage of pregnancy, thymic

weights increase or decrease with involution of the cortex (Clarke and Kendall, 1989). Normal thymic architecture is restored following birth. Treatment with luteinizing hormone-releasing hormone (LHRH) also produces a similar phenomenon in rats and mice (Greenstein *et al.,* 1987).

Nutritional status can result in thymic atrophy. Poor diet and malnutrition often result in thymic atrophy and depressed immune responsiveness (Mittal *et al.,* 1988). In part, this response may reflect increased levels of glucocorticosteroids in the sera of malnourished or undernourished animals (Adlard and Smart, 1972). Reduced immune responses can be induced by decreased amino acid uptake, vitamin B,, or zinc deficiencies (Good and Lorenz, 1992).

SERUM ENDPOINTS

Identification and quantification of serum components are often used in screens for immunotoxic events. The albumin/globulin ratio is a common determination used in toxicology studies. Because albumin is the most abundant protein in serum, fluctuations in levels may suggest altered protein synthesis (e.g., liver damage) or increased excretion (e.g., kidney damage). The globulin fraction consists of various antibody isotypes and changes may signal a derangement in synthesis (decreases) or neoplastic conditions (increases).

Immunotoxic chemicals may alter the serum immunoglobulin levels (Vos *et al.,* 1990). There may be increases or decreases in antibody levels. Measurement of antibody levels is only a screening method and gives no information on the mechanism. Assays similar to those described for human antibody quantitation (radial immunodiffusion, nephelometry, and ELISAs) are commonly used in animal studies to detect antibody levels.

The concordance between serum antibody levels and *in vivo* biological effects in the mouse is low. However, these data may be misleading. In the National Toxicology Program (NTP) validation study, 14-day studies were used to screen for low dose immunotoxicity. It is now recognized that high doses are required for antibody formation and, at a minimum, 14 days is needed to mount a response. In a rat model employing high doses administered over a long period of time, measurement of immunoglobulin levels was useful in predicting immunotoxicity induced by chemicals (Anonymous, 1995a).

REGULATORY POSITION

Regulatory agencies in the United States and Europe require the inclusion of a number of endpoints in standard toxicology studies. Weights of immunocompe-

tent organs and histopathology are required. Extensive examinations of the spleen, thymus, lymph nodes (one node covering the route of administration and another distal node covering systemic effects), Peyer's patches, and the bone marrow are required by the EPA, FDA, and OECD. Lymphocyte phenotyping is listed as an optional assay in the harmonized FIFRA/TSCA guidelines.

4

Immunogenicity and Antigenicity

INTRODUCTION

Immunocompetent lymphocytes recognize foreign material and mount an immune response directed specifically toward the foreign material. *Immunogen* is a generic term for foreign material that stimulates one or more immune effector mechanisms. Activation of killer cells or production of antibodies are possible responses to immunogens. Over the years, immunogens have been subdivided on the basis of the nature of the antibody induced and the route of exposure. The term *allergen* is given to specific pollens, foods, and proteins causing immediate allergic reactions such as asthma or food allergy.

IMMUNOGENS

Immunogens share several common attributes. Foreignness is a critical attribute for immunogenicity. The greater the phylogenetic differences between the host and the immunogen, the greater is the response. For example, human albumin injected into a mouse would generate a vigorous response. Injection of mouse albumin into a rat yields only small amounts of antibody.

Immunogens also have a defined tertiary structure that is recognized by the immune system. In most cases, the immune system recognizes discontinuous amino acids in close proximity as a result of folding of the molecule into the native configuration. Tight folding of the immunogen often reflects the presence of aromatic amino acids such as tyrosine and phenylalanine.

Elicitation of an immune response is dependent, in part, on the size of the immunogen. Minimal immune responses can be elicited by immunogens having a size of 3 kDa while the best immunogens have a size of 100 kDa. Large macromolecules are better immunogens because they are insoluble. Insoluble particles are more easily ingested and processed by macrophages.

ANTIGENS AND HAPTENS

Antigenicity denotes the ability of a processed immunogen to interact with effector cells or products of the immune system following a second exposure. Antigens interact with antibodies, induce a cytotoxic response by killer cells, and elicit hypersensitivity reactions. Most immunogens are antigens, but antigens are not necessarily immunogens.

Small chemicals (haptens) of less than 3 kDa cannot stimulate the immune system. Therefore, by definition, haptens are not immunogens. If the hapten binds to a protein (carrier) , the hapten–carrier complex becomes an immunogen that stimulates a response to the bound hapten.

EPITOPES

The molecular fragment recognized by antibodies or effector cells is called an *epitope* or *antigenic determinant.* Generally, these are low-molecular-weight hydrophilic fragments (Fig. 1) processed by phagocytic cells. In any molecule, there may be several different epitopes creating multivalent antigens. However, one epitope will be dominant over the other epitopes. The immune response is usually generated toward the dominant epitope (Table 1). However, as the immune response evolves, the predominant epitope will change by a mechanism known as *epitope spreading.*

There are several types of epitopes. Sperm whale myoglobin has five linear, sequential epitopes containing six to eight hydrophilic amino acids in the bends of the normal a-helix conformation (Fig. 3). Most of these epitopes are recognized in the native, fragmented, and extended conformations of the molecule. More frequently, linear epitopes are not accessible to antibody in the native configuration and react only with denatured forms of the molecule.

Globular proteins have different conformational epitopes created when sequential or nonsequential epitopes are brought together in close proximity by folding (Smerdou *et al.,* 1996). The nonsequential conformational epitopes of hen egg-white lysozyme are well characterized (Fig. 2). The conformational determinant shown consists of a closed loop structure formed by disulfide bonds at amino acids 64 and 80. The immune system recognizes the tertiary loop

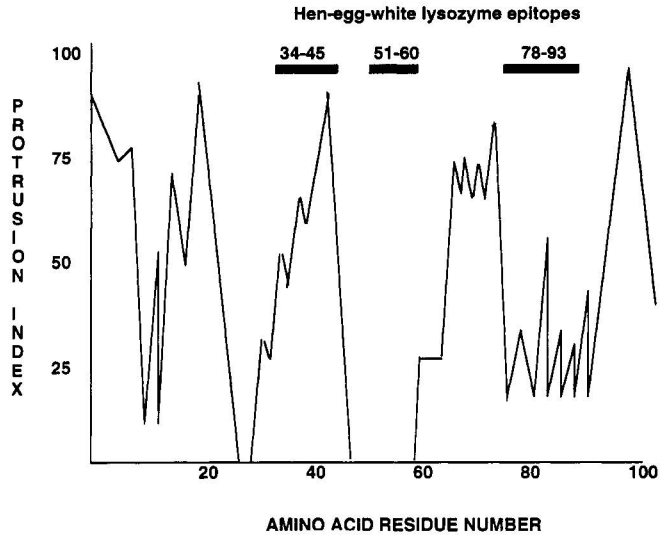

Figure 1. The relationship between epitopes and internal peptides. CD4 Th cells recognize internal peptides exposed during antigen processing by macrophages. The amino acids of hen egg-white lysozyme are plotted against the protrusion in the tertiary protein structure. The T-cell epitopes are composed of amino acids with a minimum of protrusion. Modified from *Modern Trends in Human Leukemia,* Rothbard, 1987, 7:44–66.

structure (Benjamin *et al.,* 1984) . Reduction of the disulfide bonds with mercaptoethanol disrupts the tertiary structure and the antigenicity is lost.

New epitopes can be created by proteolysis, phosphorylation, or other modifications of proteins. The alterations in the secondary or tertiary structure create a neoantigen. For example, amino acids are often added to recombinant proteins that are subsequently refolded in the laboratory. Both manipulations of native proteins can create immunogenic neoantigens.

T- AND B-CELL RECOGNITION OF EPITOPES

T and B cells recognize different epitopes. Linear, sequential epitopes are recognized by T cells. Often these epitopes are internal amino acid sequences processed by macrophages and presented on T-cell major histocompatability complex (MHC) markers. Epitopes of 7–9amino acids can stimulate T cells. Larger epitopes of 11–17amino acids can also bind to the MHC but they have a lower affinity constant. Most T-cell stimulating epitopes are amphipathic in that they contain both hydrophilic and hydrophobic sequences. The hydrophilic por-

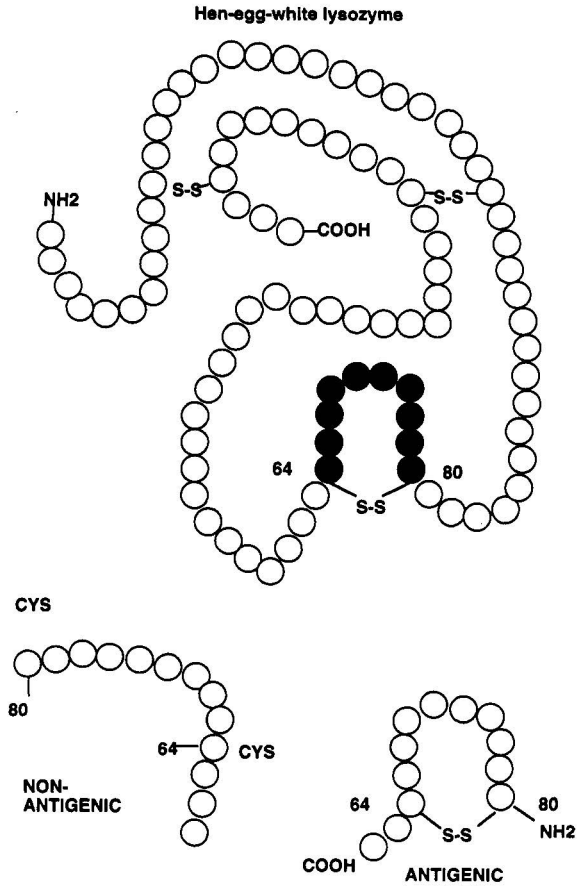

Figure 2. The conformational determinants of hen egg-white lysozyme. Binding of antibody depends on the maintenance of the tertiary epitope structure by intrachain disulfide bonds. The epitope (black circles) is created by the formation of a loop between amino acids 64 and 80. Synthetic and open and closed loop structures were reacted with antiloop antiserum. Serological reactivity was only observed when the loop structure was maintained. Modified from *Annual Review of Immunology,* Benjamin *et al.,* 1984, 2:67.

tion of the T-cell epitope reacts with the T-cell receptor whereas the hydrophobic portion called an *agretope* interacts with class I or II markers on the cell surface.

B-cell epitopes from globular proteins are located in the flexible, hydrophilic regions of an immunogen. Flexibility of the epitope ensures high-affinity binding to antibody and B cells. These epitopes may be linear or conformational. Epitope size is a reflection of the combining sites in an antibody molecule. In the

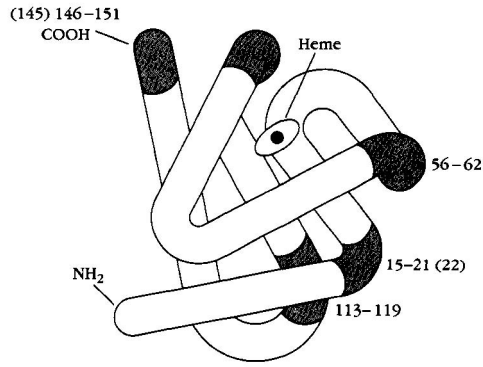

Figure 3. Diagram of sperm whale myoglobin showing B-cell epitopes. Modified from *Advances in Experimental Medicine and Biology,* Atassi *et al.,* 1978, 98:9.

case of low-molecule-weight carbohydrates or proteins, the epitope may be as small as 6–7 amino acids. Globular protein epitopes may range from 15 to 22 amino acids.

EXOGENOUS AND ENDOGENOUS ANTIGENS

Most exogenously synthesized immunogens are derived from bacteria, viruses, and fungi infecting the host. Viruses such as influenza have several different immunogenic molecules. Because the virus leaves the cell by a process of budding, the envelope surrounding the virus often contains glycoproteins and lipoproteins from the host's cell membrane. These immunogenic molecules con-

Table 1. Position of Immunodominant T-cell Epitopes and Amphipathic Segments in Selected Proteins

Antigen	T-cell epitopes	Amphipathic segments	Amphipathic score
Sperm whale myoglobin	69–18	64–78	14.2
	102–118	99–117	20.1
	132–145	128–145	15.3
Chicken lysozyme	102–118	99–117	20.1
	74–86	72–86	8.9
	81–96	96–102	13.1
	109–119	—	—
Chicken ovalbumin	323–339	329–346	18.0
Hepatitis B virus S	120–132	121–135	8.7
Hepatitis B surface antigen	38–52	36–48	7.3

tain repetitive amino acid sequences that are accessible to the immune system. In addition, the virus can produce radiating glycoproteins and proteins (hemagglutinins and neuraminidase). Influenza A virus strains are often typed on the basis of serological reactivity directed toward the hemagglutinin or the neuraminidase (Kuby, 1991).

Gram-positive bacteria have two types of antigens. Some antigens are excreted from the cell and form soluble antigens. Polypeptide exotoxin from *Corynebacterium diphtheriae* blocks protein synthesis and destroys the deep layers of the respiratory mucosa. This results in the formation of a pseudomembrane in the trachea, obstructing the airways (Davis *et al.,* 1973). Cholera toxin increases cyclic AMP in intestinal cells, resulting in chloride loss (Davis *et al.,* 1973). The chloride imbalance causes water loss and diarrhea. The exotoxin from *Clostridium tetani* reacts with the nerve endings at the neuromuscular junction, causing severe and persistent muscle contraction. In the case of toxin-producing bacteria, the immune system attempts to neutralize the toxin and destroy the bacterium.

Other antigens are part of the bacterial cell wall. The cell wall polysaccharide or peptidoglycan is comprised of N-acetyglucosamine muramic acid residues cross-linked by short glycine chains. Interspersed in the peptidoglycan are proteins and teichoic acids. There is great variation in the structure of the cell wall peptidoglycan when different species or strains of the same species are compared. These cell wall constituents are important for diagnostic and taxonomic purposes. Streptococcal strains are grouped on the basis of immunogenic cell wall constituents.

In addition to the peptidoglycan layer, some gram-positive bacteria such as *Streptococcus pneumoniae* have a polysaccharide capsule. The capsules contain repeating units of two or three sugars and may reach 140 kDa. Within the *S. pneumoniae* family, there are over 80 different strains with distinct structures and immunogenicity. However, only 14 strains are pathogenic in humans.

The gram-negative bacterial cell wall differs greatly from the gram-positive wall. There is little peptidoglycan but a large outer membrane containing lipoprotein and lipopolysaccharide (LPS). LPS consists of repeating linear trisaccharides linked to a core polysaccharide and a core lipid A moiety. The basal core and the lipid A constitute the serological specificity of the endotoxin and the associated toxicity, Attached to lipid A are odd-numbered unsaturated fatty acids. The long polysaccharide of the endotoxin exposed to the external milieu is a potent immunogen. These exposed polysaccharides (0 antigens) are often used to classify salmonella and shigella species.

When LPS is released on death of the bacterium, it acts as a potent endotoxin. Endotoxins are potent polyclonal activators of B cells with clear dose-dependent effects. Low doses of endotoxin produce LPS-specific antibodies. High concentrations of LPS stimulate many B-cell clones including autoreactive B

cells that produce antibodies reacting with host tissue. In addition, LPS has wide-ranging effects on the vascular and metabolic systems that can result in death of the host. The response to LPS is unique in that it is species dependent. Humans and guinea pigs produce antibodies to LPS but rabbits do not.

Lipids extracted from microorganisms are also immunogenic. Cardiolipin, which consists of glycerol molecules esterified with two phosphates and four molecules of unsaturated fatty acids, was extracted from the spirochete causing syphilis. Antibodies reactive with the cardiolipin form the basis of the diagnostic test for syphilis.

There are a number of endogenously synthesized antigens. Some bacteria such as mycobacteria and leishmania are intracellular parasites and live inside the endosomes of macrophages. Similarly, viruses live in the cytoplasm of the cell and interact freely with cellular components and enzymes. Viruses also use the host cell protein synthesizing capabilities to make viral proteins. Immunogenic molecules generated within the infected cells are transported to the cell surface where they interact with immune effector cells. In some diseases, immunogenic substances are liberated from the cell. For example, in systemic lupus erythematosus, highly immunogenic DNA is liberated from host cells.

There are a number of alloantigens present in the body. These are antigens that are not normally immunogenic in the host. Yet, they will elicit an immune response when administered to other members of the same species. The best studied alloantigens are the red blood cell antigens in human peripheral blood. The A, B, and O red blood cell antigens are water-soluble glycopeptides consisting of heterosaccharides attached by a glycosidic linkage at reducing ends. The molecular masses are between 200 and 1000 kDa. O blood type persons have a heterosaccharide with a fucose residue that is common to all blood types. Glycosyltransferases act on the core polysaccharide to add additional sugars. Type A antigen is created when N-acetylglucosamine is added to the terminal fucose. Addition of galactose to the terminal fucose produces a type B red cell antigen. When both galactose and N-acetylglucosamine are added to the molecule, blood type AB is created (Table 2).

Because the red blood cell antigens are alloantigens, it is possible to divide populations into universal donors or recipients. O blood type individuals are often referred to as *universal donors.* This status is possible because O blood type carries a core molecule common to all ABO antigens in all members of the species. Thus, the O antigen is not foreign to any member of the species and is not antigenic. Conversely, persons with type AB blood are considered universal recipients because they express both A and B antigens. When given either A or B blood, they do not mount an immune response because neither A nor B antigen is foreign.

The blood type antigens are not restricted to red cells. A, B, and O blood type molecules are found in a wide variety of unrelated plants and animal tissues.

Table 2. The Constituents of Human Red Blood Cell Antigens

Population	Percentage	Core polymer	Additional sugar
Type O	44	Sphingolipid	
Type A	42	Sphingolipid	Acetylglucosamine
Type B	10	Sphingolipid	Galactose
Type AB	4	Sphingolipid	Galactose and N-acetylglucosamine

Thus, the markers are heterologous in nature. Stimulation of the immune system by heterogenic antigens produces anti-A or anti-B antibodies in serum. These antibodies are termed *natural antibodies.* In the strictest terms, red blood cell markers are antigenic but not necessarily directly immunogenic.

White blood cells also express alloantigens. These antigens are glycoproteins of between 35 and 50 kDa and coded by the MHC. Because these alloantigens are present on most nucleated cells in the body, the compatibility of donor and recipient MHC antigens determines the acceptance or rejection of organ grafts between members of the same species (e.g., allografts). In humans, the *A, B, C,* and *Dr* are major loci within the MHC. Each locus is highly polymorphic with multiple suballeles. In the mouse, histocompatibility is determined by *H* genes. Like the human loci, there are major loci with multiple alleles.

HAZARD IDENTIFICATION

Immunogenicity is a critical issue in the development of peptide vaccines and recombinant growth factors and immunomodulators. Designed peptide vaccines use epitopes that cause the greatest stimulation of the immune system. Optimally designed therapeutic, recombinant or fusion proteins do not stimulate the immune system.

Attempts have been made to predict the position of antigenic sites within proteins from features related to their primary structure. These computer models measure parameters such as hydrophilicity, hydropathy, surface probability, side chain flexibility, secondary structure, and N-glycosylation sites (Carmenes *et al.,* 1989). The program PREDITOP contains 22 normalized scales that generate a file whose values represent a particular physiochemical aspect of the protein (Pellequer and Westhof, 1993). Another BASIC microcomputer program (EPIPLOT) was developed for predicting both T- and B-cell antigenic sites in proteins from their primary structure. This program uses only 13 scales known to give the best predictions of characterized, protein antigenic sites. T-cell epitope prediction is based on algorithms for amphiphilic features and sequence patterns (Menendez-Arias and Rodriquez, 1990).

Less refined methods have been developed using the assumption that hydrophilic polypeptide regions contain antigenic sites. Conversely, hydrophobic sites would be buried and inaccessible (Krystek *et al.,* 1985). In a study of 29 epitopes on four model proteins, statistical analyses showed that a segmental mobility scale and a hydrophilicity scale based on chromatographic peptide retention times gave the highest correct predictions (Van Regernmortel and Daney de Marcillac, 1988).

It is also possible to determine whether similar linear epitopes occur in other proteins. Protein data bases have been growing at a rapid rate. Currently, the Swiss-Prot data base contains 1.5×10^7 residues and the NBRF contains 1.1×10^7 residues. Others such as the National Center for Biotechnology Information, the Protein Identification Resource, and the Brookhaven Protein data bases contain smaller numbers of residues. The Brookhaven data base also has three-dimensional structures for a number of proteins. Using these data bases, it is easy to find five, six, or seven amino acid matches between unrelated proteins.

The rate-limiting step in processing of antigen and presentation to the immune system appears to be unfolding of proteins at the cleavage site. Immunodominant epitopes are associated with structurally unstable protein segments associated with highly flexible polypeptide loops (Landry, 1997). These epitopes can be measured by NMR relaxation parameters.

The instability allows structural "breathing" of the protein. In the relaxed or unstable form, the protein undergoes proteolytic cleavage and binding of the proximal C-terminus to the MHC class II marker. Additional modification or trimming of the peptide occurs in the binding cleft (Landry, 1997).

The characteristic of antigenic peptides used for the generation of vaccines has been discerned from Monte Carlo computer experiments. Peptides should have the propensity to form a-helices that do not develop coil conformations. In addition, they should have a lysine at the C-terminus (Spouge *et al.,* 1987). Moreover, the T-cell epitopes should not be segmentally amphipathic (e.g., two disjointed subpeptides one of which is hydrophobic while the other is hydrophilic).

The importance of flanking regions in the generation of antigenic epitopes is controversial. In the generation of CD8 cytotoxic T cells both *in vivo* and *in vitro,* recognition is influenced by the C- and N-terminal flanking residues. Amino acids with aromatic (tyrosine), basic (lysine), or aliphatic side chains (alanine) enhance CTL recognition. Acidic and helix-breaking amino acids (glycine and proline) inhibit recognition of the N-terminus epitope (Bergmann *et al.,* 1996). In contrast, the generation of CD4 T helper cells to HIV gp120 in recombinant proteins was dependent on the insertion position in the protein. Moreover, antigenicity was influenced by the protein region flanking the HIV peptide (Manca *et al.,* 1996).

Differences in antigenic potency are related to the size of the epitope. Linear

sequences of seven amino acids delineated T-cell epitopes. Increasing the peptide length often results in increased antigenic potency. Increased potency may be related to the ability of longer peptides to adopt the appropriate secondary structure following binding to the MHC molecule.

Although it is possible to predict antigenic fragments within molecules, it is difficult to predict immunogenicity of proteins in humans. Factors such as the presence of inflammation, local protease production, presence of molecular chaperones, and N-glycosylation influence the immunogenicity of a protein (Landry, 1997).

N-glycosylation is often critical to the expression of antigenicity. Proteins are posttranslationally modified shortly after synthesis at the ribosome and further modified when the protein is translocated through intracellular membranes (Hounsell, 1994). The addition of the oligosaccharides has a large influence on the antigenicity of the molecule. For example, patients receiving recombinant human granulocyte–macrophage colony-stimulating factor (rhGM-CSF) develop antibodies to the recombinant protein. The antibodies interact with native protein at sites normally protected by 0-linked glycosylation but are exposed in rhGM-CSF produced in yeast and *E. coli* (Gribben *et al.,* 1990).

It is likely that products of biotechnology designed for human usage will be immunogenic in most animal species. Moreover, some of the animals will form neutralizing antibodies, causing a loss of efficacy (Galbraith, 1987). Humans often develop antibodies against humanized proteins. But the therapeutic response usually persists in the presence of the antibody. This suggests that human antibodies are necessarily neutralizing.

Proper species selection may negate the production of antibodies. Animals closely related to humans may not respond to the proteins, yet they have pharmacological and metabolic activity similar to ours. Therefore, the primate is the animal of choice for testing human recombinant proteins. Testing in primates is, however, expensive and laborous.

When *in vivo* or *in vitro* models or correlates are not available, transgenic models can be used. One system is the SCID-hu model where human stem cells are placed in the mouse to create a functional human immune system (Namikawa *et al.,* 1990). Normal human ratios of CD4/CD8 are found in the blood and lymphocytes respond to mitogens, alloantigens, and anti-CD3 (Kaneshima *et al.,* 1990).

The immunogenicity of low-molecular-weight compounds requires a different approach to hazard identification. Initially, structure–activity relationships are determined with classes or families of chemicals known to be immunogenic. For example, chemicals with a structure similar to toluene diisocyanates would be flagged as a possible immunogen. Second, the chemical would be reacted with albumin in the test tube to determine whether hapten–protein conjugates are formed. Finally, the complexes would be injected into animals to determine whether hapten-specific antibodies are formed.

REGULATORY POSITION

The FDA considers immunogenicity an important issue related to biotechnology products. The agency is particularly interested in the effects of antibody formation on the pharmacokinetic/pharmacodynamic properties of the protein. Based on these data, the immune response would be classified as neutralizing or nonneutralizing. In addition, the incidence or seventy of disease, new toxic effects, or other adverse effects should be considered in the data analyses prior to submission to the agency.

In some cases, the production of antibodies may not be biologically relevant. When there is no dose–response relationship and the antibodies are not neutralizing, there may be no safety related issues. It is, however, necessary to exclude the possibility of immune complex vasculitis as a potential health-related effect. Specialized histopathological techniques can be used to identify immune complexes in the kidney or vasculature.

5

Antigen-Presenting Cells

INTRODUCTION

Antigen-presenting cells (APCs) can be divided into "professional" and "amateur" subsets. Professional APCs such as monocytes, macrophages, dendritic cells, and B cells are fully committed to antigen presentation as an integral part of their function in the afferent arm of the immune response. Other cells such as endothelial cells, fibroblasts, glial cells, pancreatic beta cells, keratinocytes, and thyroid cells present antigen only under select conditions during an immune response. These cells are considered "amateur" APCs.

APCs arise in the bone marrow, circulate in the blood, and home to different areas in the body via the lymphatic system (Katz *et al.,* 1979). Stem cells in the peripheral blood occur at a frequency. of 1 per 10^5 mononuclear cells and produce both monocytes and dendritic cells. Additional stem cell stimulation induces maturation into Langerhans cells, interdigitating cells, and follicular dendritic cells (Knight *et al.,* 1992b).

PROFESSIONAL APCs

Professional APCs share common attributes. All have the characteristic dendritic cell morphology. High levels of MHC markers, complement, Fc receptors, and adhesion factors are expressed on the cell surface. The professional APCs can present processed or native antigen to immunocompetent cells while producing interleukins such as IL-1 and IL-6 (Wacker *et al.,* 1990).

Monocytes and Macrophages

The principal professional APCs are monocytes and macrophages. Monocytes are blood-borne cells that express class I, II, and CD1 markers on the cell surface. They present processed antigen to both T and B cells. Macrophages are localized in tissue and have a different function. Marginal zone macrophages present in lymphoid tissue bind polysaccharide immunogens and present processed antigen solely to B cells.

There are two subsets of monocytes in human peripheral blood (Ziegler-Heitbrock, 1996). Over 90% of circulating monocytes are CD14+,CD16–. The remaining cells are CD14+,CD16+. Both CD14 and CD16 are biologically important receptors. CD14 is the major receptor for bacterial LPS whereas CD16 is the Fcy receptor type III (FcyRIII). Although the pathophysiological roles of the subsets are unclear, increased numbers of CD 14+,CD 16+ monocytes are found in bacterial sepsis, AIDS, tuberculosis, and solid tumors (Ziegler-Heitbrock, 1996). Because the CD 14, CD 16 cells also express high levels of MHC class 11 markers and adhesion molecules (ICAM-1), they may be potent APCs. The adhesion factors may allow rapid transit and migration into the tissue to fight disease.

Because CD14, CD16 monocytes produce TNF, IL-1, and IL-6, they act as proinflammatory monocytes. The secretion of IL-1 serves a useful purpose. IL-1 acts directly or indirectly on the temperature-regulating centers in the brain, inducing a fever by raising body temperature. Fever is a unique protective mechanism that restricts the growth of bacteria (bacteria have a narrow temperature growth range) until the immune system mounts a response.

Dendritic Cells

There are several major populations of dendritic cells. Interstitial dendritic cells are found in all major organs of the body. Interdigitating dendritic cells (IDCs) are found in areas of the lymph nodes and spleen that are rich in T cells. In fact, multicellular aggregates of T cells and dendritic cells enhance cellular interactions. These APCs express class I, 11, and CD1 molecules and present protein antigens to CD4 T cells. The IDCs are particularly important in response to foreign allografts (Shamoto *et al.,* 1993).

Follicular Dendritic Cells. Follicular dendritic cells (FDCs) are numerous in the lymph node germinal centers. They are characterized by the expression of DRC-1, Ki-M4, HLA-DR, CR1, and Clq markers on the cell surface. In addition, FDCs have receptors for the Fc portion of immunoglobulins allowing passive attachment of immune complexes (Parmentier *et al.,* 1991).

Dendrites of the FDCs contain a series of "beaded" antigen–antibody complexes called *iccosomes* (Szakal *et al.,* 1988a). In the germinal center, the icco-

somes form a complex labyrinthine structure much like layers of cheesecloth (Terashima *et al.,* 1992). Native antigen is stored in iccosomes for extended periods of time, providing a continuing source of stimulation for B cells (Gray *et al.,* 1991).

On secondary stimulation, B-cell blasts become centroblasts in the dark zone of the germinal center. The rapidly dividing centroblasts move through the FDC labyrinth (Ziegler-Heitbrock, 1996). Using the CD72 ecto 5'-nucleotidase enzyme, centroblasts attach to the FDCs, internalize the iccosomes, and process antigen for presentation to T cells or B memory cells (Airas and Jalkanen, 1996). The MHC class II molecules found on the surface of FDCs and used in the presentation of antigen to T cells are not synthesized by the FDCs but are picked up from surrounding B cells in germinal centers. Although FDCs by themselves cannot present native antigen to T cells, acquired MHC class 11–peptidecomplexes can be recognized by T cells (Gray *et al.,* 1991). Activation of T cells is mediated by HLA-I and -11 markers, ICAM-1, LFA-3, and CD44 (Kroncke *et al.,* 1996).

FDCs induce the production of a number of cytokines that activate immunocompetent cells. Interactions with other cells induce the release of IL-2, IL-3, and IL-4 (Ellis *et al.,* 1991). Human tonsillar follicular cells produce IL-7 that induces B-cell proliferation. IL-7 also influences the generation of T cells and NK cells. In higher concentrations, IL-7 also activates monocytes in a manner similar to bacterial endotoxin (Kroncke *et al.,* 1996).

Germinal centers formed by the proliferating B cells in the FDC labyrinth have a life span of 3 weeks (Kosco-Vilbois *et al.,* 1997). A reduction in antigen concentration decreases the iccosome formation (Szakal *et al.,* 1988a,b). As the iccosomes shrink, antigen in the FDC network becomes inaccessible to lymphocytes. However, some antigen may persist in isolated iccosome pockets for as long as 18 months (Kosco-Vilbois and Scheidegger, 1995).

IDCs. IDCs are found in T cell zones in lymph nodes, spleen, and thymus. Although the IDCs and Langerhans cells (LCs) are similar in morphology, they can be differentiated on the basis of surface staining. LCs express both OKT-6 and S-100 proteins whereas IDCs are S-100 protein positive but OKT-6 negative. The IDCs have the ability to process antigen and initiate T-cell proliferation in conjunction with IL-1 and IL-6. The IDCs in the thymus have been implicated in the induction of tolerance (Hsiao *et al.,* 1991).

Nonlymphoid Dendritic Cells. There are populations of nonlymphoid dendritic cells. LCs are bone marrow-derived dendritic cells that reside in the suprabasilar epidermis (Bieber, 1986; Katz *et al.,* 1979). They are identified by the presence of cytoplasmic Birbeck granules and long dendritic processes. LCs

express CD1a, CD1b, Cd1c, and class II MHC or HLA molecules and expression can be upregulated by interferon (Furue *et al.,* 1992). They are capable of phagocytosis and antigen presentation to T cells. B7–1is not normally expressed on LCs. B7–1expression can be up- or downregulated by a number of cytokines. IL-1α or -ß or IL-4 can upregulate expression of B7–1 but the most potent upregulation is mediated by GM-CSF. Conversely, IFN-γ or IL- 10 profoundly inhibit the B7–1 expression of LCs in a dose- and time-dependent manner. The down regulatory ability of IFN-γ or IL-10 neutralizes the activity of most up-regulatory cytokines (Chang *et al.,* 1995).

Chemicals or agents that cause delayed hypersensitivity reactions are handled differently than other agents activating Th2 cells. IL-6-producing LCs with surface antigen migrate from the skin to draining lymph nodes (Marchal *et al.,* 1995; Cumberbatch *et al.,* 1995). Often these cells have a distinct veiled morphology as they move toward the nodes (Wacker *et al.,* 1990). In the subcapsular space, these cells attach to the sinus floor, migrate through the interfollicular region to the paracortex, and attach to the reticular network. Antigen is presented to CD4 cells that initiate delayed hypersensitivity response (Katz, 1986; Silberberg *et al.,* 1976). LCs have the capacity to induce allogeneic T-cell activation and may play a role in skin graft rejection (Stingl *et al.,* 1980).

B Cells

B cells are able to present immunogens to T cells. In unique manner, immunogenic peptides bind with high affinity to antigen-specific antibodies on the cell surface. Cross-linking of antibodies internalizes the peptide. Peptides are degraded in the cytoplasm and presented to T cells in context with class II markers. Because the reaction is antigen specific, B cells present antigen at 100- to 10,000-fold lower levels than required for macrophage presentation. As a result of the interactions between T and B cells, B cells are stimulated to produce antibodies.

AMATEUR APCs

When activated, epithelial cells, keratinocytes, and other cells can act as amateur APCs. These cells all have inducible class II makers needed for antigen presentation. However, most lack the B7–1 and B7–2 molecules necessary for costimulation of T cells. Yet, they are able to induce immune responses by a unique costimulatory mechanism. Epithelial cells express a BB–1/B7–3 molecule, a unique isoform of the B7 family, and additional CD28/CTLA-4 counter-

receptors that act as costimulatory molecules for T cells. Usually, the epithelial cells induce Th2 responses (Nickoloff and Turka, 1994).

There is an altered cytokine profile in activated T cells following interactions with nonprofessional APCs. Activated Th2 cells produce IL-2 and IL-4 but fail to produce IFN-γ suggesting that IgE allergic antibody is the isotype produced in the immune response.

Because IFN-γ is not produced by interaction of nonprofessional APCs, the synthesis of IgE cannot be regulated (Leung, 1993). Most amateur APCs cannot synthesize IFN-γ because of a defect in a cytokine switching pathway. IL-12 production is the key switch for inducing the synthesis of IFN-γ IL- 12 consists of two protein chains with molecular masses of 35 and 40 kDa. LCs express constitutive levels of the 35-kDa protein but cannot produce the 40-kDa chain. Thus, the IL-12 is functionally defective and cannot induce the production of IFN-γ. In addition to the difference in IL-12 production, some amateur APCs may also produce IL-10 that blocks IFN-γ production by T cells (Dezutter-Dambuyant, 1994).

The absence or presence of IFN-γ determines the T-cell subset that undergoes clonal expansion. In the presence of IFN-γ Th1 cells expand and differentiate. Th2 cells are expanded in the absence of IFN-γ.

ULTRAVIOLET LIGHT AND LCs

External factors can also alter the immunological response in the skin. Ultraviolet light inactivates or destroys LCs in the skin (Alcalay *et al.,* 1989). In the absence of LCs, keratinocytes produce IL- 10 that prevents IFN-γ production. Consequently, the immune response shifts to activation of Th2 cells. This T-cell subset plays a role in the induction of IgE-mediated allergic skin reactions (e.g., atopic dermatitis) and the induction of antibody-mediated autoimmune psoriasis (Nickoloff and Turka, 1994).

IMMUNE DYSFUNCTION Of DENDRITIC CELLS

A number of dendritic cell disorders are simply characterized as abnormalities of proliferating "histiocytic cells." Disorders are either benign or malignant. Some processes such as severe histiocytosis have increased numbers of cells that can be classified as either a monocytic or dendritic disorder. Juvenile xanthogranuloma seems to be a benign proliferative disorder of macrophages. Self-healing reticulohistiocytosis is a benign disorder of LCs (Morschella and Cropley, 1990)

VIRAL INFECTIONS AND DENDRITIC CELLS

Attention is being focused on the role of dendritic cells as reservoirs for infectious HIV particles (Gerdes and Had, 1992). Normal dendritic cells are susceptible to *in vitro* infection with HIV. As the infection progresses, dendritic cells are destroyed, reducing the ability to initiate an immune response. In the human experience, HIV seropositive patients have significantly reduced numbers of dendritic cells, but patients with persistent generalized lymphadenopathy show normal numbers. Therefore, defects in dendritic cells precede the appearance of symptoms and changes in T-cell numbers (Macatonia *et al.,* 1990).

In animal studies, dendritic cells are also important in resistance to experimental HSV-1. LC presentation of virus is critical for the proliferation of T cells *in vitro* (Yasumoto *et al.,* 1986). *In vivo* studies also demonstrated that T-cell proliferation and normal LC function are related to increased resistance to HSV-1 (Sprecher and Becker, 1986).

DRUGS, CHEMICALS, AND APCs

A single systemic dose of cyclophosphamide (CY) has been shown to enhance cellular immunity in a variety of antigen models. The immunoenhancing effects of CY have been attributed to its ability to selectively abrogate suppressor cell function. However, recent data demonstrated that dendritic cells from sensitized, CY-treated mice showed longer dendritic protrusions and an enhanced accessory cell function compared with dendritic: cells from saline-treated controls. Thus, immunoenhancing effects of cytostatic drugs may occur via an effect on the dendritic cell population (Limpens er *al.,* 1991).

Anti-HIV treatment regimens also target the dendritic cell. Zidovudine (AZT) reverses a dendritic cell defect in HIV infection (Knight *et al.,* 1992a). The numbers of dendritic cells in peripheral blood of AIDS patients and the level of infection with HIV-1 were determined before and after AZT treatment. A marked rise of dendritic cells in patients given AZT was observed. In two patients, there was a significant provirus load in the repopulated dendritic cells (Patterson *et al.,* 1996).

6

Antigen Presentation

INTRODUCTION

The immune system must defend the host against diverse attacks from microorganisms, viruses and tumor cells. Some bacteria are exogenous pathogens that circulate in the blood or create foci of infections in tissue. Other bacteria and all viruses are intracellular parasites that live within the host cells. Tumor cells present a special problem for the immune system. Neoplastic cells are cells in an arrested state of differentiation with a loss of cell division control. Intracellular bacteria, viruses, and tumors create endogenous antigens.

The immune system has developed different strategies to deal with exogenous and endogenous peptide immunogens. The method of presentation is dependent on both the matrix and the nature of the antigen. For example, exogenous antigens that circulate in the blood, lymph, or external fluids are usually removed by an antibody response. An antibody response requires antigen presentation in association with MHC class II molecules and activation of both Th2 and B cells. In contrast, viral or tumor antigens develop inside cells and elicit a cytotoxic T-cell response that destroys the infected or mutant cells. Effector cytotoxic T cells are generated when antigen is presented in concert with MHC class I or CD1 family of proteins. The CD1 isoforms are a newly described family of antigen-presenting molecules that are distant cousins to the MHC markers. Isoforms of CD1 play a unique role in presenting highly conserved lipids and proteins.

Immunogens are bound to the surface of APCs by both active and passive processes. In the initial stage of the immune response, bacteria, fungi, viruses, or proteins are internalized by phagocytosis, pinocytosis, or mediated endocytosis. Phagocytosis is used to ingest large particles coated with antibody or comple-

ment fragments: membrane pseudopodia surround the large particle to create a phagosome that is internalized. Pinocytosis is characterized by nonspecific membrane invagination of particles and ingestion is proportional to the free concentration in the external milieu. Macrophages and monocytes can ingest particles by phagocytosis or pinocytosis. Most other APCs ingest antigens solely by pinocytosis. B cells utilize receptor-mediated endocytosis to internalize particles. Cross-linking of cell-bound antibodies initiates an energy-dependent process that internalizes both antibody and the antigen.

CLASS I MHC PROTEINS

Class I MHC proteins are found on all nucleated cells within the body. The structure of MHC class I molecules is characteristic of transmembrane polypeptides. All class I MHC molecules consist of a 44- to 47-kDa α chain. Chains are oriented so that the N-terminus and most of the molecule extend into the external milieu (Fig. 1). A non-MHC-coded ß2-microglobulin of approximately 12 kDa is attached to the α chain (Jones, 1997).

The major function of the class I MHC is to present immunogenic peptide fragments to CD8 cytotoxic T cells (Stern and Wiley, 1994). The peptide-binding region of the class I MHC molecule is located in the α1 and α2 regions and consists of approximately 180 amino acids. A peptide-binding cleft region is formed by two equal 88-amino-acid segments in the α1 and α2 regions. The tertiary structure is an eight-stranded, pleated sheet supporting two helix side regions. Cleft size is approximately 25 X 10 X 11 Å and binds peptides with 8–11 amino acids.

Between the α2 region and the transmembrane region is the a3 immunoglobulin-like region. This highly conserved domain has approximately 90 amino acids with a loop region linked by disulfide bonds (Wilson and Fremont, 1993). The a3 region plays an important role in the binding of the CD8 molecule necessary for the activation of T cells (Jones, 1997).

Extending from the a3 region is a short transmembrane domain of 25 hydrophobic amino acids that ends at the inner leaflet of the membrane. The cytoplasmic domain consists of 30 amino acids and contains phosphorylation sites for protein kinase A, *src* tyrosine kinase, and transpeptidation of glutaminase. Thus, the cytoplasmic region may regulate interactions with cytoskeletal elements and intracellular trafficking of MHC class I markers (Jones, 1997).

ß2-Microglobulin attaches the ß-sheet platform of the α1 and α2 regions. Structurally, it resembles the a3 immunoglobulin-like region in that it has a loop structure linked with disulfide bonds. ß2-Microglobulin stabilizes the interaction between the α chain and the immunogenic peptide.

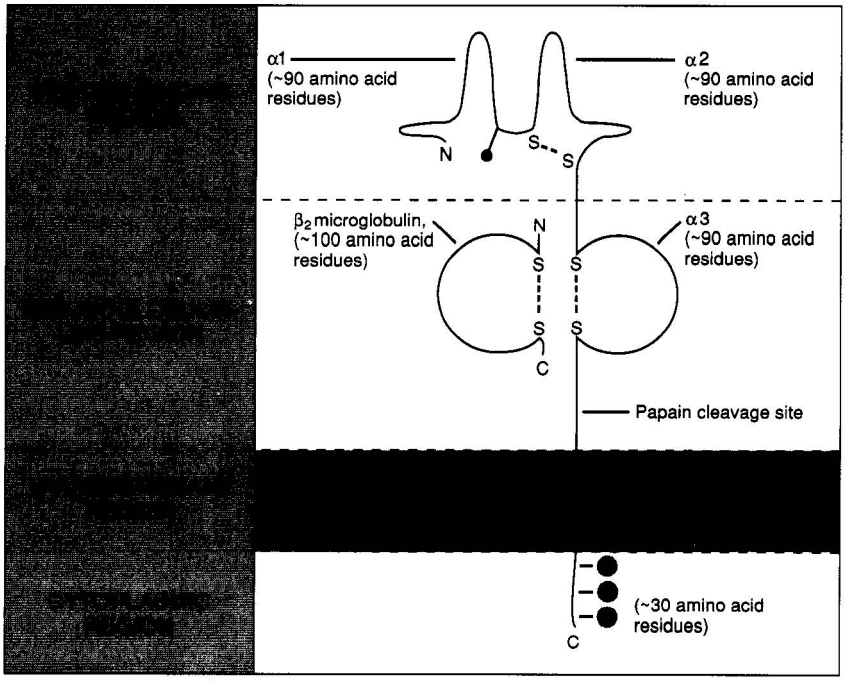

Figure 1. Schematic representation of the MHC class I protein. N and C refer to amino and carboxy termini of the polypeptide chain, S–S to intrachain polypeptide bonds, and P to phosphorylation sites. Reprinted from *Cellular and Molecular Immunology,* Abbas et al., 1994, with permission of W. B. Saunders Co.

CLASS II MHC PROTEINS

MHC class II proteins are found constitutively on B cells and dendritic cells and can be upregulated on macrophages and Langerhans cells. Each molecule is composed of two noncovalently bound α and ß chains (Fig. 2). Although both chains have the same structure, the α chain is slightly heavier (32–34 kDa) because of extensive glycosylation. Like the class I protein, the class II protein consists of a peptide-binding region, an immunoglobulin-like domain, and transmembrane and cytoplasmic regions.

In contrast to the class I marker, the peptide-binding portion is composed of the αl and ß1 region of each chain. These regions interact to form an eight-stranded ß-pleated sheet with two supporting α-helices. The ends of the peptide region are open allowing bound peptides to extend into the external milieu.

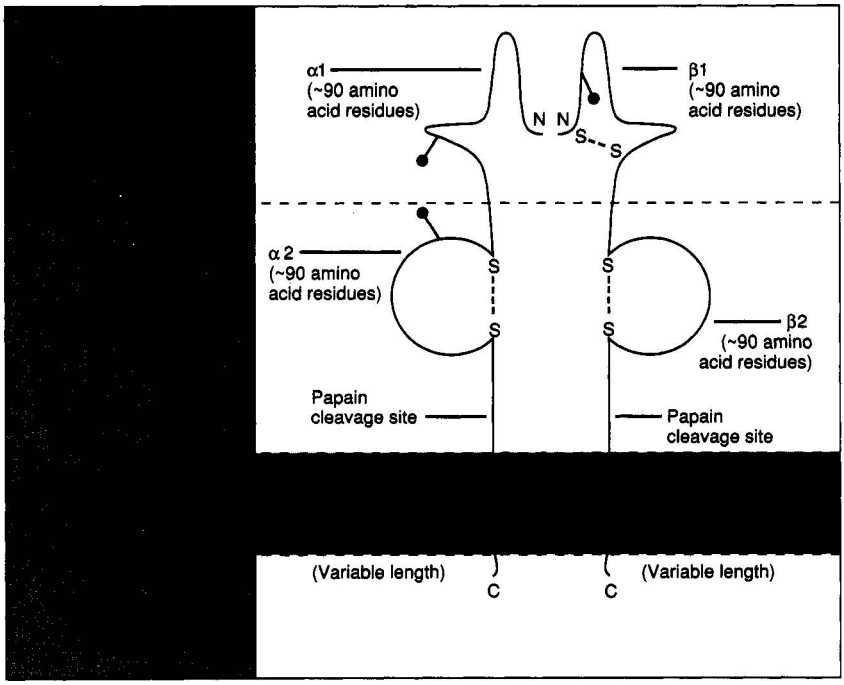

Figure 2. Schematic representation of the MHC class II protein. N and C refer to amino and carboxy termini of the polypeptide chain, S–S to intrachain polypeptide bonds, and P to phosphorylation sites. Reprinted from *Cellular and Molecular Immunology,* Abbas et al., 1994, with permission of W. B. Saunders Co.

Although the optimal binding length is 10–14 amino acids, peptides as long as 30 amino acids have been recovered from purified class II molecules.

Distal to the peptide-binding regions are the α2 and ß2 immunoglobulin-like regions. Both domains have a loop structure linked by disulfide bonds. Because of homologies in amino acid sequence, α2 and ß2 domains belong to the Ig superfamily. These domains are important in establishing noncovalent interactions between chains. Like other transmembrane glycoproteins, there are short transmembrane regions of 25 hydrophobic amino acids that span the cytoplasmic membrane. Attached to the cluster of basic amino acids at the terminus of the transmembrane region are short hydrophilic cytoplasmic tails of variable length (Schafer *et al.,* 1995).

PRESENTATION OF PEPTIDES BY CLASS I MHC MOLECULES

In the classical cytosolic pathway for endogenous antigen presentation by class I MHC proteins, the immunogenic particle is ubiquitinated and digested by proteasomes in the cytoplasm (Groettrup *et al.,* 1996). Ubiquitin, a highly conserved 87-kDa protein, binds to protein via a reaction between the €-amino group on the protein and lysine 21–23residues on the ubiquitin molecule (Mayer and Doherty, 1992). Ubiquitination occurs when additional molecules are added by reactions between lysine 48 and the carboxyl of the next ubiquitin molecule (Fig. 3). The presence of four ubiquitins targets the protein for fragmentation by the proteasomes (Schwartz and Ciechanover, 1992; Hershko, 1991a,b).

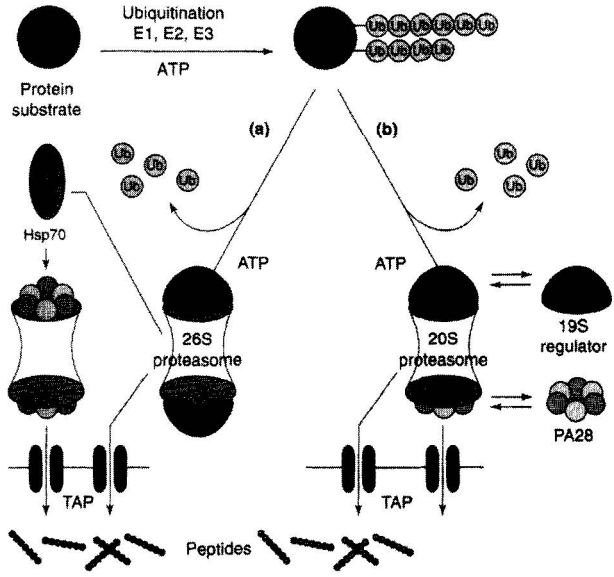

Figure 3. Fragmentation of proteins by the 20s proteasome. Two models of targeting intact proteins by the 20S proteasome. (a) Proteins are covalently linked to polyubiquitin and are deubiquitinated and unfolded by the 26S proteasome. Polypeptides are retrieved from the 26S proteasome by chaperons (e.g., heat shock protein 70) for further processing by the 20S proteasome–P28 complex. (b) Polyubiquitinated proteins are targeted for the 20S proteasome by the 19S regulator. The 20S proteasome is in dynamic equilibrium with PA28 and the 19S regulator allowing simultaneous or subsequent binding to both regulators while the polypeptides are in the lumen of the proteasome. From *Immunology Today,* Groettrup *et al.,* 1996, 17:429–35, with permission of Elsevier Science.

Proteins are fragmented by different proteasomes. A 26S proteasome deubiquitinates and unfolds the intact proteins (Fig. 3). Polypeptides then react with heat shock proteins (e.g., Hsp 70) for transport to a 20S proteasome complexed with PA28 (Mayer and Doherty, 1996; Etlinger *et al.,* 1993). The 20S proteasome consists of two α and ß subunits each with seven subsets.

When confronted with overwhelming viral antigens or high levels of interferon, three of the ß proteasome subsets (δ, MB1, and MC4) are replaced with LMP-2, LMP-7, and MECL-1 (Fig. 4). These substituted molecules are active proteases that accelerate the cleavage of proteins at the C-terminus of arginine. To prepare ligands for reactivity with the class I molecules, the 20S proteasomes cleave polypeptides on the C-terminal ends of aromatic basic and glutamic acid residues. As a consequence of cleavage, peptide fragments containing 5–15 amino acids are generated (Groettrup *et al.,* 1996).

To interact with MHC class I proteins, immunogenic fragments must be transported across the endoplasmic reticulum (ER) to the secretory compartment. Molecular chaperones such as heat shock proteins Hsp 70, gp96, or transporter associated with antigen processing (TAP) proteins are used to transport immunogens across the membranes (Fig. 5). The TAP proteins belong to a family of ATP-binding cassette proteins commonly associated with the ATP-dependent transport of proteins, sugars, and other molecules across membranes (Higgins, 1992).

The nature of the C-terminal amino acids determines whether digested peptides bind to TAP. All human peptides will bind to TAP excepting proteins with C-terminal proline and glycine. Mice bind only proteins with hydrophobic carboxyamino acids. Rats express two TAP alleles that differ in their ability to transport charged C-terminal amino acids (Howard, 1995). These alleles correspond to human and mouse which also differ in permissiveness.

In TAP transport to the lumen of the ER, the protein's hydrophobic N-terminus is trimmed so that the overall length is between 8 and 10 amino acids. This length matches the size preferred by the MHC I antigen-binding site. In effect,

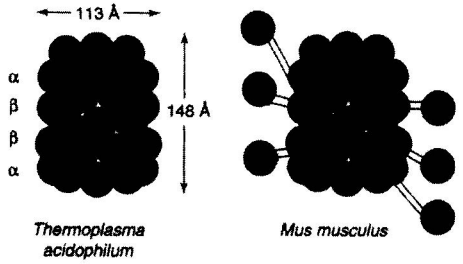

Figure 4. Model of the 20S proteasome of Thermoplasma acidophilum in the mouse. The Ta proteasome consists of 14 copies of two different subunits, namely, α and p. The mouse 20S proteasome consists of seven different α subunits and seven different ß subunits. The three ß-type subunits δ, MB1, and MC14 are replaced by LMP-2, LMP-7, and MECL-1 in IFN-γ-stimulated cells. Reprinted from *Immunology Today,* Groettrup *et al.,* 1996, 17:429–35, with permission of Elsevier Science.

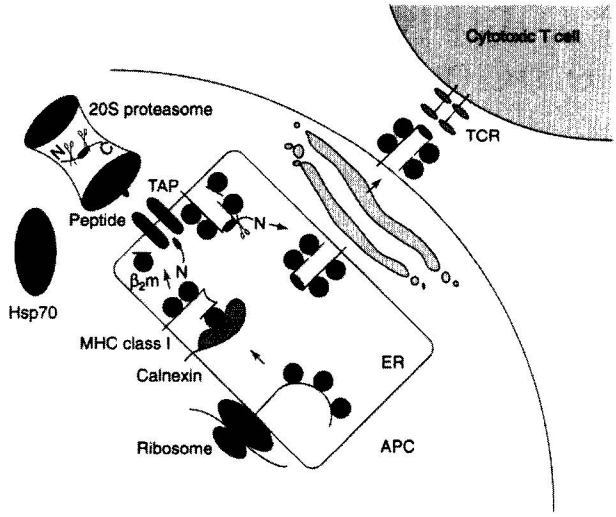

Figure 5. A model of MHC class I assembly and antigen presentation. Cytostolic peptides are degraded to peptides by 20s proteasomes. Peptides are transferred to the heterodimeric TAP transporter with the possible involvement of chaperons such as Hsp 70. MHC class I heavy chains are cotranslationally inserted into the ER where they fold and remain associated with calnexin until they form a stable complex with ß$_2$-microglobulin (ß$_2$m) and peptides imported by TAP. The C-terminus of MHC class I ligand is generated by cleavage in the proteasome while peptides longer than nine amino acids can be trimmed by resident aminopeptidases. The trimolecular complex traverses to the surface where it can be recognized by the T-cell receptor. From *Immunology Today,* Groettrup et al., 1996, 17:429–35, with permission of Elsevier Science.

TAP provides the peptide fragments for MHC I molecules. Longer peptides can be transported through the ER. Peptides with 33 amino acids have been loaded on MHC class I molecules (Howard, 1995).

As peptides are being generated, the class I molecule is being synthesized by ribosomes The molecule is cotranslationally inserted into the ER. Several proteins associate with the class I molecule to stabilize the molecule before peptide loading. After an initial folding process, the MHC class I complexes with calnexin, an 88-kDa protein (Fig. 5). Calnexin retains the partial folding of the molecule until it interacts with ß$_2$-microglobulin (ß2m) at the site of protein entrance into the ER (Soldheim *et al.,* 1997a).

Two other proteins are important in the generation of MHC I proteins. Tapasin, a recently described 48-kDa protein, bridges a trimolecular complex of two ß$_2$m/MHC class I dimers and the TAP protein. The bridging stabilizes the class I MHC and TAP complex. Although unproven, tapasin may also be in-

volved in peptide loading (Soldheim *et al.,* 1997b). Calreticulin initiates a steady-state association with the ß$_2$m/class I/TAP by interacting with the MHC I a1 domain glycosyl moiety. Final folding of the α1 protein-binding cleft induces changes in the glycosyl region. This change is detected by calreticulin, releasing both itself and the TAP protein (Howard, 1995; Soldheim *et al.,* 1997b).

Most peptides are quickly exported from the ER. Peptides can only be retained in the ER following glycosylation or reactions with class I MHC molecules (Howard, 1995). The trimolecular complex (MHC, peptide, and TAP) is transported from the ER to the Golgi apparatus. From the Golgi apparatus, the complex is transferred to vesicles that move to the cell surface. Finally, the MHC–peptidecomplex is inserted into the outer cell membrane (Soldheim *et al.,* 1997b).

Although the MHC I proteins are usually generated in response to endogenous or cytosolic antigens, monocytes and macrophages have specialized antigen-processing methods designed to present exogenous peptides to the class I MHC. Peptides are ingested by phagocytosis or pinocytosis. In the phagosome, cathepsin digestion of antigen results in small fragments of 8–10amino acids.

Interactions between the digested peptides and the class I molecules may occur by three mechanisms. Empty or non-peptide-loaded cell surface class I molecules may be internalized with the immunogen during phagocytosis. The MHC proteins can be loaded with immunogenic peptides generated in the phagosome and recycled to the cell surface. It is also possible that MHC protein recycling from the cell surface to the phagosome is a normal cellular event. Finally, newly synthesized MHC I molecules may react with the peptides in the phagosome (Rock, 1996).

These unique presentation methods for endogenous peptides provide a unique surveillance mechanism for host defense against intracellular microbes and somatic cell tumors. In defense against microbial agents, the noncytosolic presentation method provides a means to identify and destroy monocytes and phagocytes infected with mycobacteria and leishmania. The class I–bacterial peptide complexes generate CD8 cytotoxic lymphocytes that destroy the infected cells and purge the host of the cellular reservoir of infection (Rock, 1996).

These pathways may also be important in defense against somatic cell tumors. Normally, somatic tumors express suboptimal numbers of primary and secondary molecules required to generate CD8 cytotoxic responses. Ingestion of tumor cells and generation of immunogenic peptides in the phagosome, allows antigen presentation by the MHC I proteins. Moreover, costimulatory factors necessary for CD8 cell activation are present on the macrophage surface (Rock, 1996). Because of the CD8 response, both diseased somatic cells and the antigen-presenting monocytes/macrophages are destroyed. The host survives because of monocyte/macrophage regeneration from the bone marrow.

The generation of cell surface peptide-loaded class I MHC molecules is very

efficient, as only 200 MHC molecules loaded with immunogenic peptides are necessary for activation of T cells (Groettrup *et al.,* 1996). Immunogenic proteins are less than 0.01% of the total protein continuously being catabolized by the cell. Therefore, immunogenic proteins must actively compete with a wide range of different peptides for MHC binding.

PRESENTATION OF PEPTIDES BY CLASS II MHC MOLECULES

In the presentation of exogenous antigens by the endocytic pathway, presentation in concert with MHC class II molecules occurs in endosomes or phagocytic vesicles of APCs (German *et al.,* 1996; Lechler *et al.,* 1996). The two-chain MHC II molecule is synthesized independently and inserted into the vesicle (Sant, 1994). Heat shock proteins prevent interactions between the peptide fragments and the newly synthesized chains.

Like the MHC class I proteins, several ancillary proteins are required to stabilize the chains. Calnexin stabilizes the initial structures until the chains form a stable binding cleft conformation. The MHC class II proteins react with another protein, the invariant chain (Ii). This 30-kDa homodimer has a reversed transmembrane orientation and consists of a nine-chain complex of three invariant chains and three α/β homodimers. The li binding to the peptide-binding site prevents interactions between incompletely folded or denatured protein (Germain, 1995). Additional functions have also been ascribed to the invariant chain (Sant and Miller, 1994). It may be necessary for the proper folding of the α/β chains and routing of the MHC class II protein to the endosomes and lysosomes.

The MHC class II invariant chain complex is inserted into endosomes where the peptide is being degraded by exposure to a series of acid proteins in specific hydrolytic endosomal (pH 6–6.5) and lysosomal (pH 4.5–5) compartments. Once the antigen is transferred to the lysosome, 40 different acid-dependent hydrolyases can act on the molecule (Fig. 6). These acid-dependent hydrolyases include proteases, nucleases, glycosidases, phospholipases, and phosphatases (Germain, 1995). Acid proteases (cathepsins A, B, and E) that degrade peptides and cleave the invariant chain at several sites are also important in antigen degradation (Sette *et al.,* 1992).

In the late endosomes or class II vesicles (CIIV), multiple copies of the MHC II molecules are rapidly inserted. Within the endosomes, cathepsin continues to truncate the invariant chains. Final cleavage occurs in a specialized late-phase endosome, the MHC II compartment (MIIC). The resulting small class II invariant peptide (CLIP) is removed from the class II molecule (Ghosh *et al.,* 1995; Lee and McConnell, 1995). An open MHC conformation, called a *floppy* state, allows binding of the peptide and stabilization of the α/β chains.

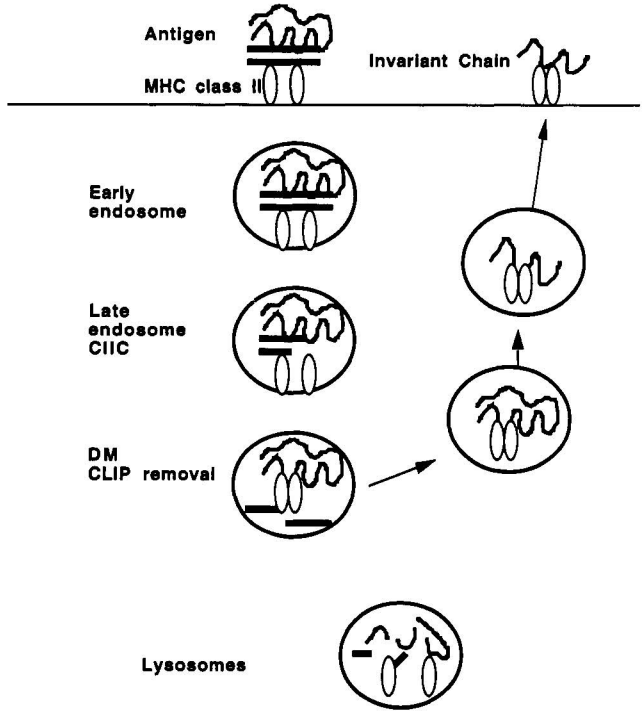

Figure 6. Summary of the MHC class II antigen processing and presentation pathway.

Subsequently, the class II complex is transported to the plasma membrane. When exposed to the neutral pH of the plasma membrane, the complex assumes a compact, stable form with a high affinity constant for the peptide. Sometimes, class II molecules with the attached CLIP are transported to the cell surface.

A second mechanism for peptide loading by class II molecules has been described. In the mechanism, surface CLIP complexes undergo endocytosis into recycling compartments. Loading of new peptides is simplified by the HLA-DM, a dual α/β chain complex having little polymorphism or protein-binding activity. DM serves as a molecular chaperon that interacts with the class II molecules after the CLIP is released, because of its instability, at the low endosomal pH. At a low pH, the HLA-DM stabilizes the peptide-binding groove of the class II molecule (Fig. 7). Stabilization allows binding of low- and high-stability peptides. The DM can exchange CLIP for peptides in a manner that follows classical Michaelis–Menten kinetics. Turnover rates of 3–12 peptides per minute have been reported for a single DM. Recently, it was determined that the "off rate" (a measure of the kinetic stability of the peptide), rather than the affinity constant, determines

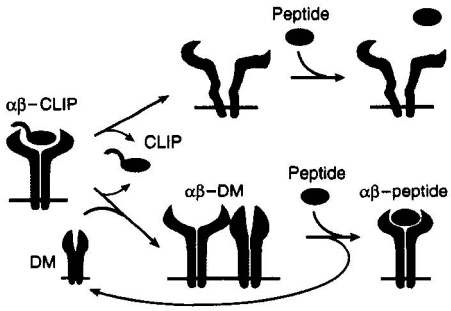

Figure 7. DM acts as a chaperone and rescues empty MHC class II dimers. CLIP dissociates from αβ-CLIP complexes by itself as a result of its low stability at low endosomal pH. In the absence of DM, the empty α/β dimers undergo unfolding and form aggregates that are no longer able to bind peptides. DM binds and stabilizes empty class II dimers until high-stability peptides occupy the groove. Reprinted from *Immunology Today,* Kropshofer *et al.,* 1997, 18:77–81, with permission of Elsevier Science.

whether the peptide is removed by the DM. Low-stability peptides have a high off rate and are released from the class II molecules. Conversely, high-stability molecules with a low off rate form stable interactions with the class II molecules (Fig. 8). High-stability peptide–class II complexes dissociate from the DM and are recycled to the cell surface (Kropshofer *et al.,* 1997).

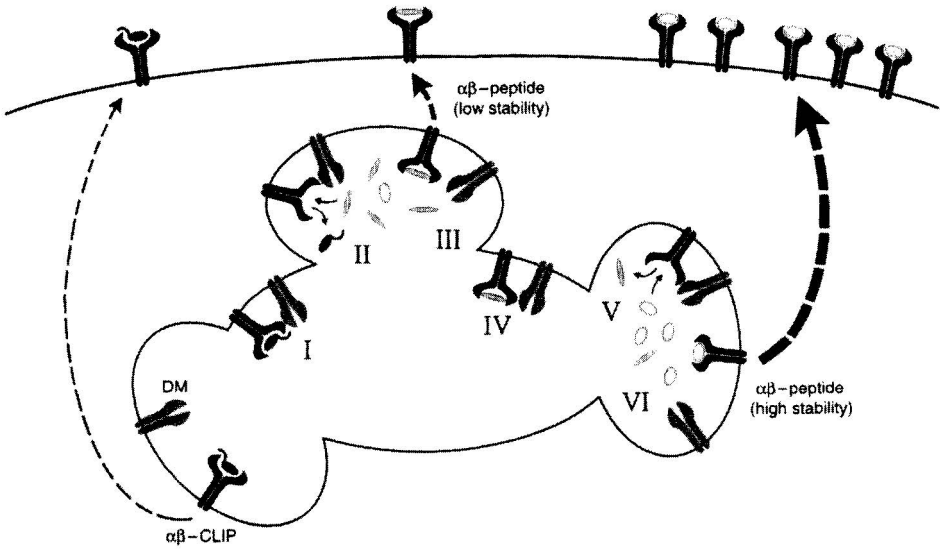

Figure 8. Low- and high-stability peptide binding in the loading compartment. DM catalyzes exchange of CLIP for peptides. Low affinity ligands are more abundant than high-stability ones and, therefore, have a higher chance of being loaded. Most of the low-affinity ligands are released and replaced with high-affinity peptides. The resulting α/β-peptide complexes further resist peptide editing by DM and accumulate on the cell surface. Reprinted from *Immunology Today,* Kropshofer *et al.,* 1997, 18:77–81, with permission of Elsevier Science.

PRESENTATION OF PEPTIDES BY CD1 MOLECULES

Proteins unrelated to the classical MHC markers can also present antigen to T cells. There are five nonpolymorphic CD1 genes in humans (CD1 A, B, C, D, and E) and two murine CDlD homologues in rodents (MCD1D1 and MCD1D2). On the basis of tissue distribution, the CD1 protein products can be subdivided into two families. Group I consists of human CD1A, B, and C isoforms. This CD1 family is expressed on Langerhans cells, dendritic cells, mantle zone B cells, and activated monocytes. Proteins in group II (human CD1D, murine MCD1D1 and MCD1D2) are expressed only on the intestinal epithelium (Beckman and Brenner, 1995; Blumberg *et al.,* 1995).

The CD1 protein is a single chain that has some sequence homology to the α3 domain and remote homologies to the α1 and α2 domains of the class I MHC markers. Unlike the classical MHC I molecules, the encoding genes are located on chromosome 1 and the gene products are nonpolymorphic (Sieling *et al.,* 1995). Like the classical MHC I markers, $ß_2$m is required to stabilize the CD1.

Antigen presentation, in association with CD1, is unique in that the TAP protein is not required for ER membrane transport. The ability of CD1 molecules to reach the cell surface without transporter interactions suggests that they require a unique set of ligands (Teitell *et al.,* 1997).

The CD1 family may represent a third lineage for presentation of unique or highly conserved intracellularly derived microbial proteins, lipids, or glycolipids (Melian *et al.,* 1996). Moreover, peptides binding to the CD1 are much longer than the typical eight to nine amino acids found in the class I binding clefts (Tangri *et al.,* 1996). Most CD1 molecules bind lipids with a hydrophobic anchor motif. Human CD1B presents the highly conserved *Mycobacterium* lipoglycan lipoarabinomannan (MLAM) (Sieling *et al.,* 1995) or mycolic acids (Beckman and Brenner, 1995). CDlC-restricted T-cell lines recognize protease-resistant mycobacterial lipids (Beckman *et al.,* 1996). Some peptides with bulky side chains and hydrophobic side chains can also bind to CD1.

In humans, CD1 isoforms present antigens to different cells. Nonpeptide *Mycobacterium* antigens are presented to CD3+, CD4−, CD8− T cells expressing the α/β TCR (Thomssen *et al.,* 1996). Peripheral blood CD1 cells present protein antigens to CD4 cells. Within a short time, specific patterns of cytokines are produced that induce Th1 cell differentiation. Peptide-specific, CD1-restricted, cytotoxic CD8 T cells can also be generated following interaction with peptide–CD1 complexes (Blumberg *et al.,* 1995).

The role of CD1-expressing cells in the intestine may differ from that of CD1 in other tissues or organs. CD1D, MCD1D1, and MCD1D2 bind a variety of antigens with hydrophobic peptides. Moreover, MCD1D1-restricted T cells are the first cells to produce IL-4 in response to antigenic insult. Thus, they drive

the response toward a Th2-driven B-cell-mediated antibody response (Beckman and Brenner, 1995) that is necessary to terminate intestinal infections.

IMMUNODEFICIENCIES AND ANTIGEN PROCESSING

Bare Lymphocyte Syndrome

Cells from patients with bare lymphocyte syndrome have low levels of CD4 cells in the peripheral blood (Table 1). Moreover, MHC class II protein is not expressed on B cells, macrophages, and dendritic cells. Occasionally, class I proteins and ß$_2$m are also not expressed.

Several genes have been implicated in the disease process. One defective gene codes for the class II transactivator (CIITA) protein. This nuclear protein has an acidic region that may act as a transcriptional activation domain for MHC 11, DR, DQ, DP proteins (Fischer and Wood, 1995). In addition, the gene may act as a cofactor in the expression of the invariant chain and the DM protein. Another defective gene codes for the 75-kDa RFX5 protein. Normally, the RFX5, in a heterodimer complex with p36, binds to the X box of MHC II gene promoters. Binding stimulates transcription of class II molecules by binding to the X box at the 5‘-end of all class II molecules (Fischer and Wood, 1995; Fischer, 1990). Most of these infants fail to thrive and are susceptible to viral and bacterial infections.

CD 1 Deficiency

An immune defect associated with defective expression of CD1A was reported (Plebani *et al.,* 1996) in a youth with “Marfan’s-like’’ syndrome. The child developed chronic ulcerated skin lesions. All cells in the inflammatory infiltrate showed normal MHC class I markers. However, dendritic Langerhans cells in the lesion failed to express CD1. Patients with self-healing cutaneous lesions express both MHC class I and CD1 (Plebani *et al.,* 1996).

Table 1. Mutations Associated with Primary Immunodeficiencies

Disease	Cell phenotype	Defective protein
Defective MHC class II	Low CD4	CIITA, RFX5
Defective MHC class I	Low CD8 and NK	TAP2

Modified from *Immunology Today,* Fischer and Arnaiz-Villena, 1995, 16510–4, with permission of Elsevier Science.

TAP Deficiency

Several patients with TAP2 deficiencies have been described. In addition to the protein deficiency, these patients have low levels of CD8 and NK cells with defective MHC class I protein expression (Fischer and Arnaiz-Villena, 1995).

INTERFERENCE WITH ANTIGEN PROCESSING

Antigen presentation can be inhibited at several levels. Site-directed protease inhibitors such as leupeptin can inhibit antigen degradation. Similar effects can be induced by chemicals diffusing across the membranes and increasing the pH of the lysosomes. Elevated pH inactivates the acid protease in the lysosome.

Antimalarial drugs such as chloroquine and hydrochloroquine are diffusible diprotic bases that elevate pH in a matter of minutes. In experimental systems, it was shown that chloroquine treated macrophages could ingest mycobacteria and listeria but could not degrade the ingested bacteria. Expression of class 11 markers can be reduced by corticosterone treatment. At concentrations as low as 2×10^{-8}M, corticosteroids can reduce the expression of class 11 markers by 50% both *in vivo* and *in vitro.*

7

Surface Interactions and Intracellular Signaling

INTRODUCTION

Previous chapters discussed the concepts of immunogenicity and antigen presentation by immunocompetent cells. The present chapter explores the concept that the immune system is activated or deactivated by surface receptor interactions and subsequent intracellular signaling. Much like starting an automobile, several signals must be given in a defined sequence to override interlocking safety systems. When the immune system is stimulated, T and B cells give each other signals in a defined pattern. Because there is a possibility of uncontrolled proliferation during activation, the immune response is tightly controlled. If an improper signal is given, cellular proliferation is prevented and the cell undergoes programmed cell death or apoptosis.

T-CELL RECEPTOR

Essential to the generation of an immune response is the T-cell receptor (TCR). The receptor has a two-chain (α and ß) transmembrane structure (Fig. 1). Both glycoprotein chains are 40 to 60 kDa in size but differ in electrostatic charge. The α chain is acidic and the ß chain is neutral or uncharged. Each chain has a variable domain (V) as an intrachain loop (100–199 amino acids) held together by two cysteine residues. Thus, there is a structural tertiary homology with the variable portion of an antibody molecule. The junction between the variable and constant domains of the TCR is linked by a joining

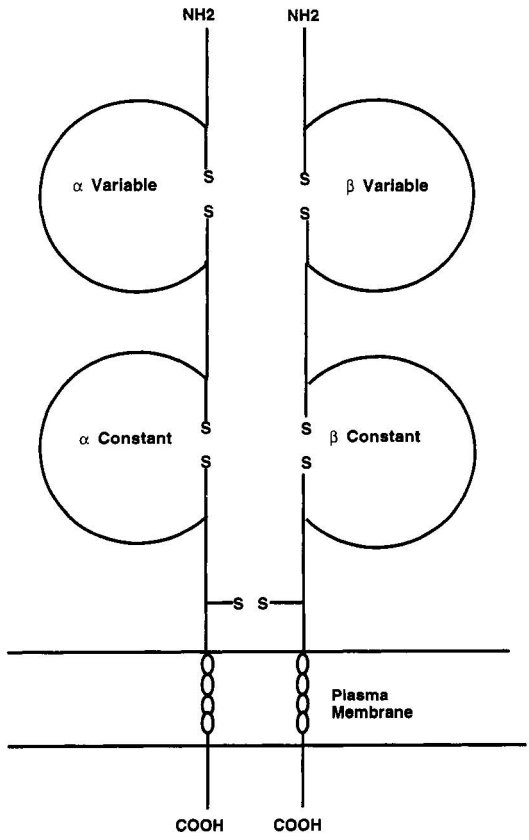

Figure 1. Schematic diagram of the αβ T-cell receptor. Each variable region contains three hypervariable regions. The structure of the γδ T-cell receptor is almost identical to the αβreceptor. Modified from *Immunology,* Kuby, 1991, W. H. Freeman & Co.

(J) region. Some ß chains also have a diversity (D) domain besides the J domain.

A constant domain (140–180 amino acids) is composed of several subregions or subdomains. There are intrachain loops similar to those found in the variable region; a hinge region with disulfide links between the α and ß chains; a transmembrane region consisting of 20–24 hydrophobic amino acids involved in activation of CD3; and a small C-terminus with no intracellular signaling capabilities.

Two different forms of the TCR have been described. The αβ(TCR2) form is found on 90–95% of peripheral blood T cells (Engel *et al.,* 1992). A second form of the receptor (TCR1) consists of γδ chains. Only small numbers of

peripheral blood lymphocytes express the γδ receptor. In contrast, high numbers of γδ receptors are found on mucosal intraepithelial lymphocytes and dendritic cells. The structures of TCR2 and TCRl differ only in the nature of the sugars attached to the hinge or constant regions and the presence of D domains in the γ chain (DeLibero, 1997).

The αβ and γδ have different functions in defense. Lymphocytes bearing the αβ receptor are dependent on interactions with the MHC class I and II proteins and respond to foreign material entering the body via the inhalation or blood routes. These lymphocytes usually recognize immunogenic linear amino acid sequences. In contrast, the γδ-expressing lymphocytes are not usually stimulated by peptides presented by the MHC. They are responsible for surveillance of the surface and bowel epithelia against infectious agents. Further, the γδ T cells may play a protective role by exerting direct lytic effects on *S. aureus* and NK-resistant tumors of the gut (DeLibero, 1997; Kabelitz, 1992).

Some γδ T cells have restricted sequences in the V domain. In humans, 1–10% of the circulating T cells express TCR Vγ9/Vδ2. These T cells recognize bacterium-derived molecules with phosphate residues. Isopentylpyrophosphate and dimethylallylpyrophosphate isolated from mycobacteria activate this population of T cells. Synthetic compounds such as alkyl phosphates are also potent activators of the γδ population.

The γδ T cells have two functions. Because they react with a wide range of cross-reactive, phosphorylated compounds from bacteria, these T cells may give the initial signal that infectious agents have entered the body. Second, γδ T cells may play a role in the early phase response to viral infections. The release of phosphorylated compounds from damaged or virus-infected cells activates and recruit γδ T cells into the area. Local release of IFN-γ and IFN-γ activates other cells (DeLibero, 1997; Kabelitz, 1992).

Origin of TCR Diversity

Because a single TCR is antigen specific, evolutionary pressures have required the development of 10^6 to 10^{13} different antigen-specific TCRs. The antigenic specificity resides in the V and J regions of the TCR. The origin of TCR diversity resides in the combinational joining of different V and J genes in αβ and γδ chains. For example, in the α chain of the TCR there are 100 different V domain genes that can be linked to 50 different J domains. Therefore, the number of possible combinations is 100 X 50 or 5 X 10^3 (Table 1).Similarly, the ß-chain genes yield 6 X 102 different combinations. It follows that 3 X 106 different TCR antigenic specificities can be generated by simple combinational joining of the V and J genes in the α and ß chains (Kuby, 1991).

Additional TCR diversity is generated by alternate joining of D genes, junctional flexibility, or N-region nucleotide addition. In the first mechanism, $V_ß$ can bind directly to either a J or a D gene creating a $V_ßJ_ß$ or a $V_ßJ_ßD_ß$ with

Table 1. Combinational Joining of Variable and J Regions

	α/β TCR		γδ TCR	
Domain	α	β	γ	δ
V	100	25	7	10
D	0	0	0	2
J	50	12	3	2
	Intrachain combinations			
	5 x 103	6 X 102	21	40
	Interchain combinations			
	3 x 106			8 x 102

Modified from *Immunology,* Kuby, 1991, W. H. Freeman & Co.

different antigenic specificities. Junctional flexibility is achieved by adding several alternate amino acids at each J domain. Six amino acids can be added at each junction, creating 5000 different combinations. The reaction is catalyzed by a terminal deoxynucleotidyl transferase. Junctional flexibility and N-region addition can generate as many as 1013 possible combinations (Kuby, 1991).

CD3–TCR PROTEIN COMPLEX

The function of the TCR is to recognize immunogenic peptides presented on MHC proteins. It has no intracellular signaling capabilities. A CD3 protein forms a complex with the TCR to activate the signaling process. CD3, a pan-T-cell marker, is a complex of γδεζη subunits (Fig. 2). The γδε chains are small (20–28 kDa) external proteins with immunoglobulin-like external domains and 40-amino-acid tails. In contrast, the ζη proteins have small external domains and long cytoplasmic domains of 113 and 150 amino acids, respectively (Altmann *et al.,* 1990).

The TCR–CD3complex is assembled in the ER by modification of the TCR N-terminus that binds the dimers of the CD3 ζζ chains in 90% of the lymphocytes. Most lymphocytes constitutively express ζη. Within the transmembrane domain, positively charged amino acids on the α (e.g., lysine) or ß (e.g., arginine) TCR chains react with negatively charged aspartic acid residues in the t[chains. The ζζ chains in combination with γε or δε are necessary for routing and inserting of the TCR–CD3complex into the cell membrane. Intracellular signaling depends on the presence of E subunit, ζη, and ζζ homodimers having antigen recognition activation motifs (ARAM). The ε subunit contains one copy of the ARAM whereas the ζζ homodimers contain three copies (Altmann *et al.,* 1990).

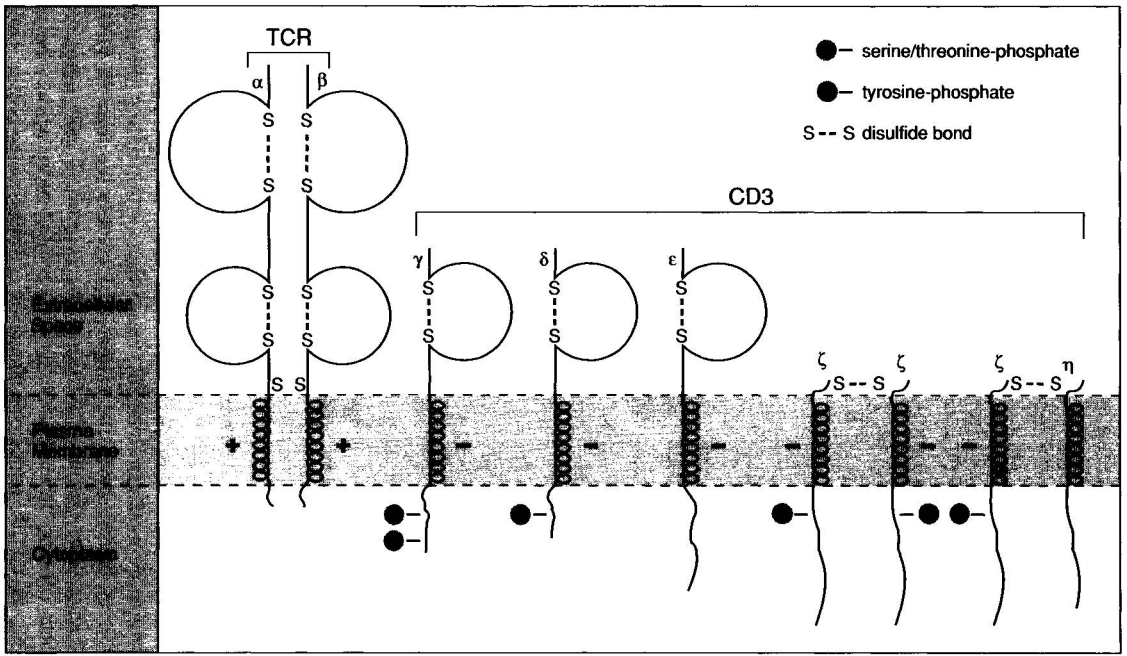

Figure 2. Schematic diagram of the αβ TCR–CD3 complex showing the αβ receptor and the CD3 complex consisting of ζζ homodimers plus γϵ, ϵδ, and ζη heterodimers. Oppositely charged transmembrane regions facilitate ionic interactions in the TCR and CD3 chains. The long cytoplasmic tails of the CD3 chains contain a common sequence, the antigen recognition activation motif (ARAM) that functions in signal transduction. Reprinted from *Cellular and Molecular Immunology,* Abbas *et al.,* 1994, with permission of W. B. Saunders Co.

CD40 PROTEIN

The CD40 receptor is a 48-kDa transmembrane protein present on B cells and APCs such as dendritic cells, follicular dendritic cells, macrophages, and hematopoietic cells. Receptor signaling is initiated by binding of CD40 to its ligand (CD40L) present on activated CD4 cells (Fig. 3). Like other members of the tumor necrosis factor (TNF) superfamily, CD40L is a homotrimer shaped like a truncated pyramid. Each CD40L binds three CD40 molecules creating multimers of CD40 on the cell surface. Multimer formation is the critical step in the initiation of intracellular signaling. CD40L signaling activates a number of different cellular pathways that upregulate the expression of the B7 costimulatory molecules. Signaling also upregulates the *bcl* protein family (Kehry, 1996). Gene products are important in the rescue of B cells from apoptosis (Fig. 4).

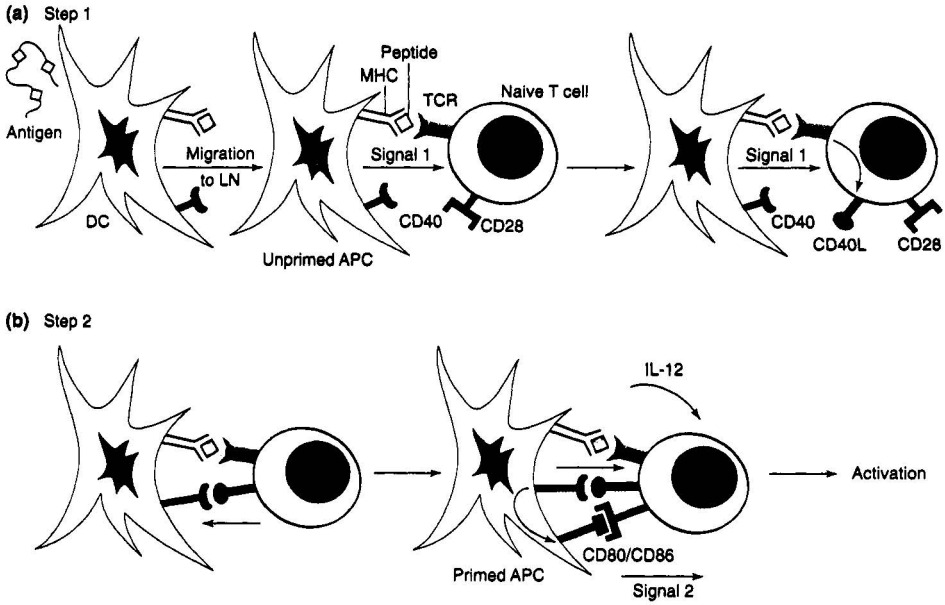

Figure 3. The role of CD40–CD40L in T-cell activation. This is a two-step model of T-cell activation. (a) Antigen-primed dendritic cells (Langerhans cells in tissue) migrate to the lymph node where they present processed antigen to T cells in the form of MHC–peptide complexes which deliver the antigenic signal. As a result, T cells upregulate CD40L on their surface. (b) The T-cell CD40L induces costimulatory activity on the dendritic cells via the CD40–CD40L interaction. Dendritic cells expressing costimulatory signals send a second costimulatory signal to T cells along with signal 1 for full activation of T cells to produce cytokines. Reprinted from *Immunology Today,* Grenwal and Flavell, 1996, 17:410–3, with permission of Elsevier Science.

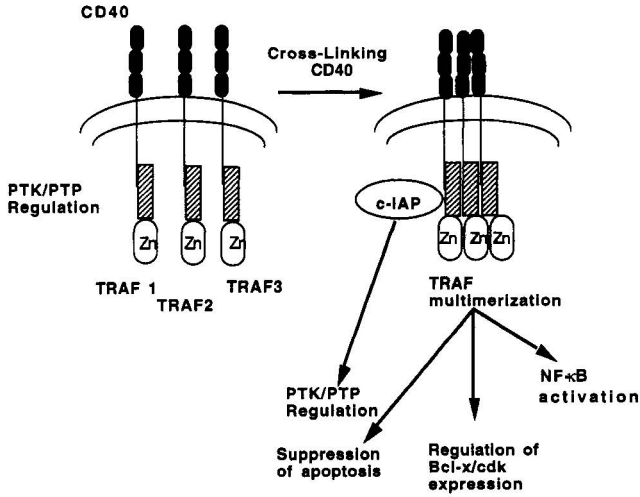

Figure 4. Modeling of CD40 signaling in B cells. Shaded areas of CD40 indicate the four extracellular cysteine-rich domains; cross-hatched areas of TRAF indicate TRAF-N and TRAF-C homology. Zn indicates the zinc ring finger and zinc finger domains in TRAF1, TRAF2 and TRAF3. TRAF3 is also known as CRAF1, CD40bp, or LAP1. Reprinted from *The Journal of Immunology,* Kehry, 1996, 156:2345–8. Copyright 1996, The American Association of Immunologists.

CD45 PROTEINS

The CD45 or LCA (leukocyte common antigen) is a surface protein found on all immature and mature leukocytes. The 180- to 220-kDa monomeric isoform serves to activate the *lck* and *fyn* kinase system by removing phosphates from regulatory proteins.

Because of alternate splicing of a single gene, two different isoforms of CD45 are expressed on T cells. A high-molecular-weight isoform (CD45RA) is expressed on naive T cells and a low-molecular-weight form (CD45RO) is found on memory T cells. Both isoforms have long cytoplasmic regions (300 amino acids) that have associated tyrosine phosphatase activity (Rao, 1991). The ligand for CD45 is unclear.

CD4 PROTEIN

The CD4 is expressed on lymphocytes having T helper/amplifier functions. It is a 55-kDa monomeric, transmembrane glycoprotein with two variable

and two constant extracellular immunoglobulin domains similar to those found on immunoglobulins (Fig. 5). CD4 also contains a small hydrophobic transmembrane region and a cytoplasmic tail of 38 amino acids. A unique feature of the transmembrane region is the presence of serine residues capable of being phosphorylated (Brady and Barclay, 1996; Harrison *et al.,* 1992).

During T-cell activation, CD4 serves as a cell adhesion molecule by binding to the constant $ß_2$ domain of the MHC II molecule. This stabilizes the TCR–CD3, MHC 11 complex and, in addition, creates oligomers of CD4 and MHC II that simplify additional T-cell activation.

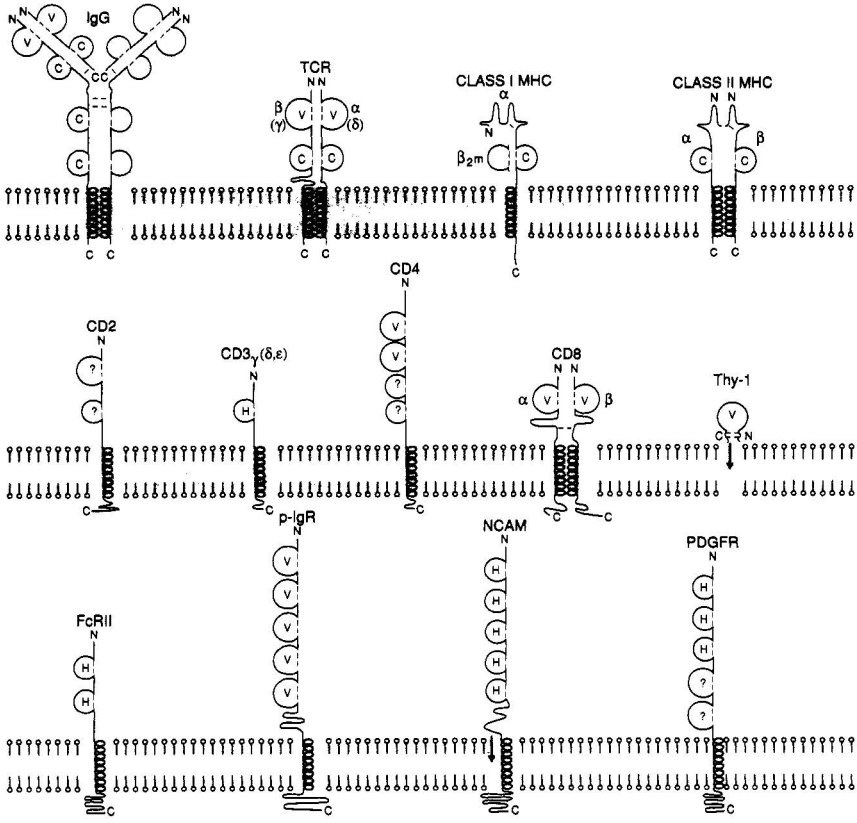

Figure 5. The Ig gene superfamily. FcRII, Fc receptor II; p-IgR, poly-Ig receptor (antibody transport receptor); NCAM, neural cell adhesion molecule; PDGFR, platelet-derived growth factor receptor. Reprinted from *Cellular and Molecular Immunology,* Abbas *et al.,* 1994, with permission of W. B. Saunders Co.

During CD4-mediated intracellular signaling, phosphorylation of serine residues in the tail activates the *lck* or p56 kinases. These kinases play a critical role in the activation of MHC 11-restricted T helper cells (Sweet *et al.,* 1991).

CD8 PROTEINS

The structure of CD8 depends on the stage of T-cell maturation and, perhaps, activation. Two distinct 32- to 34-kDaglycoproteins (CD8a and CD8ß) linked by disulfide bonds are inserted into the cell membrane (Fig. 5). Occasionally, CD8 may exist as a homodimer or associated with the non-MHC antigen-presenting CD1 molecule.

The function of CD8 is similar to that of CD4. Like CD4, CD8 serves as a cell adhesion molecule by binding to the a3 domain of the MHC I molecule on the APC. Also, CD8 may be involved in signal transduction because the cytoplasmic domain is rapidly phosphorylated following interactions with MHC I molecules. Expression of the CD8 molecule is restricted to the cytotoxic/suppressor T-cell populations (Leahy, 1995).

CD28/67

B7–1 (CD80) and B7–2 (CD86) ligands are present on activated B cells, monocytes, macrophages, and dendritic cells. Following interactions with their ligands, these proteins provide costimulatory signals that activate T cells. The isoforms of B7 activate different T-cell subsets. For example, B7–2 activates CD4 Th1 cells while B7–1 activates CD4 Th2.

CD28 is a 42-kDa disulfide-linked homodimer receptor for B7 that is present on 95% of human CD4 and 50% of CD8 lymphocytes (Hutchcroft and Biere, 1996). Activated T cells transiently express another receptor called CTLA-4 (June *et al.,* 1994).

Following ligation, CD28 is tyrosine phosphorylated by *lck* and *lyn.* Phosphorylation induces the binding of the phosphoinositol kinase (PI-3), growth factor receptor binding protein-2 (Grb-2), and IL-inducible T-cell kinase to CD28. PI-3 phosphorylates at the 3 position on the inositol ring, creating PI-3-monophosphate, PI-3,4-biphosphate, and PI-3,4,5-triphosphate.

SIGNALS INVOLVED IN T-CELL ACTIVATION

Several signals are required for T-cell activation and intracellular signaling that result in cellular proliferation and production of IL-2. Proliferation is con-

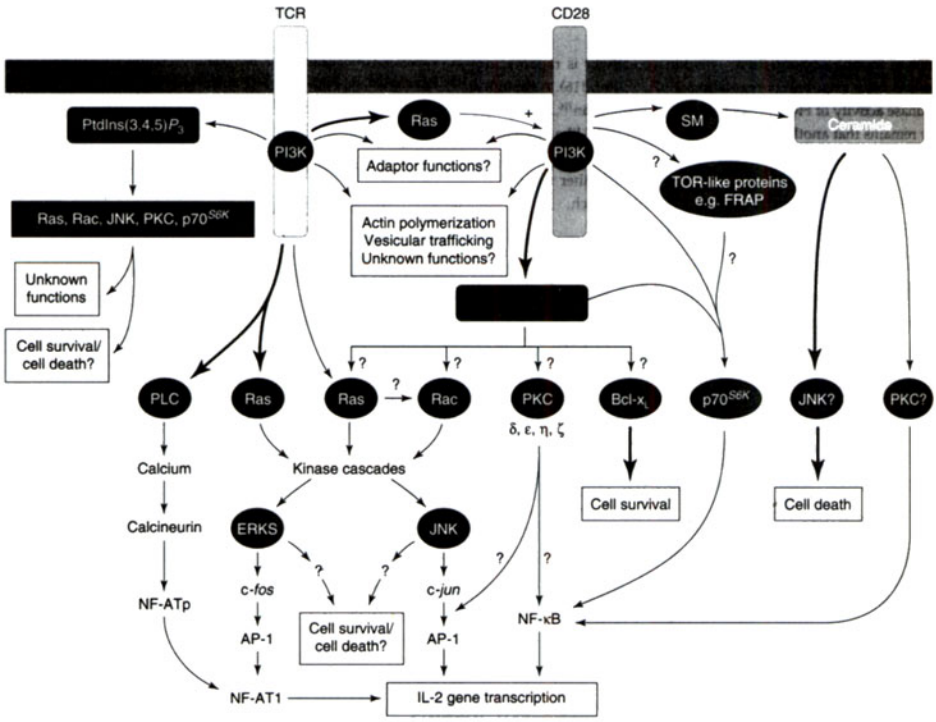

Figure 6. CD28-activated PI-3-kinase signaling. Possible sites of action of CD28 include PTKs, PLC calcium, and PI-3 kinase. Reprinted from *Immunology Today,* Ward *et al.,* 1996, 17:187–98, with permission of Elsevier Science.

trolled by TCR–CD3ZAP-70 while activation is controlled by two *src* phosphate tyrosine kinases (PTKs) called *lck* and*fyn*. *lck* is associated with CD4 andfyn is associated with TCR–CD3 (Rudd *et al.,* 1994). CD45 ligation is necessary to upregulate the activity of *lck* by dephosphorylating the regulatory tyrosine at the C-terminus (Rudd *et al.,* 1994). CD28 also plays a role in determining cell survival and cell death by activation of other enzymes (Ward *et al.,* 1996).

INTRACELLULAR SIGNALS

Proliferation is initiated by the phosphorylation of the immunoreceptor tyrosine activation motifs (ITAMs) on ζη and ζζ homodimers of CD3. Phosphorylation of the ITAMs provides docking sites for ZAP-70 (Zoller *et al.,* 1997). In turn, ZAP is phosphorylated at multiple residues including positions 292, 492,

and 493 (Zhao and Weiss, 1996). ZAP forms a trimolecular complex with phosphorylated SLP-76 and Vav, an oncogene product (Raab *et al.,* 1997). Activated or phosphorylated Vav catalyzes GDP/GTP on Rac-1 and activation of c-Jun kinase (Fig. 7). Both Rac and Jun proteins are involved in cell proliferation and cytoskeletal rearrangement (Crespo *et al.,* 1997).

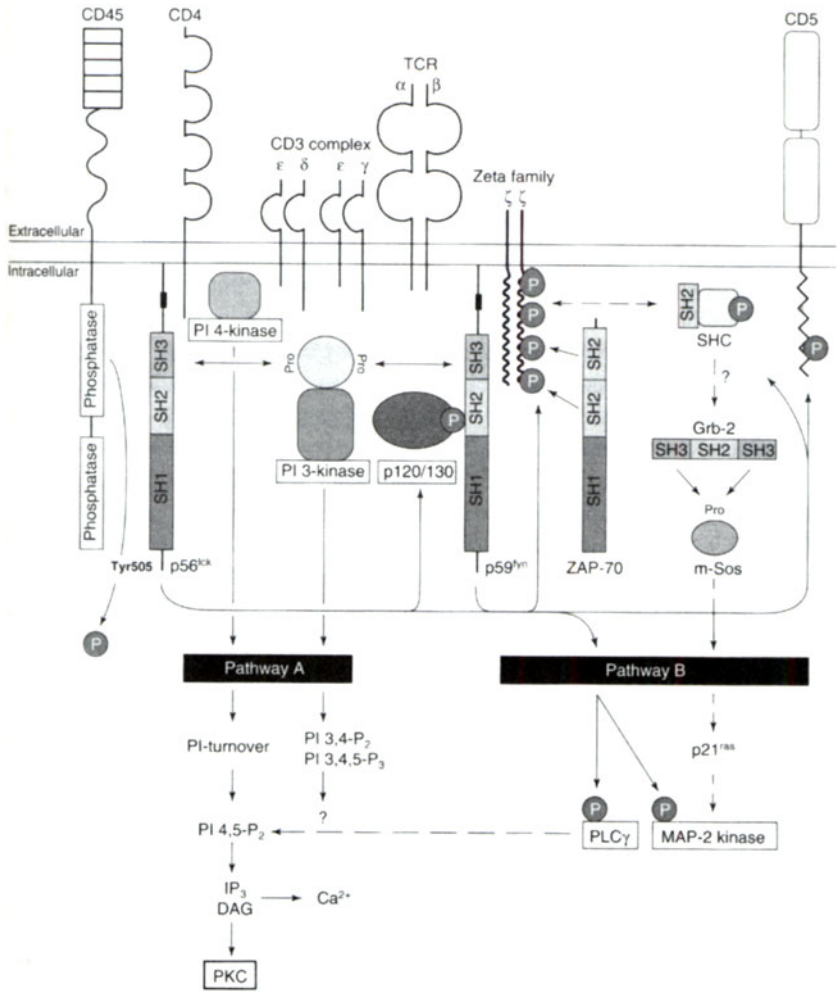

Figure 7. Multiple signaling pathways mediated by CD4 and TCR/CD3. Pathways mediated by CD4-p56lck and TCRζ/CD3-p59$^{fyn(T)}$ and associated proteins are shown. Reprinted from *Immunology Today,* Rudd *et al.,* 1994, 15:225–35, with permission of Elsevier Science.

Ligation of the TCR–CD3and CD4 activates a series of proteins that results in cellular activation by several different pathways (Fig. 4). Signaling via the ε subunit, ζη and ζζ homodimers initiates membrane protein dephosphorylation of *src* and ZAP tyrosine kinases. This activates tyrosine kinases that phosphorylate the ε and ζchains of CD3 and other proteins. Proteins with phosphate-binding sites attach to the phosphorylated proteins. Critical to the activation process is the docking of phosphatidylinositol phospholipase C (PI-PLC). PI-PLC hydrolyzes phosphatidylinositol-4,5-bisphosphate, generating inositol 1,4,5-trisphosphate (Fig. 7).

The trisphosphate moiety initiates the release of calcium from intracellular stores and increases the calcium flux across the membrane. Reaction of calcium with calmodulin activates several unique kinases and phosphatases. The calcineurin phosphatase acts on an inactive NF-ATc to create an active NF-AT. The NF-AT translocates to the nucleus where it reacts with the ARRES gene in the IL-2 enhancer region. Increased secretion of IL-2 occurs about 45 min after antigen recognition. IL-2 is critical for progression of T cells from resting G1 cells into the S phase.

In a second pathway, activation of PI-3 kinase activates the *ras* protooncogene which initiates a kinase cascade involving the ERK family of enzymes. As a consequence of the cascade, c-fos, AP-1, and NF-AT are activated. IL-2 gene transcription occurs as described above.

In a third pathway, TCR–CD3 ligation causes phosphatidylinositol 4,5-biphosphate to be hydrolyzed into diacylglycerol. Activation of protein kinase C initiates serinelthreonine protein phosphorylation together with high levels of calcium. Removal of an INK-α inhibitory protein from NF-KB allows translocation to the nucleus and binding to the κB-RE gene in the enhancer region. This gene region controls entry into cell division because it initiates transcription of the AP-1 complex containing the oncogenes *fos* and *jun.* These oncogenes regulate the production of cdk4, cyclin D, and the *myc* gene product that are necessary for cell division.

Signaling via CD28–B7is also required for T-cell activation (Rudd *et al.,* 1994). Cross-linkage of CD28 activates tyrosine kinases *lck* and *fyn.* One of the proteins phosphorylated is the p85 subunit of PI-3. Phosphorylation of phosphatidylinositols at the 3 position generates PI-3-monophosphate, PI-3,4-biphosphate, and PI-3,4,5-triphosphate. PI-3,4,5-trisphosphate activates NF-KB directly or indirectly by interaction with *sck* (Ni *et al.,* 1996). Alternatively, PI-3 kinase binds PLC γ1, growth factor receptor bound protein, and inducible IL-2 kinase. These factors are critical for growth and the production of IL-2. The protein tyrosine kinases also generate CD28RC that moves to the nucleus and binds to the CD28 RC gene in the IL-2 enhancer region.

CD28 interacts with different isoforms of B7. Interaction between B7–1and CD28 homodimers on activated Th2 cells phosphorylates *Jak1* and *Jak3.* The

activated enzymes phosphorylate and initiate homodimerization of *IL-4 stat* and *stat6*

In contrast, B7–2binds to CD28 on resting or activated Th1 cells initiating rapid clonal expansion within 72 hr and expression of the IFN-γ chain. Signaling is accomplished via *Jak1* and *Jak2* that is required for phosphorylation and homodimerization of *Stat1*. In contrast, binding of CTLA-4 to the same ligands sends a negative signal to the cell via phosphatidylinositol-3 kinase.

AUTOCRINE REACTIONS OF T CELLS

The secretion of IL-2 by CD4 or CD8 cells induces the generation of IL-2 receptors on the surface of IL-2-producing cells (autocrine activity) and non-IL-2-producing cells (paracrine activity).

The IL-2 receptor is composed of CY (p55), ß (p70–75), and γ (p64) protein subunits. The CY subunit is also known as the T activation protein (Tac). Binding of IL-2 to the α protein does not elicit a biological response. Complexes of ß and y proteins can support half-maximal growth and proliferation of T cells for an extended period of time. α, ß, and y protein complexes create high-affinity IL-2 receptors with affinity constants in the range of 10–11 M. Because of the high affinity constants, IL-2 can deliver growth signals at low concentrations.

SUPERANTIGENS

Superantigens are molecules that can, at extremely low concentrations, stimulate and activate T cells. The unique antigens cannot be processed via the usual mechanisms. Acting outside the normal binding clefts, they bind to the constant region of the MHC class Il molecules and select variable elements (Vß) of the TCR ß chain (Table 2) effectively cross-linking the two molecules (Zumla, 1992). This results in enhanced IL-2 and IFN-γ from T cells and release of IL-1 and TNF-α from monocytes. These cytokines give a negative signal for antibody production and a positive signal for T-cell activation.

Superantigens react with a small number of class II markers on the surface of APCs. Superantigen binding is sensitive to the nature of the antigenic peptide associated with the class II marker and the density of the selected peptide–MHC complex on the cell. Low-density TCR–class II complexes allow serial interactions with T cells and mimic normal antigen presentation. This suggests that the magnitude of the response to superantigens is related to the high frequency of responding cells rather than the potency of the antigen.

Enterotoxins, exotoxins, and toxic bacterial products are known superantigens. *S. aureus* strains, the major causes of food poisoning and toxic shock

Table 2. Superantigens Reactive with V_β Chains of Murine and Human T-cell Receptor

	V_β	
Superantigen	Mouse	Human
Staphylococcus		
SEA	1, 3, 10, 11, 12, 17	14
SEB	3, 8.1, 8.2, 8.3	2, 12, 14, 15, 17, 20
SEC1	7, 8.2. 8.3, 11	12
SEC2	8.2, 10	12, 13, 14, 15, 17, 20
SEC3	7, 8.2	5, 12
SED	3, 7, 8.3, 11, 17	5, 12
SEE	11, 15, 17	5.1, 6.1–6.3,8, 18
Toxic shock syndrome		
TSSTl	15, 16	2

Modified from *Immunology,* Kuby, 1991, W. H. Freeman & Co.

syndrome, have seven different enterotoxins (SEA, SEB, SEC 1, SEC2, SEC3, SED, SEE) and the toxic shock syndrome toxin (TSST1). The association between V_β domains and specific enterotoxins is shown in Table 2. Closely related gram-positive *Streptococcus pyogenes* strains actively produce four different (SPE-A, B, C, D) exotoxins that act as potent superantigens (Schlievert, 1993). Research is under way to determine the relationship between each exotoxin and the V_β domains.

Superantigens may play a role in some diseases. In rheumatoid arthritis, there is a high concentration of cells with V_β 14 T-cell receptors in the synovial fluid. Low concentration of SEA interacting with the MHC and TCR may trigger the activation of T cells (Goodacre *et al.,* 1994). Activated T cells may have broad cross-reactivity and reactivity to self antigens in the joint. Further stimulation of T cells creates populations of joint reactive T cells that cause the tissue damage characteristic of rheumatoid arthritis.

INTERACTIONS IN THE SPLEEN AND LYMPH NODES

Activation of lymphocytes in secondary lymphoid tissue depends on the route of administration and the nature of the antigen. Particulate, soluble proteins and polysaccharides in the bloodstream are trapped by macrophages in the marginal zone. However, some polysaccharides are transported to the lymphoid follicles where they activate B cells. In the spleen, the periarteriolar sheath is the center of T-cell activation and mitotic activity. Migration of T cells into the marginal zone maximizes the possibility of interactions between T and B cells.

In the lymph node, antigen is presented on dendritic cells and there is an immediate increase in lymph node blood flow with an influx of Th2 cells. These T cells react with antigen and exit the node. Within 24 hr, upregulation of adhesion factors causes retention of antigen-specific cells. Mitosis of T cells in the parafollicular area induces cytokine-producing T cells that activate other T cells. The activated T cells leave the node and circulate in the peripheral blood. B cells differentiate into antibody-producing plasma cells.

IMMUNODEFICIENCIES

There are a number of primary immunodeficiencies associated with mutated genes affecting lymphocyte signaling and activation (Table 3). A sampling of known defects is described below.

Defects in TCR Proteins

Defects in the TCR–CD3complex or select protein chains have been reported. One subject had deficient αβ T cells and an excess of γδ CD3 cells that had limited antigenic specificity. Decreased expression of CD3 was reported in two patients. One subject had one tenth the amount of normal CD3 fluorescence and a mutation in the ε chain that rendered the molecule both abnormal and unstable. A second subject with low CD3 expression had impaired responses to select polysaccharides and an IgG2 deficiency.

Select defects in synthesis or assembly of CD3 γ or CD3 ζ chains were reported in several subjects. Such γ-chain defects in CD8 cells were associated with autoimmune hemolytic anemia, antiepithelial antibodies, and increased incidence of viral pneumonia (Stiehm, 1993).

Table 3. T-cell Membrane and Signaling Defects

Membrane defect	Biological effect
IL-2Rγ (IL-2 receptor)	X-linked SCID
CD3 γ CD3 ζ deficiency	Defective T-cell activation
X-HIM (CD40L)	Defective Ig switching
Kinase defects	
ZAP-70	CD8 deficiency
ATM	Phosphatidylinositol kinase
Jak3	SCID

Severe Combined Immunodeficiency Disease

SCID can be divided into three subsets. In the X-linked form of SCID, there are profound decreases in the number of circulating T and NK cells but B cells are in normal numbers.

In another form of SCID, there is a major defect in the y chain of the IL-2 receptor that prevents binding of IL-2. The y chain is part of the cell receptor for other cytokines such as IL-2, IL-4, IL-7, IL-9, and IL-15. Therefore, defects in the IL-2 receptor result in diminished reactivity of other cytokines. Studies on IL-7 receptor-deficient mice demonstrated that the defect in the IL-7 pathway is responsible for the diminished T cell development in patients with x-SCIDS.

The IL-2 y chain is critical in the initiation of lymphocyte proliferation by signaling through the Janus-family kinase *(Jak1* and *Jak3)*. Jak3 interacts directly with the *y* chain to initiate signal transduction. In some patients, the *Jak3* protein is also defective.

A third form of SCID is associated with the absence of CD8 cells and defective ZAP-70, a cytoplasmic tyrosine kinase (Stiehm, 1993; Fischer and Arnaiz-Villena, 1995).

Defects in Signal Transduction

Defects in signal transduction are the consequence of multiple defects (Table 3). Diseases and immunodeficiency can alter membrane proteins and signaling. For unknown reasons, active rheumatoid arthritis suppresses expression of CD3 and/or CD45. Sometimes, the expression of CD45 may be reduced by 60–70%.

Internal second messenger defects were reported in a subset of patients with common variable immunodeficiency. On stimulation, these patients fail to generate inositol-1,4,5-trisphosphate. Similarly, patients with type I diabetes have a messenger defect upstream of protein kinase C.

Defect in ZAP-70 Signaling

The ZAP-70 protein kinase has been reported to cause a unique immunodeficiency. This kinase is expressed on T cells and is associated with the CD3 ζ chain of the TCR complex. TCR and ligand binding trigger the Src kinases that phosphorylate ITAMs on the ζ chain. Tyrosine phosphorylation of proteins cannot occur in the presence of a defective ZAP-70. This results in a lack of mature CD8 cells in the peripheral blood and defective activation of CD4 cells (Fischer and Arnaiz-Villena, 1995).

Defect in CD40L Protein

Mutation in the gene coding for CD40L causes hyper-IgM syndrome (X-HIM), a severe immunodeficiency. Subjects have low levels of IgG, IgA, and IgE in serum but normal levels of IgM. Other anatomic defects are also present in humans and knock-out mice (Table 4). Patients suffer from increased upper respiratory tract infections and increased susceptibility to *Pneumocystis* and *Cryptosporidium.* Recurrent infections with these organisms are typically associated with T-cell defects (Fischer and Arnaiz-Villena, 1995).

CHEMICALS INTERFERING WITH INTRACELLULAR SIGNALING

Select chemicals can inhibit intracellular signaling. Wortmannin, an inhibitor of phosphatidylinositol 3-kinase, blocks anti-CD3-induced activation of the mitogen-activation kinase *Erk2* while not affecting phorbol-ester-induced Erk2 activation (Von Willebrand *et al.,* 1996).

At the level of PLC, the phorbol ester PMA can prevent cell activation and the expected increase in intracellular cytoplasmic calcium (Whelan *et al.,* 1992). Other studies in Jurkat cells showed that herbimycin A is a specific inhibitor of PTKs in T cells. Herbimycin A inhibits both the resting and induced levels of phosphotyrosine-containing proteins, including the PLC γ 1 isozyme and the ζ chain of the TCR (Graber *et al.,* 1992).

Bacterial products can also inhibit signaling. Pyocyanine isolated from *P. aeruginosa* inhibits T-cell proliferation by decreasing the production of the critical lymphokine IL-2 and by decreasing the expression of the IL-2 receptor. Local suppression of lymphocyte stimulation by phenazine pigments such as pyocyanine may interfere with cellular immune response to *P. aeruginosa* (Graber *et*

Table 4. Defects Reported in CD40L-Deficient Humans and Mice

Impaired macrophage activation
Increased susceptibility to intracellular parasites
Reduced IFN-γ and IL-12 production
Impaired T-cell priming and responses to peptide fragments
Reduced follicular dendritic cell network
Absence of germinal centers
Impaired Ig isotypic switching

Modified from Stout and Stuttles, 1997.

al., 1992). The toxin affects the TCR–CD3 complex itself by causing a covalent modification of the CD3 ζ subunit that prevents signaling (Haack *et al.,* 1993). Cholera toxin prevents inositol triphosphate release and the rise in intracellular Ca^{2+}.

HAZARD IDENTIFICATION

Initial hazard identification studies focus on the number of total T cells and T-cell subsets present in the test sample. Lymphocyte phenotyping is a sensitive endpoint for detection of immunotoxicity. Alterations in lymphocyte subset numbers correlated with *in vivo* immunotoxicity 83% of the time (Luster *et al.,* 1992a). These data should be expressed both as percentages and in absolute numbers. While the former yields some information, the latter is the more biologically relevant endpoint.

REGULATORY POSITION

Lymphocyte phenotyping is not required for registration of chemicals under FIFRA, TSCA, and the FDA's CFSAN. Although not currently required for European registration, phenotyping may be part of the revised guidelines suggested by the RIVM of the Netherlands.

Ex vivo proliferation of T cells is the endpoint of surface interactions and intracellular signaling. Proliferation measurements serve two purposes. Assessment of the nonspecific proliferative capacity can be determined by exposure to mitogens such as phytohemagglutinin or concanavalin A. When the mitogens are used over a dose range, subtle decrements in proliferation can be detected. The capacity of memory T cells to proliferate can be probed by exposure to tetanus toxoid, mumps, or *Candida albicans.* Most subjects have memory cells directed toward one or more of these preparations.

The NTP evaluated lymphocyte proliferation assays for inclusion in a proposed immunotoxicity screen. Data from the multilaboratory comparison showed that proliferation assays were insensitive regarding detection of immune defects and not reproducible across laboratories (Luster *et al.,* 1988).

8

B-Cell Activation

INTRODUCTION

During an immune response, B cells are stimulated and activated. Activation initiates a B-cell differentiation pathway that results in antibody-secreting plasma cells. Depending on the immunogen, B-cell activation may require cytokines produced by T helper cells. Thus, the reaction is a T-cell-dependent (TD) antibody response. Other immunogens can directly stimulate B cells with or without signals provided by non-T cells. Because this response does not require T cells, it is designated a T-cell-independent (TI) antibody response.

B-CELL ACTIVATION

In both TD and TI antibody responses, the initial intracellular signaling occurs via the B-cell receptor (BCR). B cells have receptors for Ig consisting of a four-member BCR complex. Flanking the Fc portion of the cell-bound mIgM are the heterodimer-associated molecules Igα and Igß (Fig. 1). In the mouse and human, these proteins are 34- and 39-kDa glycoproteins. Each Ig contains one putative immunoreceptor tyrosine-based activation motif (ITAM) of two evenly spaced tyrosine residues within a six-amino-acid sequence. The tyrosine residues can serve as targets for src-family kinases (PTKs). Iga directly associates with tyrosine kinase *lyn.* Protein kinase *syk* is also required for communication between the BCR and phospholipase Cy (Reth, 1995). Therefore, the mIgM-associated proteins may have signaling capabilities similar to the TCR (van Noesel *et al.,* 1993).

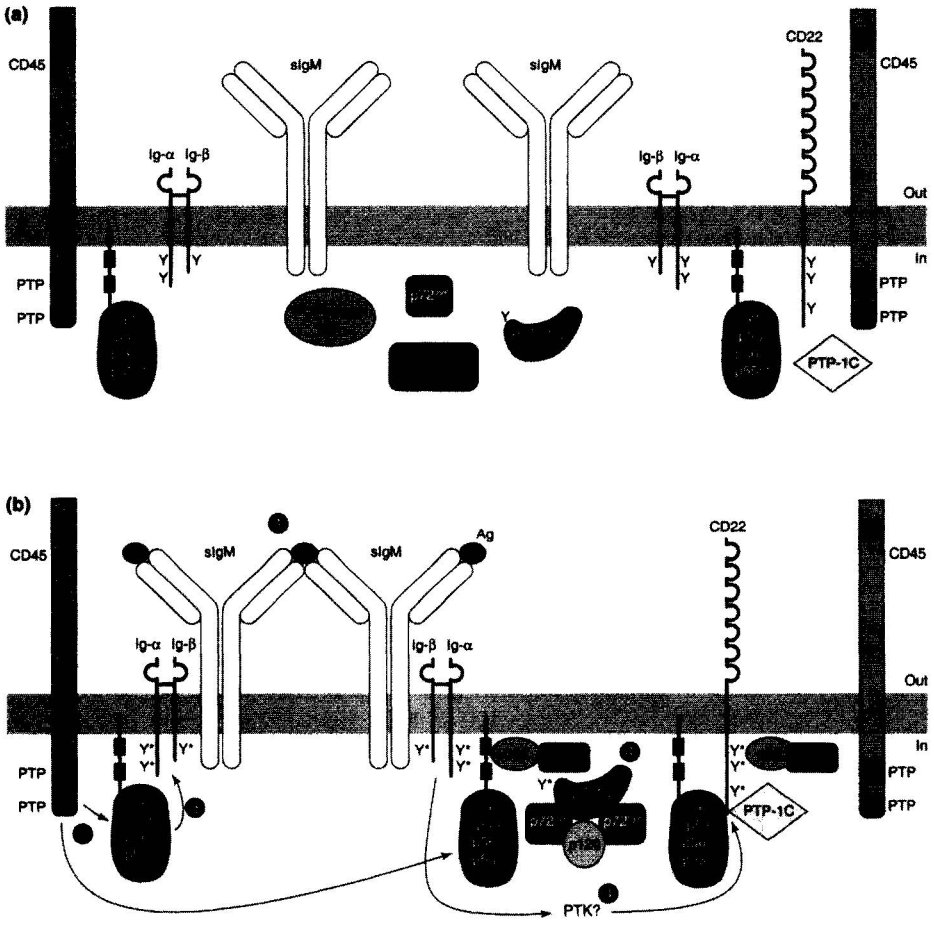

Figure 1. Interactions between the B-cell receptor complex (BCR) and associated molecules in resting B cells and in B cells activated via the BCR. (a) In resting cells, the BCR consists of surface IgM (sIgM) molecules and Igα-Igβ heterodimers, associated src-family protein kinases, and the transmembrane proteins CD22 and CD45. (b) Antigen-induced cross-linking of the BCR initiates metabolic events that result in the formation of signaling complexes. The src-family kinases (p53/p56lyn or p55blk) are activated by dephosphorylation induced by CD45. Activated p53/p56lyn or p55blk phosphorylate Igα-Igβ which can interact with p53/p56lyn or p55blk at higher affinities to activate the enzymatic process. Activated p53/p56Iyn or p55bIk also forms complexes with phosphoinositide 3-kinase (PI 3-kinase) and p72syk. This interaction may send a mitogenic signal to B cells or regulate B-cell trafficking. Reprinted from *Immunology Today,* Law *et al.,* 1994, 15:442–8, with permission of Elsevier Science.

T-CEL L-DEPENDENT B-CELL ACTIVATION

After the T cell interacts with processed antigen on APCs, a tight complex of T and B cells is formed. Complex formation is followed by a unique orientation of the Golgi apparatus and the microtubules toward the B cell.

Complex formation also causes a redistribution of surface proteins. The TCR, CD4, and LFA-1 become redistributed and aggregate at the junction of the T and B cell membranes. It is known that CD4 and LFA-1 are adhesion molecules that bind to ICAM on the B cell surface. The interactions between the adhesion molecules may increase the avidity of the T–Bcell interactions, and allow more efficient signaling between cells and a directed secretion of cytokines.

The interaction between T and B cells also leads to the expression of new proteins necessary for B-cell activation. One protein involved in the activation process is CD40. This transmembrane glycoprotein consists of 40- and 48-kDa heterodimers and has four repeating cysteine-rich domains. Based on structural homologies, CD40 is considered to be a member of the TNF superfamily. Other members of the TNF family are tumor necrosis factor and Fas. It has been postulated that the TNF family of proteins regulates cellular proliferation and apoptosis (Noelle *et al.,* 1992).

Activated T helper cells express gp39, the CD40 ligand (CD40L). CD40L is a 33- to 35-kDa glycoprotein consisting of three noncovalently bound proteins (Fig. 2). Interactions between CD40 and gp39 are independent of antigen and MHC interactions. Interactions between CD40 and its ligand cause B cells to

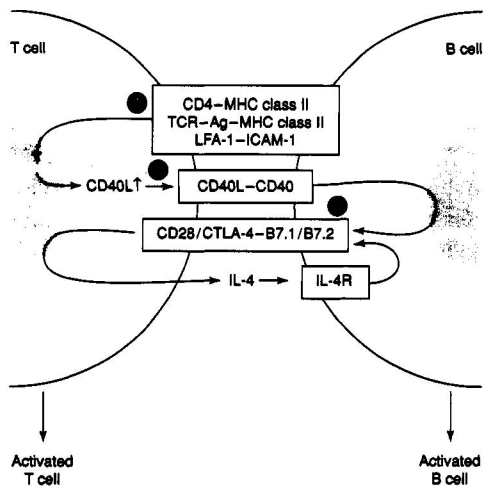

Figure 2. The sequential expression of CD40L, B7.1, and B7.2 on T and B cells. T and B recognition requires MHC-restricted interactions involving ligation of the TCR (stage 1). This leads to upregulation of CD40L expression on T cells and interaction with CD40 on B cells (stage 2). Signaling via CD40 in combination with T-cell-derived IL-4 binding to the IL-4 receptor results in the expression of B7.1/B7.2 (stage 3). This results in B cells becoming competent APCs that can activate T cells by binding of CD28/CTLA-4 to B7.1 /B7.2. Reprinted from *Immunology Today,* Dune *et al.,* 1994, 12406–10, with permission of Elsevier Science.

undergo rapid growth that prepares them for stimulation by cytokines (Durie *et al.,* 1994).

Intracellular signaling is also a function of CD40. TRAF3 (CD40bp, CRAF1) and TRAF2 bind to the cytoplasmic domain of CD40. The TRAF family of enzymes upregulates other tyrosine kinases (e.g., *src* and *syk)* that rescue B cells from apoptosis.

Apoptosis of B cells is prevented by the upregulation of the protooncogene bc1-2 and family members bc1-x (long). Interactions between CD40 and B cells in an antigen recognition combination upregulate the apoptosis-inducing Fas ligand on B cells. In activated B cells, reaction of Fas with the ligand fails to induce apoptosis. In contrast, interaction of CD40 with resting, non-antigen-activated or bystander cells activates the Fas ligand and the intracellular nuclease bax-2. Interaction of the Fas ligand with Fas and the degradation of nucleic acid cause a rapid apoptotic event.

Other signals are necessary for elicitation of a TD antibody response. Following stimulation of the BCR, interactions occur between CD45 on T cells and CD22 on B cells. Tyrosine phosphorylation of CD22 allows binding of *syk* and signal transduction. The recruitment of *syk* is essential to the activation of phospholipase Cγl and PI-3 kinase. The latter kinase may associate with the BCR–mIgM by interacting with *src* kinases such as *lyn* (Law *et al.,* 1994).

Activation of the protein tyrosine phosphatase and protein tyrosine kinases (e.g., *src* and *syk)* upregulate nuclear transcription factors including NF-KB, AP-1, and NF-AT. Removal of an InK-α inhibitory protein from NF-KB allows translocation to the nucleus and binding to the κB-RE gene in the enhancer region. This gene region controls entry into cell division and it initiates transcription of the AP-1 complex containing the oncogenesfos and *jun.* These oncogenes regulate the production of cdk4, cyclin D, and the *myc* gene product that are necessary for initiation of cell division.

TD Antigens

Several immunogens elicit a classical TD response. The production of antibodies to sheep red blood cells requires interactions between T cells, B cells, and macrophages plus the production of cytokines. In humans, pokeweed mitogen-induced stimulation and proliferation of B cells requires the presence of small number of T cells and macrophages.

THYMUS-INDEPENDENT B-CELL ACTIVATION

Many immunogens can directly cross-link the BCR–mIgMcomplex. Generally, these immunogens have multiple identical epitopes or repeating subunits and are not normally processed in the antigen presentation pathways.

With some exceptions, these immunogens fall into two groups. The first group consists of polysaccharides, glycolipids, and nucleic acids. The second group consists of various lipopolysaccharides (LPS) liberated from the cell walls of gram-negative bacteria.

Using an alternate nomenclature, these immunogens are designated TI type 1 or 2 depending on the necessity for second signals in B-cell activation. Type 1 TI immunogens such as LPS, neisserial porin proteins, and bacterial DNA do not need a second signal to initiate antibody production (Snapper and Mond, 1996). With the type 1 antigens, the nature of the response is dose dependent. For example, low concentrations of LPS initiate production of anti-LPS antibody. At high doses, LPS is a polyclonal B-cell stimulator producing antibodies to a variety of antigens.

Type 2 polysaccharides and glycolipids stimulate B cells but require a small number of macrophages and NK cells producing IL-12 and IL-15. These cytokines direct the differentiation and recruitment of Th1 cells that secrete IL-2. In addition, polysaccharide-activated NK cells secrete IFN-γ and GM-CSF. These cytokines directly stimulate proliferation of B cells. Antibodies resulting from type 2 immunogen stimulation are usually low-affinity, anticarbohydrate antibodies called *natural antibodies* (Snapper and Mond, 1996).

Often TI immunogens have attached complement components. When complement is part of the complex reacting with the BCR, other molecules can associate with the BCR and assist in the signaling process. CD19, CD81 (receptor for complement fragment C3d), TAPA-1, and Leu-13 form multimolecular complexes with the BCR (Fig. 3). The CD19, CD81, Leu-13 complex functions as a signal transduction complex for the complement receptor (Tedder *et al.,* 1994). Phosphorylation of serine kinase is the major signaling pathway. Alternatively spliced forms of the *src* kinase *lyn* form stable complexes with CD19 initiating intracellular signaling (van Noesel *et al.,* 1993). The CD19–CD21–TAPA-1 mechanism is distinct from the BCR signaling. Signaling does not require the *syc* protein for activation and, by virtue of nine tyrosine residues in the CD19 tail, it can bind similar and related kinases (Fig. 4).

REGULATION OF THE B-CELL RESPONSE

Regulation and/or termination of a B-cell response involves the CD40L receptor and the presence of antigen. Cross-linking of mIgM by antigen stimulates the B cell and initiates the expression of Fas protein on the cell surface. Fas interaction with the FasL causes apoptotic death of B cells. Apoptosis is prevented if the antigen-stimulated cell receives a second signal provided by CD40–CD40L interactions. Fas expression is not prevented. Rather, the signal renders the B cell resistant to the effect of FasL interactions (Scott *et al.,* 1996).

Signaling via CD40 in combination with T-cell-derived IL-4 binding to the

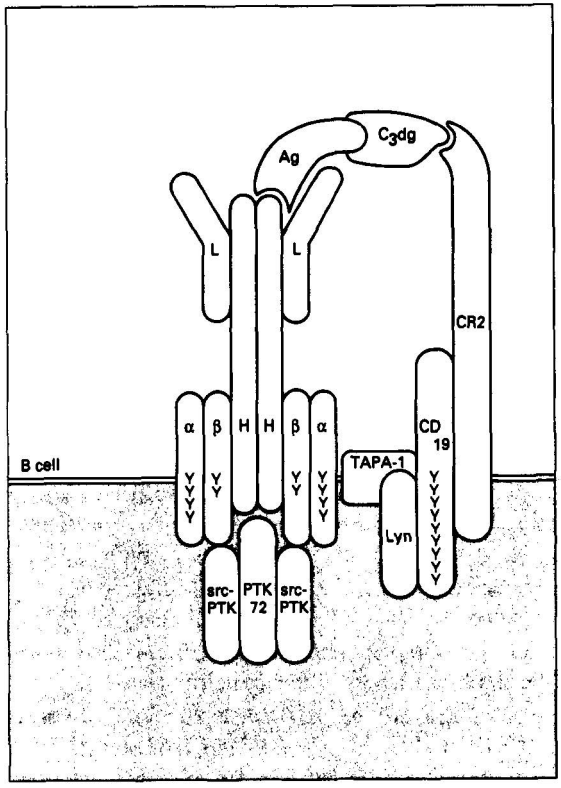

Figure 3. Model for dual antigen recognition by B cells. A complex of antigen (Ag), complement (C3dg), and the BCR induces multimerization of the complex and aggregation with CD19–TAPA-1 –CR2 complex. Multimerization allows cross-phosphorylation and activation of the src-like protein tyrosine kinase PTKlyn and PTK72. Activated forms of these phosphorotransferases may be responsible for complexing to second messenger proteins via phosphorylation of tyrosine residues (Y) in the cytoplasmic regions of Igα-Igß and CD19. Reprinted from *Immunology Today,* van Noesel *et al.,* 1993, 14:8–12, with permission of Elsevier Science.

IL-4 receptor on B cells upregulates the expression of B7–1 and B7-2. Additional interactions between CD28 and CTLA-4 renders the B cell a fully competent immunogen-presenting cell. In addition, the CD28–ligand interaction stimulates the T cell to produce additional cytokines necessary for antibody production, germinal center expansion, isotypic switching, and induction of memory cells.

CD40 may also play a role in regulating late phases of the immune response. When clonal expansion has reached a maximum late in the response, the interac-

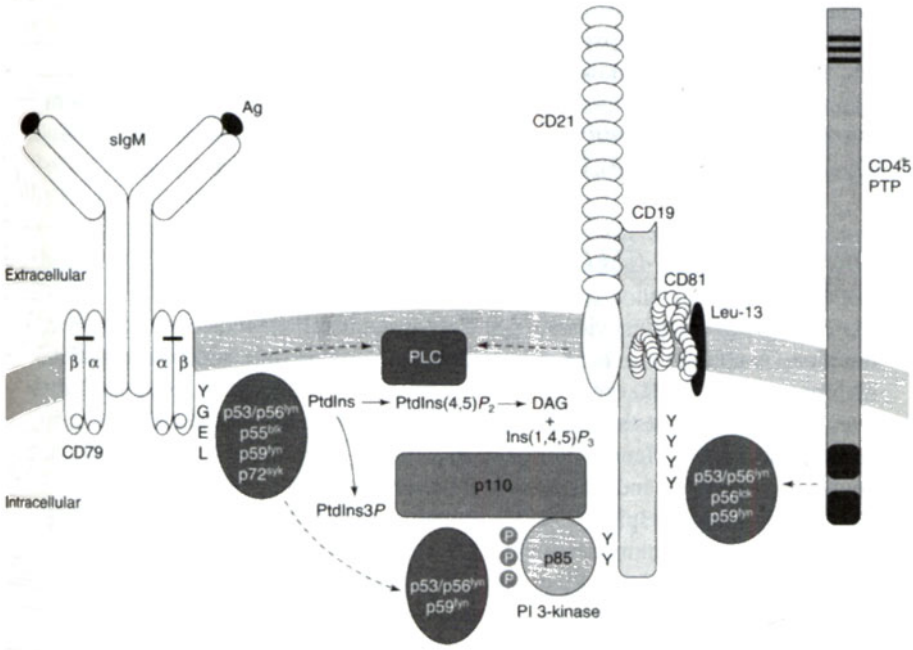

Figure 4. Proposed signal transduction pathway activated by CD 19–CD21 receptor complex. Dashed arrows indicate potential signal transduction pathways. Reprinted from *Immunology Today,* Tedder *et al.,* 1994, 15:437–41, with permission of Elsevier Science.

tion of CD40 and CD40L causes an upregulation of the Fas on unstimulated or naive B cells. At the same time, TCR activation of immature T cells and Th1 cells initiates the expression of Fas and the production of FasL. The latter reacts with Fas to induce B-cell apoptosis. T cells that do not interact with B cells via the CD40 ligation commit suicide via FasL-induced apoptosis. Similarly, stimulated B cells that do not receive the CD40 signal are "murdered" by T-cell-produced FasL. In this manner, further clonal expansion of T cells is limited (Scott *et al.,* 1996).

CYTOKINE PRODUCTION BY B CELLS

B cells are potent cytokine producers. On a cell-to-cell basis, B cells produce cytokines in amounts similar to or higher than monocytes or T cells (Pistoia, 1997). B-cell-produced cytokines have paracrine effects on various cell types or autocrine effects on B cells. Examples of paracrine effects are (1) macrophage or follicular dendritic cell activation by IFN-γ, TNF, IL-6, or GM-CSF; (2) NK cell activation by IFN-γ, IFN-α, or IL-12; (3) recruitment of inflammatory cells by

IL-8, G-CSF, or GM-CSF; and (4) costimulation of T-cell growth by IL-1α, IL-1ß, TNF, and IL-6 (Pistoia, 1997).

B-cell-derived cytokines can also stimulate other B cells in an autocrine manner. Early B-cell growth can be stimulated by recombinant (r) IL-7, while late B-cell growth and differentiation are stimulated by rTNF, rLT-α, rIL-2, rIL-4, rIL-10, and rIL-13 (Pistoia, 1997)

DRUGS AND B-CELL ACTIVATION

A number of drugs, vitamins, and environmental chemicals can alter B-cell function. Amphotericin B (AmB) and two of its derivatives (N-Fru AmB and N-thiopropionyl AmB) are potent activators of B lymphocytes. The two derivatives are more potent than the parent compound and act by induction of membrane polarization (Henry-Toulme *et al.,* 1989). Retinoic acid, the parent compound of vitamin A, induces a 50-fold increase in IgM synthesis (Ballow *et al.,* 1996; Ballow and Weiping, 1995) in human cord blood stimulated with *S. aureus* Cowans I strain. Dioxin treatment of B cells increases the resting intracellular calcium level and induces kinase activity. These signals render the B cells less responsive to antigen or mitogen stimulation (Karras and Holsapple, 1994)

Some flavinoids have profound effects on the function of immune and inflammatory cells. This family of chemicals have multiple effects on cells including inhibition of the B-cell secretory process, mitogenesis, cell–cellinteraction, and expression of adhesion molecules (Kandaswani, 1992).

HAZARD IDENTIFICATION

B cells undergo a defined series of events prior to production of protein antibodies. Stimulation of the BCR in concert with other receptor–ligandalters membrane flux. Increased membrane permeability allows calcium to enter the cell where it regulates or activates cellular processes. Fluorescent Indol- 1AM dye can be used to demonstrate increases in calcium flux (Ranson *et al.,* 1986). After activation, B cells undergo a rapid proliferative event that induces differentiation into antibody-producing plasma cells. B-cell proliferation can be measured as [^{3}H]thymidine incorporation following stimulation with bacterial LPS endotoxins, pokeweed mitogen (PWM), or *S. aureus* Cowans I strain.

The nature of the test species often determines whether the response is TD or TI. Endotoxins are usually T cell independent in both mouse and human. Murine mitogenesis induced by PWM is thymus independent. In contrast, PWM stimulation of human cells requires the presence of a small number of T cells, macrophages, and cytokines (Greaves *et al.,* 1974; Keightley *et al.,* 1976). Most

of the B cells responding to PWM are IgD– B cells considered resting memory cells. Only a small number of uncommitted IgD+ cells producing IgM are stimulated (Jelinek *et al.,* 1986). Bacteria such as *S. aureus* Cowans I also stimulate proliferation in B cells. Proliferation of B cells occurs in a TI manner, but cytokines are required for B-cell maturation into APCs (Falkoff *et al.,* 1982). Both IgD– and IgD+ B cells respond to *S. aureus* Cowans I with proliferation of IgG, IgA, and IgM antibodies.

Rat B cells pose a technical problem in B-cell proliferation studies. They do not respond vigorously to most B-cell mitogens including endotoxins (Vos *et al.,* 1984). The most potent rat B-cell mitogen is a water-soluble extract from *Salmonella typhimuriurn* (Minchin *et al.,* 1990). However, cells respond to this mitogen in a unique manner. Responses are observed at extremely low levels and there is a plateau effect. Only one study has used *S. typhimuriurn* to study the effect of chemicals on the immune system (Smialowicz *et al.,* 1991).

REGULATORY POSITION ON B-CELL ACTIVATION

Proliferation induced by LPS endotoxin is a common endpoint in toxicology studies. However, the assay is not very sensitive because cells can recover function in the 3–5 days necessary to induce proliferation. Moreover, the assay is not reproducible in the laboratory, making the data difficult to interpret. Because of the technical difficulties, there is only a 50% concordance between decrements in LPS-induced proliferation and those in biologically relevant *in vivo* effects (Luster *et al.,* 1992a; 1993). Despite the lack of sensitivity in the lymphocyte proliferation assays, the FDA's Center for Food Safety and Applied Nutrition (CFSAN) has included a B-cell proliferation assay in a screening protocol for product safety assessment. In evaluation of immunotoxic potential, both reduced and elevated proliferation are of interest to the Center (Hinton, 1992).

9

Antibodies

INTRODUCTION

Antibodies or immunoglobulins are protein globulins found in the serum and various other external secretions; their production is the result of stimulation by an immunogen. They can react specifically with the antigen stimulating synthesis. In the older literature, production of antibodies is termed a *humoral* response. There are several different antibody families or isotypes. Each isotype appears at a different point in time during an immune response and has different functions.

STRUCTURE OF ANTIBODIES

Antibodies are glycoproteins with two pairs of heavy and light peptide chains. The heavy (H) chains have a molecular mass of approximately 50 kDa each. Disulfide linkages at critical cysteine residues link the heavy chains. Other disulfide bonds link light (L) chains (25 kDa) to the H chains. An immunoglobulin molecule has one pair of identical H chains and one pair of identical L chains (Fig. 1). The antibody combining sites are located at the N-terminal end of the heavy and light chains and each immunoglobulin molecule has at least two antigen reactive sites. The C-terminus of the H chains can bind to receptors on the surface of B cells, monocytes, and other APCs.

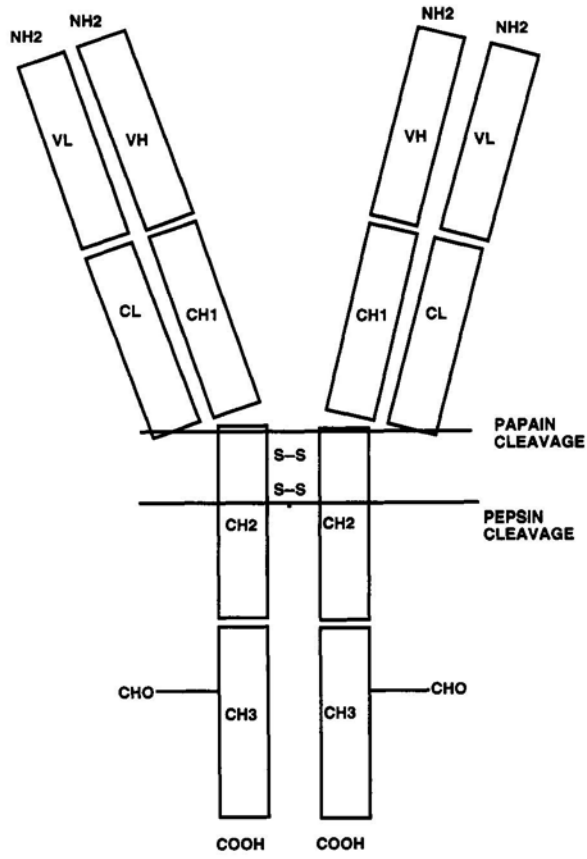

Figure 1. The basic dimer (H–L) structure of an immunoglobulin.

Enzymatic Cleavage of An tibodies

The functional components of antibodies have been delineated by enzymatic digestion. Papain cleaves the H chains at a point above the interchain disulfide linkage. Two functional subunits called *Fab* fragments are liberated. The Fabs consist of the entire L chain and the N-terminal end of the H chain. Antibody combining sites for antigen are contained within the Fab fragment. Because the two Fab fragments are not linked together by a disulfide bond, the Fabs are univalent and can bind antigen separately. The remaining portions of the two H chains are linked together to form a unit called the *Fc fragment.* The Fc portion of antibodies binds to receptors on the surface of immunocompetent cells.

Pepsin cleaves the antibody at a different point on the protein. The C-terminus or Fc portion is digested below the disulfide bridge creating a molecule with no Fc portion and functionally bivalent antibody combining sites.

ANTIBODY ISOTYPES

Several different antibody isotypes, differing both in structure and in biological function, have been described. In the older literature, antibodies were called *gamma globulins* or *7 S globulins* based on the rate of migration in an electrophoretic field or sedimentation rates in an ultracentrifuge, respectively.

There are four major antibody isotypes differentiated by the chemical structure of the H chain (Fig. 2). Therefore, γ H chain antibodies are termed IgG, μ chains are found in IgM antibodies, α chains are a component of IgA antibodies, and IgE antibodies have ε chains.

In addition to the H chains, there are two types of L chains: kappa (κ) or lambda (λ). A single antibody molecule is composed of isotypic H chains and

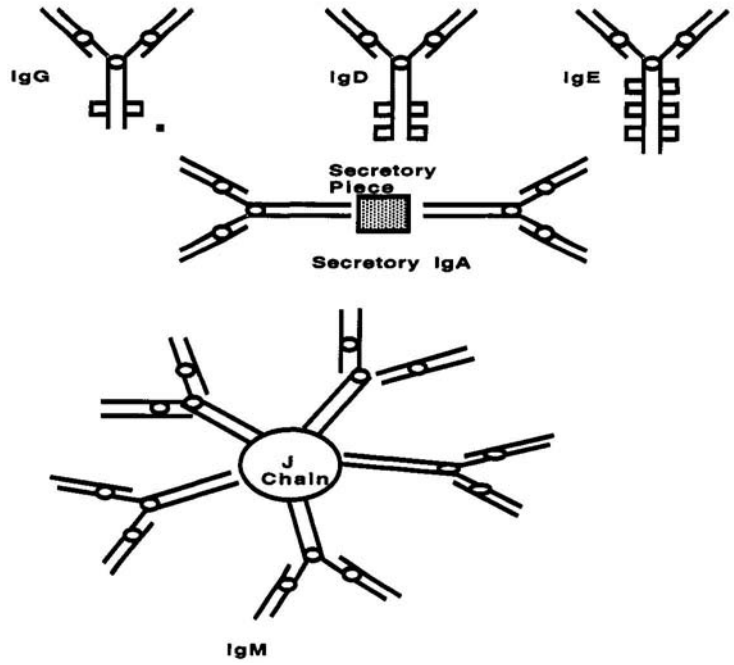

Figure 2. Schematic of antibody isotypes in blood and saliva. Blocks denote glycosylation points.

either κ or λL chains, but not both. In a normal antibody population, antibodies of the same isotype will have κ or λchains.
Based on amino acid sequencing studies, antibody structure can be broken down into variable and constant portions. The N-termini of the H and L chains are variable and are designated the V_L and V_H portions of the antibodies. Conversely, the C-termini of the H and L chains have a constant amino acid structure. The L chain has only one constant domain but the γ and μ chains have three different constant domains called C_H1, C_H2, and C_H3. The α and ε chains have an additional constant C_H4 domain at the C-terminus.

Immunoglobulin M

IgM antibody is produced in the early phase of the immune response. This isotype is the first line of defense against extracellular blood-borne pathogens. IgM is composed of five basic units of heavy chains (μ) and light chains (κ or λ) covalently linked together by a peptide known as the *J chain.* The five-unit IgM molecule has a molecular mass of 970 kDa. Because IgM has ten antigen-combining sites, it can, on a mole-to-mole basis, bind more antigen than other isotypes. However, the antigen-binding affinity is much lower than for other antibody isotypes. In the serum, the normal range for IgM is 85–350 mg/100 ml.

Heterologous antigens and natural antigens elicit an IgM response. These antigens include rheumatoid factors, cold agglutinins, heterophile antigens, single- and double-stranded DNA, normal isoagglutinins of human blood groups, leukoagglutinins, thyroid antigens, *Salmonella typhosa* "O" antigens, and the Wassermann antigen found in the spirochete causing syphilis.

Immunoglobulin G

The IgG class of immunoglobulins is produced during a secondary (anamnestic) immune response. This antibody class possesses a greater antigen-binding affinity than IgM. IgG antibodies consist of two H chains and two L chains and have only two antigen-combining sites. IgG accounts for nearly 70–75% of the total antibody found in serum. Normal adult serum levels range from 640 to 1350 mg/100 ml.

There are four subclasses of IgG that vary in the size of the hinge region, position of interchain disulfide bonds, and molecular mass. Subclass IgG_3 is 170 kDa, which is higher than for most IgG molecules and reflects a longer γ chain with more cysteine residues. All other IgG subclasses have a molecular mass of 146 kDa.

The IgG subclasses have different biological functions and have different roles in the immune response. IgG_1 and IgG_3 are usually produced in response to protein antigens (Table 1). Functionally, they can activate the classical comple-

Table 1. Functional Characteristics of IgG Subclasses

	Receptor binding	Complement activation
IgG 1	+	+
IgG2	−	−
IgG3	+	+
IgG4	−	−

ment pathway and bind to cellular Fc receptors. IgG_2 and IgG_4 are produced in response to carbohydrate immunogens. They do not activate the complement pathway and cannot react with Fc receptors (Hammarstrom and Smith, 1986).

Human infants do not begin to produce their own IgG in appreciable amounts until about 7 weeks of age, although at birth they possess high amounts of maternal IgG acquired transplacentally. The maternal IgG in the neonate has a half-life of approximately 45 days.

Production of the IgG subclasses varies with age. IgG_1 and IgG_3 are usually produced early in life. However, because of a developmental block, IgG_2 is often not produced until infants are 18 months of age.

This developmental block is taken into consideration in pediatric immunization strategies. Vaccinations for haemophilus and pertussis are usually performed at 18 months of age. The IgG_2 directed toward the carbohydrate antigens on pertussis and haemophilus is critical to the development of protective immunity.

Immunoglobulin A

IgA, consisting of α heavy chains, makes up about 15–20% of the normal pool of immunoglobulins. It is found in different forms in the serum or external secretions. IgA found in blood is either the basic H–L units or dimers of basic H–L units bound together by J chains.

In humans, there are two subclasses of IgA (IgA1 and IgA2). In the serum, 90% of the IgA is IgA 1, whereas 60% of the IgA in secretions is IgA2. Tears and colostrum contain the highest concentration of IgA2.

The secretory (s) form of IgA is found in body secretions such as saliva, tears, and colostrum. sIgA in external fulids consists of the basic H–L dimers with an attached secretory piece (70 kDa). The secretory piece, a glycoprotein member of the Ig superfamily, is coiled around the Fc portions of the dimers. It functions to inhibit enzymatic degradation of the sIgA in the external or internal, acid environments.

sIgA antibodies serve as the first line of specific defense against microorganisms that invade the respiratory and gastrointestinal tracts. This antibody isotype is particularly important in defense against *Salmonella, Vibrio cholerae,* and *Neisseria* species. IgA does not fix complement by the classical pathway. It does not pass the placenta and so is not detectable at birth, although free transport piece may be present. Human infants are capable of synthesizing IgA at 2–3 weeks of age.

Immunoglobulin D

IgD accounts for less than 1% of the total circulating immunoglobulin pool (3 mg/100 ml serum). Although its role in serum is uncertain, IgD is an important part of B-cell differentiation prior to steps leading to class-specific clone development.

Immunoglobulin E

The IgE class has a chemical structure similar to IgG in that it consists of the basic H–L structure. Its molecular mass is higher than IgG because the heavy chains (ε) have an extra constant domain (C_H4). IgE is also unique in having a high carbohydrate content—12% versus 2–3% for the IgG subtypes.

IgE is associated with immediate allergic reactions such as asthma, rhinitis, and urticaria. Using Fc ε receptors, IgE attaches to fixed mast cells, and basophils in the blood.

IMMUNODEFICIENCIES

There are a number of familial syndromes associated with hypogammaglobulinemia (Table 2). Many of these syndromes reflect defects in isotypic switching or production of specific antibody isotypes.

Selective IgA Deficiency

IgA deficiency is the most common of all immunodeficiencies. Depending on the country, the incidence is between 1:400 and 1:3000. There is an increased incidence of IgA deficiency in subjects with defects in chromosome 18, connatal rubella syndrome, and ataxia telangiectasia (Mietens, 1983). Associated with the deficiency are recurrent bacterial infections, bronchitis, sinusitis, pneumonia, allergies, and autoimmune diseases (Schaffer *et al.,* 1991). B-cell differentiation arrest preventing isotypic switching from IgG to IgA is the central defect in the immunodeficiency.

Table 2. Familial Syndromes Associated with Hypogammaglobulinemia

X-linked agammaglobulinemia
Common variable immunodeficiency
Immunodeficiency with hyper-lgM
Cyclic neutropenia
Shwachman syndrome
Ataxia telangiectasia
Reticular dysgenesis
Short-limbed dwarfism
Partial albinism and immunodeficiency
WHIM syndrome

Modified from *Pediatric Research,* Stiehm, 1993. 33:S2-7, with permission of Pediatric Research.

Common Variable Immunodeficiency

CVID is a major antibody immunodeficiency characterized by recurrent upper, lower respiratory tract infections and, in a few patients, granulomatous disease (Cunningham-Rundles, 1994). Over 20% of the patient populations also develop autoimmune diseases (Vetrie *et al.,* 1993).

Based on the number and function of B cells in the peripheral blood, patients can be segregated into three groups. A small group of patients lacks B cells in the blood and has only scattered bone marrow B cells. These subjects are classified as Bruton's type X-linked agammaglobulinemia (XLA). The major phenotypic defect is a mutation in the Bruton's kinase gene *(Btk).* Over 120 point mutations of *Btk* have been described (Vihinen *et al.,* 1995).

Btk is activated following cross-linking of surface immunoglobulin. A pleckstrin-homology domain binds to G-protein subunits and the proline-rich portion binds to the *src* homology domains of the *src* protein family. In addition to controlling isotypic switching, the *Btk* gene controls other critical functions such as light chain VDJ rearrangements and differentiation of B cells.

The second patient group has reduced numbers of B cells and defects in the synthesis of IgG and IgM. These patients present with granulomatous disease resembling sarcoid. Granulomata may be found in the spleen, lymph nodes, lungs, skin, and brain. The histology is characteristic of noncaseating epithelial granuloma (Spickett *et al.,* 1990).

A third group of patients are categorized as hyper-IgM (HIM). Subjects have recurrent upper and lower respiratory infection, otitis, diarrhea, and ulcers. Patients do not respond to normal vaccines (Notarangelo *et al.,* 1992). From a mechanistic perspective, HIM results from mutations in the CD40L (gp39) that preclude interactions with CD40.

B cells from these patients do not produce either IgG or IgA. IgM can be produced *in vitro* but not *in vivo.* Because only IgM can be produced, there are recurrent upper respiratory infections and infections with opportunistic pathogens such as *Cryptosporidium* or *Pneumocystis carinii.* Moreover, some IgMs are autoantibodies reacting with host red cells, leukocytes, and platelets. The destruction of these blood cells leads to secondary immunodeficiencies and reduced resistance to infections (Fischer and Arnaiz-Villena, 1995).

DISEASES AND ANTIBODY SYNTHESIS

Different clinical conditions and diseases affect the synthesis of antibodies. Acute and chronic viral infections, especially those with Epstein–Barr virus and cytomegalovirus, can decrease synthesis. Similar decreases have been reported in subjects with collagen vascular disorders such as SLE. Malignancies of the immune system (e.g., leukemia, lymphoma, myeloma, and thymoma) also decrease the numbers or function of antibody-secreting cells.

BACTERIAL EVASION OF THE ANTIBODY RESPONSE

Some microorganisms can circumvent the effects of antibodies. Bacterial serine protease activity cleaves human IgA1 at the hinge region. Cleavage separates the monomeric combining sites from the Fc, thus inactivating the antibody. Highly active proteases are produced by the three leading causes of bacterial meningitis (e.g., *H. influenzae, N. meningitis,* and *S. pneumoniae)* and urogenital pathogens.

Other bacteria frequently change the surface immunogens presented to the immune system. Pilins, which are required by *N. gonorrhoeae* for attachment to epithelial cells, constantly mutate and change the protein structure.

ENVIRONMENTAL CHEMICALS AND ANTIBODY PRODUCTION

The effects of metals and environmental chemicals on antibody production have been reported using mice, rats, guinea pigs, and rabbits (Tables 3 and 4). With few exceptions, the mouse is the most sensitive species. Guinea pigs and rabbits were the least responsive to the immunosuppressive effects of chemicals.

Table 3. Select Metals that Alter Antibody Production

	Mouse	Rat	Guinea pig	Rabbit
Lead	+	+	−	+
Cadmium	+	+	−	+
Mercury	+	−	−	+
Selenium	+	+	−	−
Nickel	−	+	−	+
Zinc	+	−	−	−

Modified from *Immunology and Allergy Practice,* Koller, 1985, 7:13–25.

MONOCLONAL ANTIBODIES

Murine Monoclonal Antibodies

Early experiments by Kohler and Milstein (1975) showed that it was possible to produce antibodies in the test tube by fusing immunized murine splenic lymphocytes with an antibody-producing cell line (Kohler and Milstein, 1975). Technology has been modified and extended so it is also possible to produce antibodies following rabbit–mouseor hamster–mouse fusions (Kohler and Milstein, 1975; Raybould and Takahashi, 1988).

Monoclonal antibodies represent a homogeneous reagent useful in both *in vitro* and *in vivo* studies. Each antibody has the same structure, affinity, and avidity for the antigen that elicited its production. Because the antibodies are

Table 4. Select Chemicals that Alter Antibody Production

	Mouse	Rat	Guinea pig	Rabbit
TCCD	+	−	+	−
Tetrachlorodibenzofuran	+	−	−	−
Pentachlorophenol	+	+	−	−
Polychlorinated biphenyl	+	+	+	+
Polybrominated biphenyl	+	−	−	−
Benzo[a]pyrene	+	−	−	−
Dimethylbenz[a]anthracene	+	−	−	−
Methylcholanthrene	+	−	−	−
Urethane	+	−	−	−
Phorbol acetate	+	−	−	−
Lindane	+	−	−	−
Toxaphene	+	+	−	−

Modified from *Immunology and Allergy* Practice, Koller, 1985, 7:13–25.

produced in cell lines, the latter represent an infinite source of reagents. Cell lines can be frozen for several decades and reconstituted to produce antibody.

The technique for fusion and selection is extremely simple but tedious and time consuming. Briefly, immunized murine spleen cells are fused with a tissue-culture-adapted cell line from the same or alternate species (e.g., mouse, rabbit, or hamster). Hybrid cells are positively selected by the addition of hypoxanthine, adenine, and thymidine (HAT) to the culture medium. Only hybrid cells have a nucleic acid salvage pathway that will grow in the HAT medium. Surviving cells are expanded and cloned to a single cell level. Cloning is required to ensure that the antibody is secreted from the progeny of a single cell.

Cells are cloned in soft agar and each clone is transferred to a 96-well microtiter plate. Poisson statistics show that less than 22% of the wells have growing cells when cells are seeded at 0.3 cell/well. If the cloning efficiency is 100%, then 88% of the wells would have a single cell. Wells containing a single cell are expanded and tested for antibody production. Normally, it takes 4 to 6 months to generate a reagent grade monoclonal antibody.

Humanized Monoclonal Antibodies

Murine monoclonal antibodis have been used in the treatment of several malignancies. Howver, efficacy was often reduced because the murine protein was immunogenic in humans. To reduce the immunogenicity of monoclonal antibodies, humanized monoclonal antibodies have been constructed (Shearman *et al.,* 1991: Adair *et al.,* 1994). Using the genomic fragments from a single round of PCR, humanized antibodies can be produced by inserting the murine H and L chain hypervariable V regions into a mammalian expression vector containing the human κ and γ IC exons (Shearman *et al.,* 1991). Generally, IgG2 is used in the formation of humanized antibody because it does not fix complement or bind to cells via the Fc receptor.

Bispecific Monoclonal Antibodies

Bispecific antibodies that have two different antigenic specificities can be created by hybridoma fusion or fusion of antibodies production by classical hybridomas. In the hybridoma fusion method, two hybridoma cell lines are fused to produce a quadroma. Typically, the technology takes advantage of drug marker cell lines having distinct drug sensitivities or chemical fusion [e.g., HAT(s)/Neo(r) and HAT(r)/Neo(s) are fused, positively selected in the proper media (De Lau *et al.,* 1989). and expanded either *in vivo* or *in vitro.]* Although the production of antibodies is simple, purification of bispecific antibodies poses a major technical challenge. Only 5–15% of the total antibody population is bispecific, reflecting the fact that coupling of homologous H chains (De Lau *et al.,* 1991) occurs at a much higher frequency than two different H chains. This

small amount of bispecific antibody must be separated from most of the antigenically inactive but structurally similar antibodies.

Chemical Synthesis of Bispecific Antibodies. Monoclonal antibodies, Fab' or $F(ab')_2$ fragments can be coupled to form bispecific antibodies. The most common linkage method involves covalent binding via SH groups in the hinge region or ε amino groups. Cross-linkage of antibodies or Fab' fragments can be facilitated by succinimidyl-3-(2-pyridyl-dithiol)-propionate (SPDP). Although the reaction readily activates SH groups, a population of randomly cross-linked antibodies with different specificities is created. This limits the utility of the SPDP-linked antibodies (Fanger *et al.,* 1992). Increased specificity can be achieved by linkage of different purified Fab' fragments with 5,5'-dithiobis(2-nitrobenzoic acid) or o-phenylenedimaleimide (Glennis *et al.,* 1987; Brennan *et al.,* 1985). Using o-phenylenedimaleimide, it is possible to add a third fragment to form trispecific antibodies (Tutt *et al.,* 1991). Bi- and trispecific antibodies have limited clinical use because they have increased immunogenicity and reduced *in vivo* half-life.

ADVERSE EFFECTS OF ANTIBODY ADMINISTRATION

Polyclonal, monoclonal, and chimeric antibodies have been used for treatment of cancers, autoimmune diseases, clotting disorders, infectious diseases, and graft rejection (Fanger *et al.,* 1992). Administration of all forms of antibodies evokes an antibody response after 1–2 weeks of treatment. Because the murine antibodies are more foreign to the human host, responses occur with a greater frequency and a higher intensity compared with the responses to humanized antibody.

The immune responses directed toward murine and humanized monoclonal antibodies are directed toward different antigenic sites. Murine antibodies are directed toward both idiotypic and isotypic markers. The interactions between the induced antibody and idiotypic markers in the monoclonal antigen-combining site block binding to the target molecule (idiotypic reactivity) while other antibodies reacting with isotypic markers enhance clearance from the body. Responses to the humanized antibody are normally directed toward idiotypic markers in the H and L chain variable region. Because the isotypic markers in humanized antibodies are not recognized as foreign, there is usually no isotypic response.

The Activation Syndrome

A group of side effects generally associated with the first administration of antibodies is termed the *activation syndrome.* Within 2–8 hr following antibody administration, patients exhibit high fever, chills, nausea, vomiting, and diarrhea.

A small group of patients develop severe respiratory symptoms and hypotension characteristic of an anaphylactoid reaction (Revillard *et al.,* 1995; Ronnenberger, 1990).

The frequency and intensity of the activation syndrome vary with the antibody preparation. Monoclonal antibodies directed toward CD3 and Cdw52 evoke intense reactions (Friend *et al.,* 1989; Revillard *et al.,* 1995). Milder reactions were observed with the administration of 33B3.1, an antibody directed toward the IL-2 receptor protein (Soulillou *et al.,* 1990). No adverse effects were observed following injection of anti-CD1 la/CD18 (Revillard *et al.,* 1995).

Because it evokes unique and unexpected adverse effects, the monoclonal anti-CD3 is the most studied antibody preparation. This antibody is effective as prophylactic therapy for treatment of acute graft-versus-host rejection following bone marrow transplantation and acute organ graft rejection. The most common dose regimen for prophylactic prevention of graft rejection is 5 mg/kg for 7–14 days. Like most antibody preparations, administration to humans caused the activation syndrome side effects. Following the first dose, patients developed fever (75%). chills (57%), tremors (10%), dyspnea (21%), and chest pain (21%). Other symptoms such as nausea and vomiting occurred in approximately 11% of the patients. Population studies have established the frequency of serious side effects following application of monoclonal antibodies to treatment of cancers. The overall frequency of side effects in a large test population is usually about 1–2% (Table 5).

The mechanism by which the anti-CD3 induced the activation syndrome has been partially defined. Cross-linkage of the CD3 activates the T cell that begins to produce IFN-γ. Interferon-activated monocytes produce TNF-α. When com-

Table 5. Frequency of Immunological Side Effects Produced by Administration of Monoclonal Antibodies for Treatment of Cancers

Side effect	No. of cases	Total no. of patients
Serum sickness	I	553
Anaphylactoid reactions		
Intestinal carcinoma	5	195
Chronic lymphocytic leukemia	I	45
Neuroblastoma	1	4
Bronchospasm		
Intestinal carcinoma	2	195
Cutaneous T-cell lymphoma	2	40

Modified from *International Journal ofBiological Markers.* van der Linden *et al.,* 1988. 12:147–53.

bined with IL-8 production, TNF-α activates monocytes, neutrophils, platelets, and endothelial cells. Adherence of immunocompetent cells to the endothelium increases coagulation, thrombosis, and vascular permeability. Mediators released from monocytes cause fever and chills (Drees, 1991).

Aseptic Meningitis

An unexpected side effect of anti-CD3 monoclonal antibody therapy was aseptic meningitis, which occurred in 2% of the treated populations (Drees, 1991). It is noteworthy that the symptoms of meningitis quickly abate despite continued treatment with the monoclonal (Drees, 1991).

Although the etiology of the aseptic meningitis remains undefined, two possible mechanisms have been put forth. One mechanism proposes that the CD3 marker is present on both lymphoid cells and cells of the central nervous system. Binding of antibody and complement activation results in lysis of lymphocytes and brain cells. This is followed by an inflammatory response causing the meningitis. A second hypothesis involves phagocytosis of brain lymphocytes, release of mediators, and recruitment of inflammatory cells to the brain.

Serum Sickness

Delayed toxicity is also a feature of continuous antibody administration. Serum sickness may develop following 2–4 weeks of administration. The patient may present with a constellation of symptoms including urticaria, malaise, arthralgias, and generalized lymphadenopathy. Symptoms are caused by the deposition of immune complexes in organs and capillaries (Dillman, 1988).

HAZARD IDENTIFICATION

Static Measurements of Immunoglobulins

Determination of antibody levels in plasma or serum may be helpful in defining an immune defect and can be determined by a number of different assays or endpoints. Total globulin concentrations or globulin/albumin ratios yield crude estimates of total antibody levels. Immunoglobulin isotype levels can be ascertained by many different methods such as radial immunodiffusion assays, enzyme-linked immunosorbent assays (ELISAa), and laser nephelometry (a measure of turbidity related to antigen–antibody complexes). The sensitivity of method used to measure serum immunoglobulin levels is shown in Table 6.

In the case of IgG and IgA determinations, normal levels in the serum or external fluids do not exclude the possibility of an immune defect. There may be

Table 6. Sensitivity of Methods used to Identify and Quantitate Immunoglobulins in Serum of Plasma

Assay	μg antibody N/ml
Ouchterlony double diffusion	3.0–20
Gel diffusion	0.2-1.0
Radioimmunoassay	0.00001 –0.001
ELISA	0.00001-0.001

Modified from *Manual of Clinical Immunology,* Rose and Friedman, 1986, American Society for Microbiology.

alterations in specific IgG or IgA subclasses that predispose to select disease processes.

The determination of total IgE levels can be helpful in the diagnosis of certain diseases involving alterations in the synthesis of IgE (e.g., bronchopulmonary aspergillosis and hyper-IgE syndrome). In these diseases, the levels of IgE may approach those observed in patients with IgE myelomas (de Shazo *et al.,* 1987).

Levels of serum IgE are usually only detectable with radioimmunoassay (RIA) or ELISA technology. The RIA can employ either a competitive or a noncompetitive binding assay. Although elevated levels of the IgE isotype have been associated with immediate hypersensitivity reactions, there is little evidence that IgE plays a role in secondary immunodeficiency.

A reduction in antibody levels may be related to increased degradation of antibody or decreased synthesis of immunoglobulin. Degradation rates are expressed in terms of antibody half-life. Increased antibody catabolism can be easily demonstrated using radiolabeled immunoglobulin tracer studies. In the peripheral blood, the IgG antibody population has an average half-life of 23 days. Of the IgG subclasses, only IgG3 has a shorter half-life (8–9 days). The IgM and IgA isotypes have a relatively short half-life of 5–6 days (Burrell *et al.,* 1992). Caution must be exercised in the interpretation of data. Loss of immunoglobulin occurs in intestinal disorders affecting the integrity of the mucosal layer.

Induction of Antibody Production

Human Experience. Serum antibodies induced by immunization to common infectious agents can also help in the diagnosis of secondary immunodeficiency. Human serum should contain antibodies directed toward common childhood vaccine antigens such as diphtheria, tetanus toxoid, measles, and polio.

ELISAs can determine the presence and/or the quantity of each antigen specific antibody.

Sometimes, it is necessary to determine whether the patient can respond to vaccination with unique antigens such as keyhole limpet hemocyanin or ϕX174, a well-studied bacteriophage. Because humans are not normally exposed to these antigens, a primary response is measured.

In some immunodeficiencies, the patient cannot respond *de novo* to antigenic stimuli or certain forms of the antigen. As mentioned above, keyhole limpet hemocyanin is used to measure primary responses. Similarly, primary T-cell-independent responses can be determined by immunization with a pneumococcus vaccine (Schiffman, 1983). The anamnestic response can be measured by immunization with a T-cell-dependent antigen (diphtheria and tetanus toxoid). Immunization protocols can be modified to determine whether the subject can respond to soluble or insoluble antigens.

Some immunodeficiencies are associated with reduced numbers of B cells in the blood. B cells can be enumerated by flow cytometric techniques using fluorescein-labeled antibodies directed toward B-cell-specific surface antigen (Lovett *et al.,* 1984). The distribution of B cells secreting IgM, IgA, or IgG can also be determined by flow cytometry. Using anti-immunoglobulin isotype-specific antisera, it is possible to determine the various isotype-specific B-cell subsets producing antibodies. Peripheral blood B cells can be significantly altered by splenectomy, administration of insulin, active infection, or xenobiotic contact (During *et al.,* 1984).

The Animal Experience. In mice and rats, the capacity of B cells to produce antibodies can be measured in the classical Jerne plaque-forming culture (PFC) assay. This assay measures the primary IgM response following immunization with sheep red blood cells (SRBCs) (Jerne-Nordin, 1963; Cunningham and Szenberg, 1968). Four days after a second immunization (the optimal time period for synthesis and secretion of antibody), animals are sacrificed and isolated splenic cells are mixed with sheep cells and suspended in agarose (Jerne-Nordin, 1963; Cunningham and Szenberg, 1968). Antibodies diffusing from the B cells react with the immobilized erythrocytes. When guinea pig complement is added to the agarose, the antibody coated sheep cells are lysed leaving a clear zone or plaque around the antibody-producing B cells. Data from the direct assay are expressed as IgM PFC/106 cells and IgM-producing cells per spleen (Burrell *et al.,* 1992).

The production of antibodies to SRBCs is a sensitive indicator of immunotoxicity. The concordance between ex vivo SRBC PFC and *in vivo* decrements in immune response is 78% (Luster *et al.,* 1992a).

Although simple in design and implementation, technical factors can influence the data. Animals used as splenic donors must be free of endemic murine viruses that reduce the ability of the B cells to proliferate *in vivo.* Second, only

select production lots of fetal calf serum will support the ex vivo antibody production. In addition, SRBCs from different animals vary with respect to immunogenicity. Therefore, the same lot of SRBCs should be used for the *in vivo* and *ex vivo* portions of the assay. Finally, guinea pig complement can often lyse red cells in the absence of antibody. Complement must be carefully screened before use in the assay (Mishell and Dutton, 1966).

To avoid problems associated with development and enumeration of PFC, an ELISA to measure antibodies to SRBCs has been developed (Temple *et al.,* 1993). In the assay, SRBC membranes are prepared by KCl extraction. As serum can be stored frozen for extended periods before analyses, the ELISA has great advantages over the PFC assay, which must be performed on the same day as sacrifice.

There are distinct differences in data interpretation from ELISA and PFC assays. The PFC assay measures the total number of B cells in the spleen producing antibody on a per cell basis. A negative result in the PFC assay does not exclude an immunotoxic event in other immunocompetent organs such as the thymus or lymph nodes. In contrast, the ELISA measures the total production of antibodies from all immunocompetent cells and decrements in antibody levels or "titer" may be reflective of an immunotoxic effect. The ELISA is a valid screening tool to test the effects of chemicals and pharmaceuticals and the PFC assay may be more useful in mechanistic studies (Anonymous, 1995a).

REGULATORY POSITION ON IMMUNOGLOBULIN ANALYSES

Static Measurements

Only the Center for Food Safety and Applied Nutrition requires the electrophoretic analyses of serum proteins. This includes the quantification and relative percentages of IgG, IgA, and IgM in control and treated animals.

Antigen-Specific Antibody Responses

Centers in the FDA and the EPA also require the petitioner to determine the effects of the test substance on responses to SRBCs (a T-cell-dependent antigen). It is expected that the OECD will also include this endpoint in the next version of the 407 Guidelines. The FDA will also accept antibody data from studies using human tetanus toxoid vaccine as an immunogen (Vos, 1977; Hinton, 1992).

Some agencies request information concerning test chemical effects on antibody responses to T-cell-independent antigens. Immunization with Ficoll (a branched polysaccharide), haptenated Ficoll, polyvinylpyrrolidone, lipopolysac-

charide endotoxin, and human pneumococcal vaccine have been used in mice and rats (Hinton, 1992; Benson and Roberts, 1982). Using standard human sera provided by the FDA Center for Biologics, responses of the test species can be compared with human responses (Schiffman, 1982).

Traditionally, the SRBC PFC assay has been used to assess antibody production in rats and mice. All agencies now consider the ELISA for antigen-specific antibody as a reliable, sensitive, and reproducible technique for detection of antigen-specific responses to SRBCs.

Monoclonal Antibodies

For monoclonal antibodies used in human clinical trials, the FDA requires a detailed description of the immunological properties. The description includes antigenic specificity, complement binding capability, and unintentional reactivity toward human tissues or organs different from the target organ. Cross-reactivity can be assessed in a variety of human tissues using appropriate immunohistochemical techniques.

Biotechnology-Derived Proteins

Most products derived by biotechnology will be immunogenic in animals. Therefore, the FDA requires measurement of antibodies directed toward the biotechnology-derived product, usually as an endpoint in repeat dose toxicity studies. For regulatory purposes, the appearance of antibodies is correlated with changes in the pharmacokinetic or pharmacodynamic parameters of the test compound. These data allow antibody classification as either neutralizing or nonneutralizing.

The antibody response is considered insignificant when certain criteria are fulfilled. The criteria are (1) there is no dose-related immune response to the biotechnology-derived product, (2) the response is variable and occurs in only a small number of test animals, and (3) pharmacological or toxicological effects occur only in a small number of animals.

10

Immunological Memory

INTRODUCTION

Immunity to viruses such as measles, yellow fever, and polio has shown that the immune system can remember (anamnestic response) a previous insult and that the immunity is long lived. Once a person recovered from the initial infection, he or she could not be reinfected later in life (Ahmed and Gray, 1996). Also, the data showed that viral immunity persisted without reexposure to the virus (Sprent, 1994).

Although immunological memory of T and B cells is accepted as dogma (Cerottini and MacDonald, 1989), there is considerable debate over the mechanisms involved in the generation and maintenance of memory cells. There are two opposing viewpoints. One viewpoint postulates that memory is related to long-lived, resting memory cells that do not require antigen for survival. The opposing view is that memory is the product of continued stimulation by low doses of antigen (Sprent, 1994).

AFFINITY MATURATION OF MEMORY CELLS

T Cells

The generation of immunological memory depends on the positive selection of T cells with high-affinity antigen receptors. During the initial stages of an immune response, intense proliferation generates T cells having high- and low-

affinity TCRs. When antigen is abundant, T cells having low-affinity receptors are expanded by proliferation. Therefore, the low-affinity T cells are dependent on high antigen concentrations for activation and survival. When antigen levels become limiting, T cells with low-affinity TCRs disappear because of (1) loss of contact with antigen, (2) the expression of the Fas ligand that induces apoptosis, (3) loss of contact with growth-promoting cytokines, (4) cellular exhaustion related to hyperstimulation of T cells, or (5) irreversible homing to secondary lymphoid tissue (Sprent, 1995).

As the antigen concentration decreases, a Th1 cell activation predominates. T cells with high-affinity TCRs have a preferential advantage because they effectively compete for low concentrations of antigen. Activation of T cells by low protein concentrations activates Bcl-2 genes that protect the cell from programmed cell death (Sprent, 1995; Collee *et al.,* 1990). In this manner, high-affinity T cells are expanded.

This model of high-affinity memory T cells has been challenged. Not all high-affinity cells survive following the primary response, suggesting that other factors must play a role in memory cell generation. Other studies demonstrated that endogenous superantigens generate high-affinity T cells and tolerance rather than memory (Sprent, 1995b).

B Cells

Generation of B-cell memory involves long-term exposure to antigen, antigen–antibody complexes, and the presence of complement fragments. Follicular dendritic cells form long, beaded extensions called *iccosomes.* Iccosomes bind only antigen–antibody complexes produced early in the immune response. Retained antigens maintain immunological specificity and native molecular mass. Initial binding of B cells to the antigen–antibody complexes requires the B-cell Fc receptors for immunoglobulin and complement. B-cell binding via the complement receptor is especially important in memory cell generation. Abolition of C3 function or decomplementation inhibits the trapping of antigen–antibody complexes (Pepys, 1976), proliferation of B cells (Klaus and Humphreys, 1977), and development of B-cell memory.

Separate B-cell populations may be involved in antibody synthesis and B-cell memory. When B cells with low-affinity B-cell receptors (BCRs) are stimulated by antigen, they produce low-affinity antibodies. Stimulated B cells with high-affinity BCRs ($K_d = 10^{-11}$) undergo proliferation without differentiation into plasma cells. Within a short time, the high-affinity B cells (memory precursors) migrate into the marginal zone (Figure 1) in the spleen (Williams and Nossal, 1966; Gray *et al.,* 1986).

In the marginal zone, these memory cell precursors begin hypermutation of the antibody genes. Hypermutation introduces random changes in the H and L

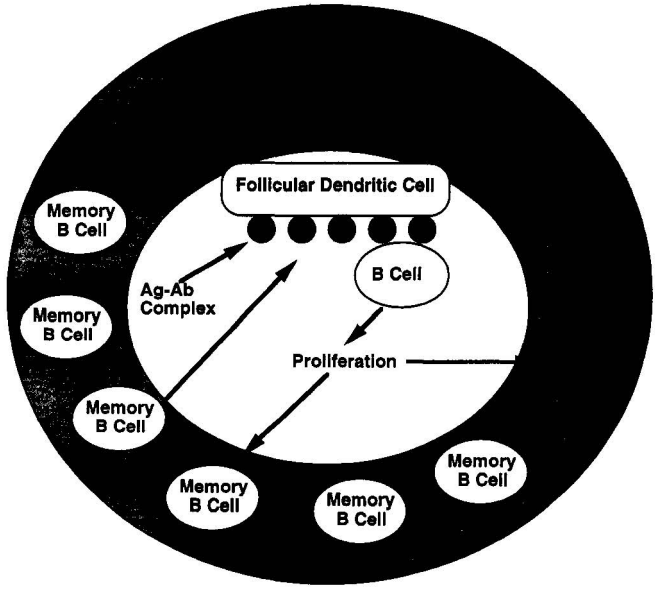

Figure 1. Memory B-cell generation and circulation in the germinal centers. Antigen–antibody complexes bind to Fc receptors and complement components C3 on the follicular dendritic cells. Mature, antigen-specific B cells are trapped in the germinal center. The B cells undergo a rapid proliferative cycle followed by apoptosis. Selected memory cells migrate to the marginal zone. Antigen stimulation mobilizes the memory cells to reenter the germinal center and proliferate.

chains. The "key mutations" introduced into the V regions of the H and L chains occur at a rapid rate. Mutations are introduced at a rate of 103 bp–1per generation in both the H and L chains. Based on a standard V region length of 600 bp, this leads to calculations of two mutations per generation (Berek and Milstein, 1987; Berek and Ziegner, 1993). Two weeks after initiation of the immune response, 50% of the generated antibodies have "key mutations" in the variable region (Berek, 1992). Moreover, many of the antibodies have high-affinity constants for antigen.

It is not clear whether continued hypermutation occurs during the memory response. Some studies suggest that hypermutation occurs with each new wave of antigenic stimulation in the primary, secondary, and tertiary responses. Continued hypermutation would result in higher affinity antibodies and increased numbers of memory cells with increased affinity for the antigen. Other studies, using hybridomas, derived from the same clone, showed that hypermutation is not associated with class switching to IgG or restimulation of B memory cells (Siekevitz *et al.,* 1987).

PHENOTYPIC MARKERS ON MEMORY CELLS

T Cells

Based on the expression of surface markers, there are four different types of T memory cells. One population expresses the phenotype of an activated T cell ($CD45RB^{lo}$, L-$selectin^{lo}$, CDM^{hi}). This population may persist for extended periods or revert to a memory cell with a naive T-cell phenotype ($CD45RB^{hi}$, L-$selectin^{hi}$, $CD44^{lo}$). These putative memory cells may persist for months (rodents) or years (humans) in the peripheral blood (Beverley, 1990; Sprent, 1994; Tough and Sprent, 1995b). The commonly recognized "true" memory cells express $CD45RO^{hi}$, L-$selectin^{lo}$, $CD44^{hi}$ and are usually long-lived resting cells. A subset of cells ($CD45RO^{hi}$, L-$selectin^{lo}$, $CD29^{hi}$, $CD44^{hi}$) responds maximally to recall antigens and provides help to B cells in the production of IgG (Morimoto and Schlossman, 1993).

Many of these cells can divide without losing the naive cell phenotype. Therefore, cells with the naive T-cell phenotype may be the progeny of antigen-specific memory cells.

B Cells

Despite intensive investigation, the phenotypes of memory B cells are not fully delineated. Several different phenotypes have been described. Like T cells, the progeny of memory cells can revert to a phenotype expressed by naive cells (IgM+ IgD+ HSA^{int}). This memory subpopulation produces low-affinity antibody with loose specificity. Another population producing high-affinity antibodies expresses IgM+ IgD– HSA^{hi}. Although there is general agreement that both phenotypes have a memory transfer capability, there is controversy over the continued proliferation capacity of one or both memory cell populations.

Other isotypes are also expressed on memory cells. Surface IgG is expressed on a small population of memory cells. These cells are precursors of cells producing IgG isotypes. For example, memory cells expressing IgG1 synthesize and secrete only IgGl following antigenic stimulation (Coffman and Cohn, 1977; Yefenof *et al.,* 1977).

Virgin B cells or memory cells can be discriminated by the expression of the surface antigen J11D. Primary B cells express high levels of the antigen whereas B memory cells express small amounts of J11D, high levels of ICAM-1, and increased numbers of complement receptors (Klinman and Linton, 1988).

Memory cells capable of producing antibody recirculate in the serum and the lymph fluid (Collee *et al.,* 1990). The half-life of circulating B memory cells is estimated at 40 days.

MAINTENANCE OF MEMORY

The persistence of antigen and the nature of the antigen-presenting cells are important for memory generation. Antigen may persist on some dendritic cells for extended periods of time. For example, human serum albumin can persist intact in lymphoid follicles for months (Tew and Mandel, 1978). Some dendritic cells provide a matrix for interactions with high-affinity T cells and processed antigen. Follicular dendritic cells in the germinal center do not bind free antigen, reducing their ability to stimulate TD responses. Yet the same cells provide an extremely effective method for stimulating B cells via localization of immune complexes. Cross-reacting antigens may also play a role in the maintenance of memory. In TD responses, low concentrations of cross-reactive antigens may stimulate memory T cells (Beverley, 1990b). In the case of TI antigens, the role of cross-reactive antigens is more complex.

It was always assumed that TI did not produce memory cells and continued stimulation would not evoke a secondary response. It is now appreciated that TI antigens can produce memory cells, but they do not respond to homologous antigen challenge. Stimulation of B memory cells and the production of IgG antibodies is possible if the cells are challenged with a heterologous form of the antigen. For example, memory cells produced by TI antigens can be stimulated by challenge with a TD form of the same antigen (Lite and Braley-Mullen, 1981). Conversely, TI antigens can stimulate memory B cells induced by TD antigens (Braley-Mullen, 1975).

Because some memory cells express activated T-cell markers, it is unclear whether this memory cell subset requires antigen for the continued generation of memory T cells. In some adoptive transfer systems, memory cells decay rapidly in the absence of antigen (Sprent, 1994; Collee *et al.,* 1990). In contrast, studies of T-cell immunity to viruses suggest that priming antigen is not necessary for the long-term maintenance of CD8 memory.

The generation of memory cells to viruses may, however, be unique. Memory cells express increased numbers of cytokine receptors. Bystander stimulation of T cells by interferon causes proliferation of $CD44^{hi}$ CD8 cells. These cells then revert to long-lived memory cells with a phenotype intermediate between naive and activated T cells ($CD45RB^{hi}$, L-$selectin^{hi}$, $CD44^{hi}$). Accumulating data suggest that cytokines play the key role in the CD8 maintenance process following viral infections (Beverley, 1996).

HAZARD IDENTIFICATION

Immunization with antigens known to elicit memory and an anamnestic response can be used in hazard identification. By definition, most of these antigens are TD antigens such as SRBCs or tetanus toxoid. Immunizations are

widely spaced so that the IgM and IgG responses can be measured in ELISAs or, in the case of SRBCs, the PFC assay.

REGULATORY POSITION ON IMMUNOLOGICAL MEMORY

The regulatory agencies have not shown an interest in the effects of chemicals or pharmaceuticals on immunological memory.

It is assumed that effects on the memory cell population could be defined by conventional histopathology. Guidelines for immunotoxicity testing of biochemical pesticides (FIFRA, subsection M) do, however, require the petitioner to determine test compound effects on both IgM and IgG responses to a TD antigen.

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Complement

INTRODUCTION

Antigen and antibody can interact with one another to form large lattices in vessels. Eventually, these complexes settle out in the small capillaries of various organs. Removal is facilitated by phagocytic cells drawn into the area by chemotactic proteins. Complement fragments are the major source of chemotactic factors. These biologically active fragments are liberated following reactions between bound antigen and antibody.

Complement is a series of 9 or 11 proteins that interact in a cascade effect liberating many fragments that have disparate functions (Table 1). Fragments are chemotactic, release histamine from basophils or mast cells, or stimulate the production of antibodies by B cells. The complement cascade can be activated by antigen–antibody complexes (classical pathway) or a unique alternative mechanism that does not require the presence of antibody (Table 2).

CLASSICAL COMPLEMENT PATHWAY

Recognition Complex

After the interaction with the antibody (either IgG or IGM), multimeric complement component C1 binds to the Fc portion of the antibody. Only one molecule of IgM or two molecules of IgG are required to activate the three subunits of C1 (Fig. 1).

Table 1. Characteristics of Proteins Involved in the Complement Pathways

Protein	Molecular weight	Serum concn. (μg/ml)
C1q	410,000	70
C1r	85,000	50
C1s	85,000	50
c4	210,000	300
c2	110,000	25
C3	190,000	1200
C5	190,000	70
C6	120,000	60
C7	110,000	55
C8	150,000	60
C9	70,000	60

Complement component C1 consists of three different proteins. C1q is composed of three polypeptide chains with a triple-helical "collagenlike" N-terminus and a globular structure at the C-terminus. C1q interacts with the antibody at the hinge region. Changes in C1q conformation activate the calcium-dependent enzymatic activity of dimeric C1r. C1r is cleaved autocatalytically to a 28-kDa molecule with serine esterase activity (Fig. 1). Using the serine esterase activity, C1r cleaves C1s into another 28-kDa protein with esterase activity (Muller-Eberhard, 1975).

Activation Complex

Activated C1s acts on multiple C4 and C2 molecules in the serum to liberate biologically active fragments (Fig. 2). C4 is a large (210 kDa) trimeric polypep-

Table 2. Proteins of the Alternative Complement Pathway

Name	Molecular weight	Serum concn. (μg/ml)
C3	190,000	1200
Factor B	93,000	200
Factor D	24,000	1
ß1H	150,000	560
Properdin	224,000	20
C3b inactivator	88,000	34

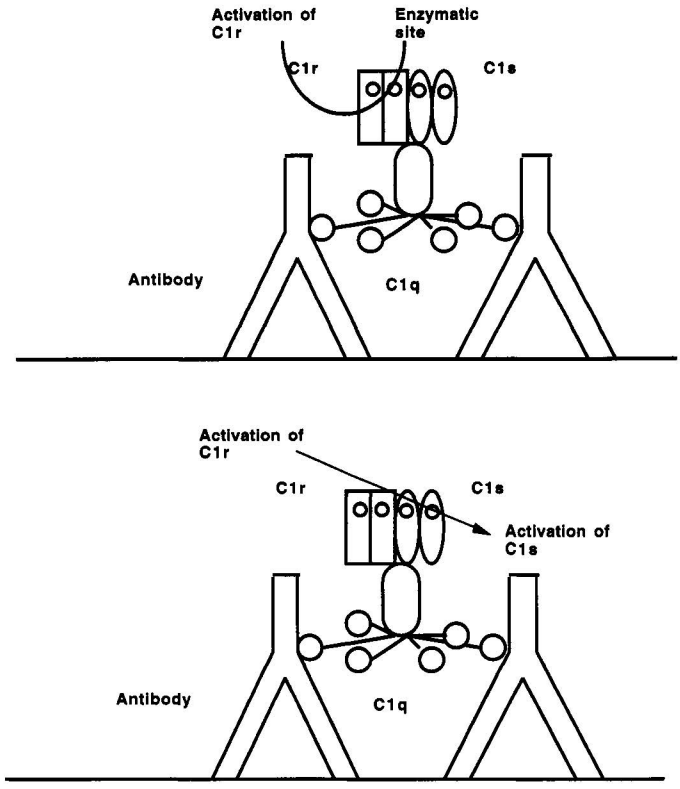

Figure 1. Activation of the C1 complex.

tide containing α and ß chains. The soluble C4a (9.0 kDa) binds to amino groups on proteins. Although the biological significance of C4a is unclear, it does act as an anaphylatoxin that nonimmunologically liberates histamine from mast cells and basophils. The released histamine increases vascular permeability and smooth muscle contraction.

C4b has a different function. It has an unusual thioester group in the α chain. When attacked by amino and hydroxyl groups, an unstable C4b is created. Some metastable C4b undergoes esterification, forming bonds with carbohydrates on the cell surface or amide bonds with proteins (Fig. 3). The function of the C4 cleavage is to localize the C4b in clusters on the cell surface near the site of the antigen–antibody interaction (Ruddy *et al.,* 1972a).

Native C2 is a 110-kDa polypeptide that binds to the cell-bound C4b in a reaction requiring magnesium. C2 is cleaved by C1s into C2a and C2b. C2b (35 kDa) diffuses into the external milieu and activates C2 kinins. A larger (75 kDa)

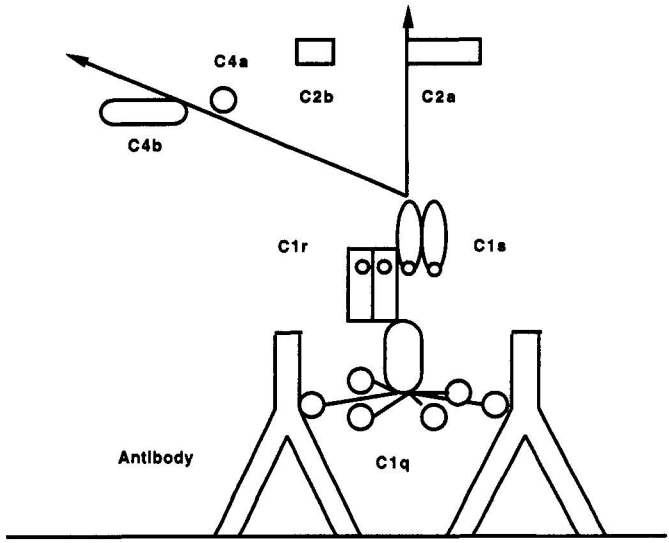

Figure 2. Cleavage of complement components C4 and C2.

fragment (C2a) remains in contact with the C4b to create a C3 convertase enzymatic complex (Muller-Eberhard, 1968).

The C3 convertase is used to amplify the classical complement cascade (Fig. 3). This 195-kDa dimeric protein has α and ß chains. C4-induced cleavage of C3 α chain liberates C3a, a 9-kDa fragmen. This small fragment is an anaphylatoxin. Compared with C4a anaphylatoxin, C3a is 20–30 times more biologically active. Like the C4 molecule, the large (186 kDa) C3b α chain has a unique thioester that is attacked by amino and hydroxyl groups.

The metastable C3b (see Fig. 7) binds to the cell surface, forming a complex with C4aC2b. Binding of C3b is highly efficient in biological terms. Between 5 and 10% of the total C3b binds to the cell surface (Ward, 1972).

Membrane Attack Unit

The C4bC2aC3b complex serves as a C5 convertase that cleaves the 190-kDa heterodimer C5 (Fig. 4). Cleavage of C5 liberates an 1 l-kDa C5a fragment and a two-chain 180-kDa C5b. Following interactions between soluble C5a and mediator containing cells (basophils, mast cells, and platelets), histamine can be released. Thus, C5a acts as an anaphylatoxin inducing contraction of smooth muscle and increasing vascular permeability. C5a is the most potent anaphylatoxin (100–1000 times more potent that C3a) in the complement cascade. In addition, C5a increases the movement of neutrophils (chemokinesis) and stimulates

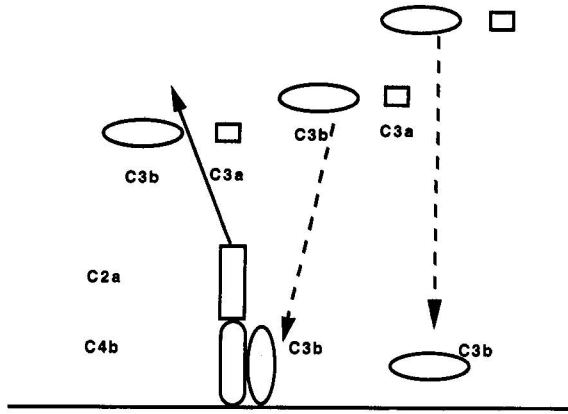

Figure 3. Formation of C3 convertase.

migration (chemotaxis) of polymorphonuclear leukocytes and monocytes into the area. C5b binds to the cell surface (Muller-Eberhard, 1971).

Interactions between C5 and C6 create a stable complex on the cell surface (Fig. 5). Binding of the C7 component creates a tightly binding trimolecular, lipophilic complex C5C6C7 via insertion of C7 into the hydrophobic cell membrane. C8 is a 155-kDa trimeric polypeptide with α, ß, and γ chains. The 22-kDa γ chain is inserted into the lipid bilayer, further increasing the stability of the C5C6C7C8 complex. Following polymerization of four C9 molecules into a cylindrical form, C9 is inserted into the membrane near C8 forming the C5C6C7C8C9 complex (Fig. 5). Occasionally, 12 to 15 C9 molecules polymerize, forming "poly C9."

Insertion of C9 creates lytic pores in the membrane. The pores allow the exchange of water and ions, resulting in osmotic lysis of the cell. Alternatively,

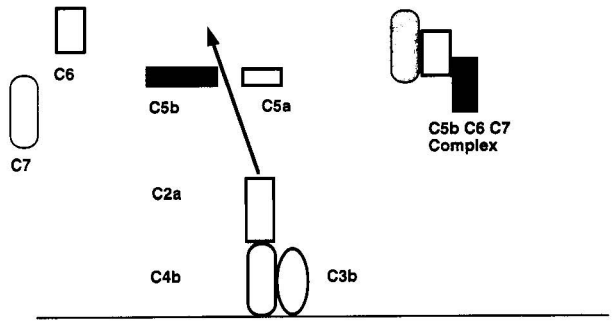

Figure 4. Cleavage of C5 and interactions with C6–9.

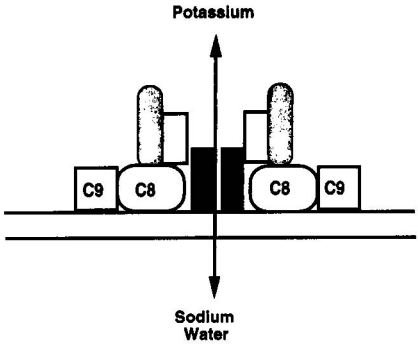

Figure 5. Pore formation and lysis of sensitized sheep red cells.

pore formation may allow high concentrations of calcium to enter the cell, causing cell death (Muller-Eberhard, 1975).

ANTIBODY-INDEPENDENT CL ASSICAL PATHWAY ACTIVATION

Select proteins, carbohydrates, viruses, and bacteria can activate C1q in the fluid phase and without antibody. Polyanions such as polyinosine, polyguanilic acid, lecithin, and sphingomyelin are potent activators. C-reactive protein (CRP) can also activate C1q indirectly following interactions with polyanions.

Proteins that are part of the innate immune defense system also activate C1q. Mannose-binding protein (MBP) and membrane-associated serine protease (MASP) activate complement because they mimic the structure of C1q (Turner, 1996).

Viruses can indirectly activate the classical pathway. Oncornaviruses such as the Moloney leukemia virus and vesicular stomatitis viruses have a membrane receptor for C1q (Volanakis, 1975) that activates C1q

CONTROL MECHANISMS IN THE CLASSICAL PATHWAY

Control of the Recognition Complex

C1 serine protease inhibitor (INH) abrogates the activity of C1s and C1r. INH mimics the normal C1 substrate (Fig. 6). After cleavage, C1 INH uses a thioester linkage to covalently bind to C Is and C1r and inhibits enzymatic activity of the C1 recognition unit (Ziccardi and Cooper, 1979; Bauer, 1986).

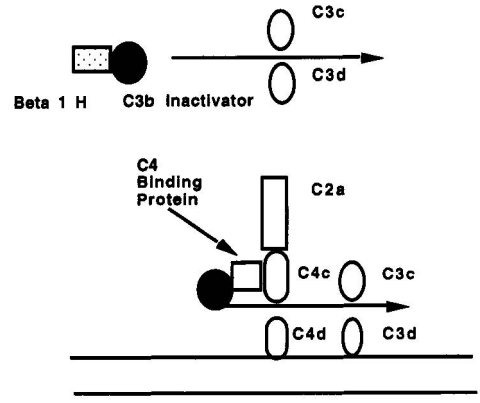

Figure 6. Control proteins for the classical pathway.

Control of the Activation Complex

Several control proteins inhibit the activation complex. Decay-accelerating factor (DAF) prevents activation or accelerates dissociation of the C3 convertase (Cooper, 1973). Other proteins that increase the dissociation of complexes are the C4 binding protein (C4bp) and the type 1 complement receptor (CR1) (Seya *et al.,* 1995). C4bp accelerates the decay of the C4bC2a portion of the C3 convertase. Dissociation of the C3 convertase is enhanced by CR1 together with factor I that cleaves C3b and C4b into inactive fragments.

Cleavage and inactivation of C4b depends on several proteins including membrane cofactor protein (MCP) which is a cofactor for factor I (Frank and Atkinson, 1975). Factor I has serine esterase activity that cleaves C4b into soluble C4c and C4d that cannot participate in the formation of the C3 convertase. Factor H inactivates both C3b and C4b but requires CR1 and MCP as cofactors (Muller-Eberhard, 1975).

Control of the Membrane Complex

Two regulatory proteins control the activation of the membrane attack complex (MAC). Both restrict the lysis of bystander cells during the complement cascade. Homologous restriction factor (HRF) has been given a CD59 designation. This protein binds to the C8 component and inserts itself into the plasma membrane via phosphatidylinositol linkages. C9 insertion is prevented and cells cannot be lysed. The second protein—vibronectin or S protein—complexes with soluble C5bC6. Thus, membrane insertion of the MAC is prevented (Muller-Eberhard, 1975).

ALTERNATE COMPLEMENT ACTIVATION PATH WAY

Activation Complex

Particulate polysaccharides (e.g., inulin, zymosan), bacterial lipopolysaccharide, measles-infected cells, and virus-transformed cells can directly activate the complement system at the level of C3. This alternative pathway, which does not involve antibody, is a primitive defense mechanism that has a unique C3 amplification step (Fearon *et al.,* 1975).

In normal serum, metastable C3 in the serum undergoes a slow spontaneous hydrolysis of an internal thioester bond (Fig. 7). C3b binds to the cell surface of bacteria and a unique 93-kDa single-chain protein (Factor B) creates complexes of C3B. The C3B complex acts as a priming convertase. Factor D, a 25-kDa serine protease, cleaves Factor B into a soluble 33-kDa Ba fragment and a cell-bound 63-kDa Bb fragment. The C3bBb complex becomes an unstable C3 convertase. Addition of a 220-kDa properdin that binds to C3b stabilizes the complex and enhances the association with Factor B (Fig. 8).

The amplification step in the alternate pathway is unique in that C3b is a component of the C3 convertase and a product of the reaction (Ruddy *et al.,*

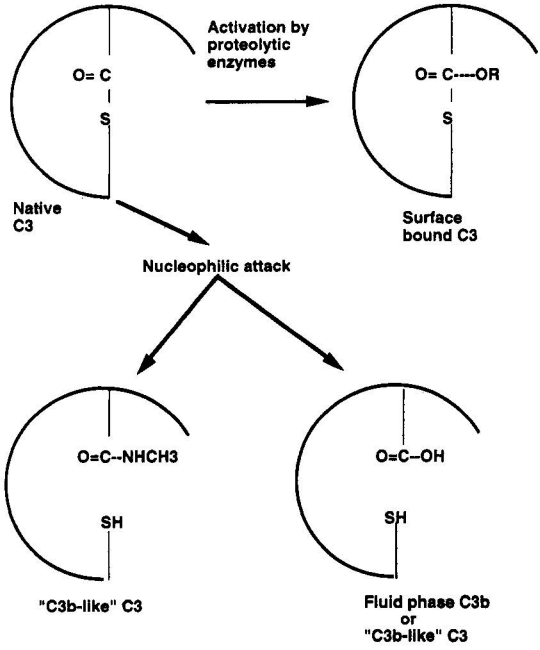

Figure 7. Schematic of metastable C3b.

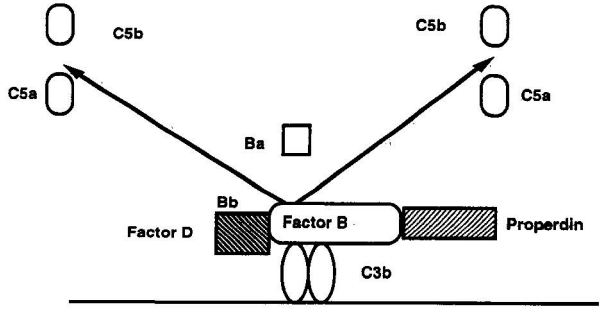

Figure 8. The C3 convertase in the alternative pathway.

1972a,b). Surface-bound C3bBb cleaves the α(Arg-77–Ser-88) chain of C3 to generate C3b that binds to the cell and forms additional C3 convertase complexes. Some C3b liberated by the novel convertase binds to the complex itself creating a C3BbC3b. The new complex acts as a C5 convettase that hydrolyzes the α chain (Arg-74–Arg-75) liberating C5a and C5b (Osler and Sandberg, 1973).

CONTROL MECHANISMS IN THE ALTERNATE PATHWAY

Similar proteins control the classical and alternate pathways. Factor H competes with factor B for binding to C3b and accelerates the decay of the alternate C3 convertase C3Bbb (Fearon *et al.,* 1975). In addition, factor H cleaves and inactivates C4b when cofactors such as CR1 and MCP are present (Fig. 9).

Binding of the cofactors is dependent on the concentration of sialic acid residues on the cell surface. High concentrations of sialic acid on the cell surface increase the binding of Factor H and other cofactors. Low concentrations of sialic acid favor the binding of Factor B and potentiation of the alternate complement activation pathway. Other protein accelerating decay proteins (e.g. Factor H) inhibit the binding of C3B to Bb (Muller-Eberhard, 1971, 1975). Factor H binds

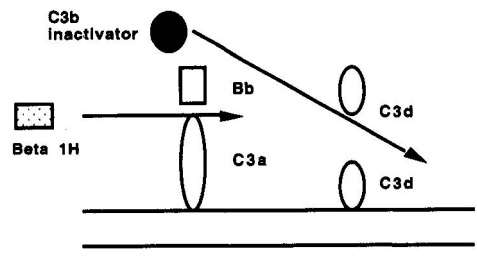

Figure 9. Control proteins in alternative pathway.

strongly to highly sulfated glycoconjugates such as dextran sulfate, heparin, chondroitin sulfate A, and carrageenan. Binding depends on the number, orientation, and polymeric arrangement of sulfate groups (Meri and Pangburn, 1994).

CELLULAR RECEPTORS FOR COMPLEMENT

CR 1 Complement Receptor (CD35)

CR1 is present on a number of blood cells including polymorphonuclear leukocytes, monocytes/macrophages, follicular dendritic cells, glomerular epithelial cells, T and B cells. The receptor binds C3b, iC3b, and C4b. Interactions between complement fragments and the cellular receptors initiate endocytosis and phagocytosis of bacteria. In addition, the CR1 receptor serves to regulate complement activation via its role as a cofactor in the cleavage of Factor I (Thomsen, 1988).

There are four different polymorphic forms of CR1 that range from 190 to 280 kDa. All members of the family are single-chain membrane proteins with multiple, arranged, short consensus units (SCRs) of 65–70 amino acids. Binding of C3b and C4b is associated with the second SCR. This ensures that binding of the complement fragments to CR1 occurs at a distance from the cell surface (Berger *et al.,* 1981).

CR2 Complement Receptor (CD21)

CR2 is a single-chain integral membrane glycoprotein. It binds iC3b and C3dg that are liberated when Factor I cleaves C3b. CR2 is present on follicular dendritic cells in the germinal centers and serves to trap antigen–antibody complexes and activate memory B cells. On naive B cells, CR2 receptor is found associated with CD19 and TAPA-1 protein. This complex is necessary to deliver activation signals at low levels of antigen (Sim *et al.,* 1986).

CR3 Complement Receptor (Mac-1, CD17 b/CD1 8)

CR3 is found on monocytes, neutrophils, NK cells, and dendritic cells. Structurally, CR3 has homologies with the integrin family of cell surface proteins. CR3 consists of two noncovalently linked α (165 kDa) and ß (95 kDa) chains designated CD18. The CR3 ß chain has homology with the ß chains of lymphocyte function-associated antigen (LFA-1) and p150, 95. Because of the similarity to LFA-1, monocytes and neutrophils can attach to the vascular epithelium via ICAM-1 without complement activation.

Phagocytosis and killing of xenobiotics are initiated by two distinct mechanisms. Like other complement receptors, CR3 binds iC3b liberated by Factor I

cleavage and initiates phagocytosis and intracellular killing of bacteria. CR3 binds lectins present on the surface of *Saccharomyces, Staphylococcus epidermiditis,* and *Histoplasma capsulatum* without the necessity for complement activation (Berger *et al.,* 1981).

MICROBIAL ABUSE OF THE COMPLEMENT SYSTEM

Bacteria and viruses can use complement receptors to initiate penetration into cells. CR1 serves as a receptor for a number of pathogenic bacteria including *Mycobacterium, Leishmania, Legionella,* and *Babesia.* CR2 is necessary for binding and infection of B lymphocytes by Epstein–Barrvirus.

HIV spreads by attachment to CR1, CR2, or CR3. As it reacts with the host cell, the virus is protected from complement lysis by a coating of host membrane inhibitor of reactive lysis (CD59), and decay-accelerating protein (DAF, CD55). In addition, HIV gp41 (necessary for direct fusion of the virus and host cell membrane) is coated with factor H, an inhibitor of alternate complement pathway activation.

Microbial attachment to cofactor proteins is also used as a means to penetrate cells. Measles virus and *Streptococcus pyogenes* Group A attach and infect cells via the complement membrane cofactor protein. In the case of the streptococcus, a cell wall protein called M protein attaches to MCP on the surface of keratinocytes. Complement lysis of the bound bacterium is prevented by a coating of factor H.

MICROBIAL COMPLEMENT INACTIVATION

Microbes have developed unique mechanisms to circumvent or inactivate the complement cascade. It is well documented that high concentrations of sialic acid on the cell surface increase the binding of factor H and inhibit the complement cascade. Type III, Group B streptococcus, K1 *E. coli,* and Group B and C *Neisseria meningitdis* (potent pathogens in humans) have highly developed capsules rich in sialic acid residues. Other bacteria such as *Pseudomonas* secrete elastase that inactivates C3a and C5a reducing the local inflammatory response in tissue. Parasites *(Trypanosoma cruzi)* can inactivate the complement cascade by a 97-kDa protein called T-DAF, This protein has 27% homology with the classical DAF. Other parasites *(Leishmania major* promastigotes) simply release the membrane complex from the cell surface. Viruses can also inactivate the complement pathway. The vaccinia virus produces a vaccinia control protein (VCP) that is homologous to the 60-amino-acid sequences at the N-terminus of C4b binding

protein. Interaction between VCP and complement components blocks the activation of C3 and C4.

COMPLEMENT DEFICIENCIES

Recognition Complex Deficiencies

Hereditary angioneurotic edema (HANE) is caused by a deficiency of C1 INH that allows deregulated activation of C1. The defect can result from either production of normal amounts of dysfunctional C1 INH or a lack of the C1 INH protein. Uncontrolled complement activation results in fluid accumulation in the larynx and skin lasting from 24 to 72 hr. Swelling may occlude the air passages and become life threatening. In some subjects, complement activation occurs in the intestinal mucosa causing vomiting and diarrhea (Bartholomew and Shanahan, 1990).

C1 INH is also a major inhibitor of the clotting pathway. Attacks of HANE are associated with episodic elevations of the Hageman factor that converts plasminogen to plasmin. Plasmin acts on C2 liberating a fragment with kinin activity. Also, activation of serine proteases cleaves kininogen to form bradykinin via activation of kallikrein and factor XII. Both bradykinin and kinin increase vascular permeability and produce edema (Bartholomew and Shanahan, 1990).

Defects in Accessory Proteins

Some subjects lack DAF, HRF, and CD59 that normally inhibit C3 convertase formation. In this syndrome—called Paroxysmal nocturnal hemoglobinuria (PNH)—these regulatory proteins have a defective glycosyl phosphatidylinositol anchor protein, preventing binding to the cell membrane. Because red blood cells are usually the target of complement activation, subjects have recurrent, intravascular red cell hemolysis (Bartholomew and Shanahan, 1990).

MICROBIAL INFECTIONS AND COMPLEMENT DEFICIENCIES

Defects in the early components (C1, C4, C2, or C3) of the classical pathway render subjects susceptible to pyogenic infections with gram-positive bacteria such as streptococci and staphylococci. Defects in Factor D or properdin in the alternate pathway are associated with recurrent infections with pyogenic bacteria or neisseria species. Infections with neisseria are also associated with defects in C5–9 components of the MAC.

Table 3. Chemicals and Pharmaceuticals that Inhibit the Complement System

Compound	Treatment	Inhibition
Glutaraldehyde	Anti-inflammatory	C3a, C5a
Dapsone	Leprosy	C142
Chloroquine	Leprosy	C142
D-Penicillamine	Arthritis	c4
Glycosaminoglycan polysulfate	Multiple diseases	C4 and factor B
Cyclosporin A	Organ rejection	CH_{50}
Dioxin		CH_{50} and C3
Pentachlorophenol		CH_{50} and C3
Sodium azide		CH_{50}

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CHEMICALS, DNA, AND COMPLEMENT ACTIVATION

The complement cascade can be activated by many chemicals, polymers, and modified DNA fragments. Common chemicals involved in complement activation are shown in Tables 3 and 4.

Complement can be activated by the administration of antisense DNA oligonucleotides (ODNs). Phosphorothioate analogues of ODNs have been used as antiviral (Agrawal, 1992), anticancer (Ratajczak *et al.,* 1991), and antiparasite (Rappaport *et al.,* 1992) agents. In rats and mice, there are no adverse effects following administration of ODNs. In monkeys, however, acute pulmonary hy-

Table 4. Chemicals Increasing Complement Levels in Serum

Chemical	CH_{50}	C3
t-Butylhydroquinone	−	+
4-Chloro-o-phenylenediamine	+	+
Diethylstilbestrol	+	−
ß-Estradiol	+	−
m-Nitrotoluene	−	+
Pyran copolymer	+	−
4,4'-Thiobis(6-t-butyl-m-cresol)	+	−

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potension is observed following infusion of phosphorothioate ODNs at doses of 5 mg/kg. Deaths in monkeys occurred at doses of 10 mg/kg. The pulmonary response is independent of ODN length, sequence, or target tissue (Black *et al.,* 1994). Because the changes were associated with decreased total serum complement levels accompanied by the appearance of C5a split product, the response is believed to be an anaphylactoid reaction (Galbraith *et al.,* 1994).

Gene delivery vehicles complexed with DNA can also activate the complement system. Commonly used complexes are DNA with polylysine of varying lengths, transferrin, polylysine, poly(amidoamine) dentrimer, and poly(ethyleneimine) dentrimer.

The complement activating properties depend on the chain length and the ratio of polycation to DNA (charge ratio). Activation can be reduced by treating the complex with poly(ethylene glycol) that modifies the DNA complex surface (Plank *et al.,* 1996).

Biomaterials used in a number of different applications can activate complement. Commercially available catheters made from polyamide, polyethylene, polyurethane, and polytetrafluoroethylene all activate complement. Activation is most prominent with polyamide. The least active substance is polyurethane (Kuroki *et al.,* 1995).

Biomaterials involved in hemodialysis often result in the activation of complement. Polystyrene derivatives with isolated sulfonate groups adsorb serine proteases and factor D. Normal serum complement can be activated by interaction of C3 with the polystyrene derivative complexed with factor D. Factor H can be absorbed to the polystyrene, causing an immediate dysregulation of the complement cascade (Pascual *et al.,* 1993).

HAZARD IDENTIFICATION

Two methods are used for assessing complement function. One method measures the functional activity while the second quantitates the amount of C3 protein in the serum (Lin and White, 1995). The functional, hemolytic activity of complement can be determined by relating the amount of hemoglobin released from antibody-coated erythrocytes following reaction with serum. Using the Van Krogh equation, the volume of serum necessary to lyse 50% of the coated red cells (CH_{50}) under standard conditions is determined (Lin and White, 1995).

The choice of antibody-coated target cells differs with different sources of complement. Human, guinea pig, and dog complement will lyse SRBCs.

Mouse complement cannot lyse sensitized SRBCs because, for unknown reasons, surface proteins inactivate the early complement components. Mouse complement readily lyses rabbit red cells coated with antibody (Van Dijk *et al.,*

1980). Microtiter complement assays for hemolytic complement (CH_{50}) were developed in the early 1980s (White and Anderson, 1984).

In the second immunochemical assay system, antibody to the C3 complement is reacted with serum producing antigen–antibodycomplexes. Complexes increase the turbidity of serum in a manner proportional to the amount of C3 in the sample (Lin and White, 1995). Radial immunodiffusion can also be used to measure C3 levels.

REGULATORY POSITION ON COMPLEMENT

Testing the effects of chemicals on the complement cascades or synthesis of complement components is not mandated by the EPA. This is because there is no validated method for determining complement function

During the time that the NTP/NEHS was validating immunotoxicity testing methods in mice, it was believed that the mouse had a nonfunctional complement system. Therefore, complement assays were not included in the NTP mouse validation studies. Only recently were data presented to show that the mouse had a fully developed complement system (Van Dijk *et al.,* 1980).

The Center for Biologics Effects Research within the FDA has included complement endpoints in screens for preclinical safety evaluation of biotechnology products. CBER has responsibility for safety assessments of monoclonal antibodies, recombinant proteins, vaccines, and gene therapy modalities.

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Phagocytosis and Intracellular Killing

INTRODUCTION

As a consequence of some immune responses, antigen–antibodycomplexes are formed in the vasculature or the tissue compartment. Circulating poly: morphonuclear cells, monocytes, and tissue fixed macrophages (collectively called *phagocytes)* are able to collect and digest the complexes. Digested molecules are recycled to the external milieu. In effect, phagocytes are the garbage collectors of the immune system. They tidy up the dross following immunological reactions.

Phagocytic cells are recruited into areas of inflammation by chemoattractants. Chemoattractants can be produced by a number of different immunocompetent cells, endothelial cells, and as a consequence of the complement cascade. Often the inflammatory reaction occurs in the tissue compartment outside the vasculature. Phagocytic cells have devised unique mechanisms to dissociate the endothelium and allow transmembrane migration of macrophages and T cells.

IMMUNE COMPLEXES AND COMPLEMENT ACTIVATION

In the early phase of the antibody response, there is an excess of antigen compared with the antibody concentration. Small and intermediate size complexes are formed when there is excess antigen. Complement is usually not activated because there are few antibodies associated with the complexes. As the antibody concentrations increase, the complexes become larger. When the ratio

of antigen to antibody is optimal, a large latticework complex is formed. The insoluble complexes activate the classical complement pathway with the liberation of chemotactic factors.

Because of a high hydrostatic pressure, large complexes usually settle out in the kidneys or the synovia. If the antigen is cationic, binding to the basement membrane of the capillary vessel creates the potential for a more destructive effect.

TRANSMEMBRANE MIGRATION

Phagocytes often must leave the vessels by transendothelial migration and enter the tissue compartment. The passage through the endothelium requires the disassembly and remodeling of both the endothelium and basement membrane. This process is simplified by the secretion of matrix metalloproteinases (MMPs), a family of 11 proteases. These enzymes have a wide spectrum of activity on collagen, laminin, and gelatin.

Synthesis of the MMPs is upregulated by cell–matrix interactions (e.g., contact with collagen) or cell–cell interactions. Neutrophil upregulation is dependent on interactions between neutrophil molecules (e.g.. $ß_2$-integrin, L-selectin) and ICAM-1, E- and P-selectin on the endothelium (Carulli, 1996; Goetzl *et al.,* 1996).

Because the MMPs have collagenase, laminase, and gelatinase activity, they disassemble and remodel connective tissue during an inflammatory response (Woessner, 1991; Emonard and Grimaud, 1990). Transient disassembly allows the transmembrane migration of T cells and macrophages into tissue. Macrophages secrete MMP1, 2, 3, 9 and a unique elastase designated MMP12.

In addition, the MMPs act on the membrane form of TNF to release active TNF-α. At the same time, TGF-$ß_1$ and insulin growth factor are released from the degraded connective tissue.

PHAGOCYTIC CELL Fc RECEPTORS

Following interactions with the endothelium, the neutrophils express a number of specific receptors including Fc receptors for immunoglobulins (Table 1). Three classes of receptors are expressed by phagocytic cells: FcγRI (CD64), FcγRI (CD32), and FcγRIII (CD16). Most receptors are hetero-oligomeric complexes consisting of nine membrane-associated FcRs and three soluble FcrγRs coded by eight genes. The receptors are transmembrane proteins with similarities to the BCR and TCR recognition proteins. Some receptors have "immunoglobulin-like" domains. High-affinity FcγRI (CD64) contains three immu-

Table1. Cellular Receptors on the Surface ofPhagocytic Cells

Receptor	Location	Ligand
CR1 (CD35)	Activated PMNs Follicular dendritic cells	C3b, iC3b, and C4
CR2 (CD21)	Follicular dendritic cells	iC3b and C3dg
CR3, Mac-1, CD11b/CD18	Monocytes Activated PMNs Dendritic cells	iC3b
FcγRI (CD64)	Monocytes/macrophages Activated PMNs	Human IgG1 and IgG3 Mouse IgG2a Oxidative burst
FcγRII (CD32)	Monocytes/macrophages Activated PMNs	Polymeric IgG Respiratory burst
FcγRIII (CD16)	Macrophages	IgG2a and IgG3

noglobulin-like domains in the extracellular region. FcγRII and FcγRIII have only two immunoglobulin domains and a lower binding affinity for IgG (Deo *et al.,* 1997).

Genetic polymorphism among the Fc receptors is common. Alternate forms of FcγRII (CD32) differ by a single amino acid (arginine or histidine) at position 131 in the second Ig domain creating FcγRIIa-R131 or FcγRIa-H131(Fig. 1). The low-affinity receptor FcγRIIIalso has two alternate forms: FcrγRIIIa and FcγRIIIb. Subtypes of both the FcγRIIIa and b receptors have been identified. The two allotypes of FcγRIIIa contain either valine or phenylalanine at position 158. Allotypes of FcγRIIIb-NA1 or NA2 differ in five nucleotides that result in different glycosylation patterns (Deo *et al.,* 1997)

INTERNALIZATION OF OPSONIZED PARTICLES

Using receptors for the Fc portion of IgG or complement fragments (Table 1), phagocytic cells attach to the bacteria–antibodycomplexes. Bacteria can also attach to the surface receptors without a requirement for opsonization. The CD11/CD18 integrin receptors on phagocytic cells recognize corresponding adhesion molecules on micrabial (e.g., *Klebsiella pneumoniae* capsule) surfaces (Ofek *et al.,* 1995).

Cross-linking two receptors (complement or Fc) triggers intracellular signaling. Intracellular signaling depends on the receptor γ, ß, or ζ chains that contain a 26-amino-aid immunoreceptor tyrosine activation motif (ITAM). Several ITAMs are phosphorylated by *src* protein tyrosine kinases (PTKs) which allow interactions with *syk* kinases (Strzelecka *et al.,* 1997).

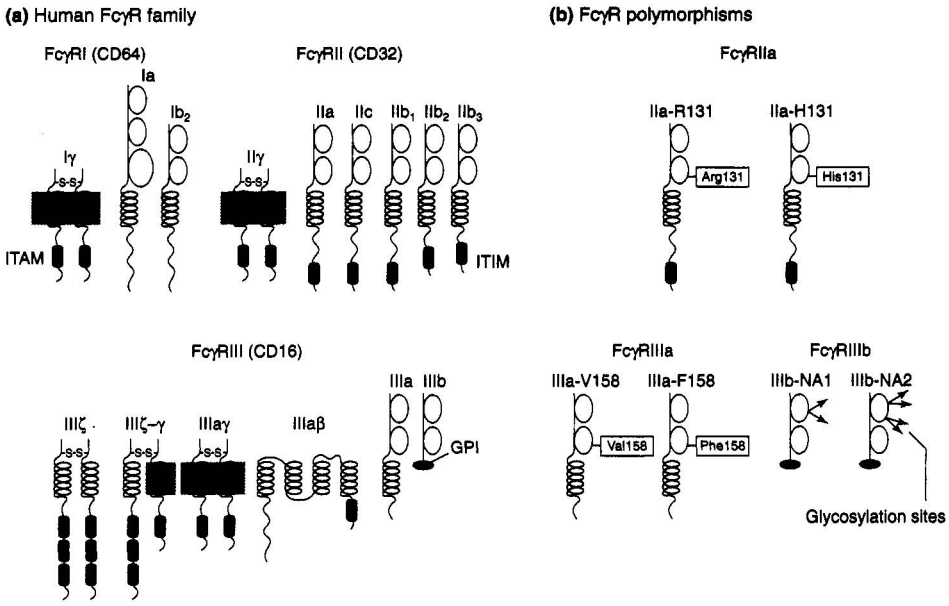

Figure 1. The human FcγR receptor family. The ligand-binding a chain contains extracellular regions comprising disulfide-bonded immunoglobulin (Ig)-like domains. FcγRI has three Ig domains, the others have two Ig domains. FcγRIa, FcγRIIa, and exist as oligomeric complexes with associated FcRγβ chains that contain immunoreceptor tyrosine-based activation motifs (ITAMs). FcγRIIb contains an inhibitory molecule (ITIM). There are two allotypic forms of human FcγRIIa that are distinguished by the presence of arginine (FcγRIIa-R131) or histidine (FcγRIIa-H131). The two allotypes of FcγRIIIa contain either valine or phenylalanine at position 158. Reprinted from *Immunology Today,* Deo *et al.,* 1997, 18:127–133, with permission of Elsevier Science.

As a consequence of cellular signaling, antigen–antibody complexes are ingested by phagocytosis, pinocytosis, or receptor-mediated endocytosis. During phagocytosis of large particles or bacteria, membrane pseudopodia surround the opsonized particle to create a phagosome. In contrast, particles can be ingested by non-receptor-mediated membrane invagination or pinocytosis. Ingestion is proportional to the free concentration in the external milieu.

Macrophages and monocytes ingest antigen by either phagocytosis or pinocytosis. In contrast, neutrophils use pinocytosis to ingest the opsonized complexes and create a phagosome.

RESPIRATORY BURST

Digestion and intracellular killing requires energy derived from a respiratory burst (Baggiolini and Wymann, 1990). Energy is generated from the classical Embden–Meyerhof pathway and the hexose monophosphate shunt (HMS) (Fig. 2). In actively respiring cells, 40% of the energy is provided by the HMS. In the initial stages of the respiratory burst, O_2 reacts with oxidases that use

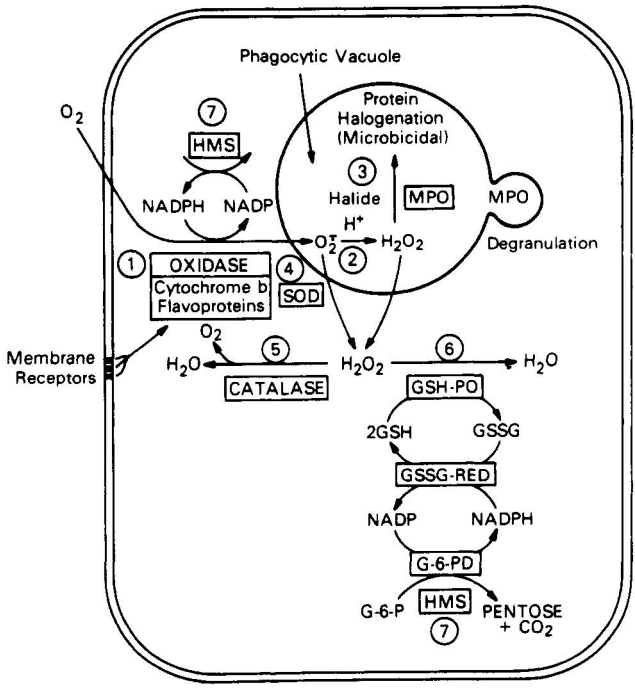

Figure 2. Summary of the pathways for oxygen metabolism in polymorphonuclear leukocytes: the major pathways for oxygen metabolism in polymorphonuclear leukocytes during the respiratory burst. Subsequent to membrane stimulation, molecular 0, undergoes a single-electron reduction to superoxide ($O\cdot_2^-$), a reaction catalyzed by an oxidase that uses reduced NADPH as a substrate. The $O\cdot_2^-$ is further reduced by spontaneous dismutation to hydrogen peroxide (H_2O_2). Myeloperoxidase is deposited into the phagosome where it catalyzes microbicidal reactions using H_2O_2 and halide ions as a substrate. These reactions lead to halogenation of cellular and microbicidal proteins. The O_2^- that escapes from the phagocytic vacuole is reduced to H_2O_2 at an enhanced rate by the enzyme SOD. Reprinted from *Review of Infectious Diseuses,* Root and Chen, 1981, 3565–98, with permission of the University of Chicago Press.

NADPH as an electron donor and cytochrome b588 to generate superoxide ($\cdot O_2^-$). Superoxide formed during the respiratory burst is released into the phagosome, dismutated to hydrogen peroxide (H_2O_2), or reacted with NO·. All three chemical species are toxic to bacteria.

Dismutation of $\cdot O_2^-$ to H_2O_2 occurs by various mechanisms. In the phagocytic vesicle, reductions in pH result in the release of protons that accelerate the rate of spontaneous dismutation to H_2O_2. Enzymatic degradation of $\cdot O_2^-$ by superoxide dismutase (SOD) is also used to generate H_2O_2. The latter activity is found in various mammalian cells having either Cu. Zn, or Mn in the cytosol or mitochondria.

Hydrogen peroxide is toxic to ingested bacteria, host cells and tissue. It disrupts cell membranes and reacts with DNA to cause extensive genomic damage.

The $\cdot O_2^-$ can participate in other reactions to generate additional oxidants more reactive than the superoxide. Transition metal (Fe^{3+} and Cu^{2+})-catalyzed Haber–Weiss reaction generates both hydrogen peroxide and hydroxyl radicals. The proposed reaction sequence (Christen *et al.,* 1996) is as follows:

$$M^{n+} + O_2 \rightarrow M^{n(n-1)} + O_2$$

$$M^{(n-1)} + O_2 \rightarrow M^{n+} + \cdot OH + OH^- \text{ (Fenton reaction)}$$

$$H_2O_2 + O_2 \rightarrow O_2 + \cdot OH + OH^- \text{ (net reaction)}$$

The OH–radical generated during the reaction acts on polyunsaturated fatty acids with several double bonds and initiates a chain reaction. Consequently, carbon centered radicals react with 0_2 to generate hydroperoxides. In the presence of iron, the lipid peroxides decompose to form cytotoxic aldehydes and reactive gases. Peroxides and aldehydes react with the bacterial and host cell membranes to disrupt cell function.

Alternatively, $\cdot O_2^-$ can react with NO– to form peroxynitrite anion (ONOO–)or the conjugate acid (ONOOH). Once the peroxynitrite is protonated, it decomposes rapidly to form hydroxyl radicals, protons, and peroxynitrous acid (ONOOH+). The reaction (Christen *et al.,* 1996) is as follows:

$$\cdot NO + \cdot O_2^- \rightarrow ONOO^-$$

$$ONOO^- + H^+ \rightarrow ONOOH \rightarrow NO_3 + H^+ \text{ or } ONOOH$$

The reactive peroxynitrite molecule or the conjugate acid react with many different substrates. Peroxynitrite oxidizes sulfhydryl groups while peroxynitrous acid hydroxylates phenolic compounds, peroxidates lipids, and oxidizes sugars

and DNA. Peroxidation of proteins containing sulfydryl groups (cysteine) or basic amino acids (histidine, lysine) is also common.

Monocytes and PMNs also contain a myeloperoxidase (MPO) which is secreted into the phagosome from primary granules (Table 2). In the presence of MPO, hydrogen peroxides and halides act as substrates for the formation of hypohalous acids. Because Cl^- is the major halide present in the body, hypochlorous acid is usually formed (Christen *et al.,* 1996):

$$H_2O_2 + Cl^- + H^+ \rightarrow HOCl + H_2O$$

Hypochlorous acid has both beneficial and detrimental effects. The acidic environment in the phagosome is toxic to ingested bacteria and has a beneficial effect in host defense. However, hypochlorous acid and proteases leak from the phagocytic cell and cause tissue injury or the generation of mutagenic molecules. Protease activity continues unabated because the acid also inactivates $ß_2$-macroglobulin, the major inhibitor of protease activity.

Tissue or extracellular proteins are readily halogenated by hypochlorous acid. For example, alanine decarboxylation and deamination results in the generation of ammonia, carbon dioxide and reactive aldehydes that are toxic to cells. If iodide is the halide, tyrosine containing proteins are halogenated. Reactions between primary amines and hypochlorous acid also result in the formation of chloroamines that are highly mutagenic (Christen *et al.,* 1996):

$$RNH_2 + HOCl \rightarrow RNHCl + H_2O$$

Table 2. Constituents of Primary and Secondary Granules

	Primary granules	Secondary granules
Microbicidal	Myeloperoxidase	Lysozyme
	Lysozyme	
Neutral serine proteases	Elastase	Collagenase
	Cathepsin G	
	Proteinase 3	
Acid hydrolases	N-Acetylglucosamine	
	Cathepsin D	
	Cathepsin B	
	ß-Glucuronidase	
Miscellaneous	Lactoferrin	
	Vitamin B12 binding protein	
	Cytochrome B	

Modified from *Annual Review of Medicine,* Gallin, 1985, 36:263–74. with permission of the *Annual Review of Medicine.*

Late in the intracellular killing sequence, enzyme-containing primary granules called lysosomes fuse with the phagosome membrane. The granules contain neutral serine proteases, acid hydrolases, and lactoferrin (Table 2). Lysozyme and other acid hydrolases attack the bacterial cell wall at the ß1,4 glucosidic linkage between acetyl muramic acid and N-acetylglucosamine residues. Neutral serine proteases degrade collagen and elastin.

Neutrophils also have secondary intracellular granules. Stored in the secondary granules of neutrophils and macrophages are a unique family (HP- 1,2,3) of protein defensins. Defensins are small, circular, cationic proteins (29–35 amino acids) with a beta sheet structure. Like the C9 component of complement, defensins form ion channels in the membranes of *S. aureus, S. pneumoniae, E. coli, P. aeruginosa, H. influenzae,* and *M. tuberculosis.* The altered flux of calcium and potassium through the membrane results in bacterial death (Nicolas and Mor, 1995; Boman, 1996).

MODULATION OF THE RESPIRATORY BURST

The respiratory burst can be modulated by extracellular or intracellular mechanisms. One extracellular modulation mechanism involves the inactivation or chelation of copper and iron that mediate the Haber–Weiss reaction.

Ceruloplasmin, lactoferrin, and transferrin are proteins that bind 2 moles of metal ion per mole of protein, thus reducing the ions available for facilitation of chemical reactions. Ceruloplasmin binds copper while lactoferrin and transferrin bind iron without the release of free radicals (Urban *et al.,* 1995).

In a second regulatory pathway, intracellular enzymatic degradation of superoxide and hydrogen peroxide can also terminate the respiratory burst. SOD reduces the $\cdot O_2^-$ to H_2O_2 and O_2. The enzyme catalase further reduces H_2O_2 to H_2O and O_2.

Toxic accumulations of H_2O_2 can also be prevented by glutathione reductase. Oxidized glutathione is reduced using NADPH as the electron donor to form H_2O and O_2. In turn, the NADP is reduced by hexose monophosphate metabolism. Hydroxyl radicals are similarly reduced by glutathione peroxidase (Urban *et al.,* 1995).

NITRIC OXIDE PRODUCTION

In a system independent of the respiratory burst, phagocytes can induce NOmediated cytotoxicity by activation of inducible (iNOS) and constitutive (cNOS) isoforms of nitric oxide synthase (NOS). Using this oxidative pathway, L-arginine can be converted to citrulline with the liberation of nitric oxide ($\cdot NO$).

In turn, NO reacts with O_2 to form a number of additional reactive intermediates such as dinitrogen trioxide (N_2O_3) or dinitrogen tetroxide (N_2O_4) (Dietert *et al.,* 1997). The reaction sequence for the generation of N_2O_3 is as follows:

$$2 \cdot NO + O_2 \rightarrow N_2O_3 + 2 \cdot NO_2$$

$$\cdot NO + \cdot NO_2 \rightarrow N_2O_3$$

$$N_2O_3 + H_2O \rightarrow 2HNO_2 \rightarrow 2NO_2^- + 2H^+$$

The N_2O_3 readily reacts with water to form nitrites. Nitrites are further oxidized to form nitrates. Besides the formation of nitrates, reaction products can interact with secondary amines or amides to form mutagenic N-nitrosoamines or N-nitrosoamides (Marletta, 1988).

Nitric oxide and the reactive nitrogen intermediates play a role in host defense. Nitric oxide kills tumor cells (Stuehr and Nathan, 1989) and bacteria (Adams *et al.,* 1990) by modifying iron sulfhydryl complexes within mitochondrial enzymes. Proposed mitochondrial enzyme targets are aconitase (Drapier and Hibbs, 1988), NADH reductase, and select ubiquinone oxidoreductases (Granger and Lehninger, 1982).

ADVERSE HEALTH EFFECTS

Chronic infection or inflammation is associated with adverse health effects. MMPs play a role in the pathophysiology of experimental disease models. In animal models of rheumatoid arthritis, MMPs may contribute to cartilage and bone destruction, synovial proliferation, and periosteal bone formation (Firestein, 1992). In experimental allergic encephalomyelitis (EAE) in mice, MMPs appear in the spinal fluid and alter the normal permeability of the blood–brain barrier (Goetzl *et al.,* 1996).

Reaction products from the oxidative burst and the nitric oxide pathway are harmful to the host. During the oxidative burst, reaction products such as $\cdot O_2^-$ and H_2O_2 leak from phagocytic cells and kill cells in the surrounding tissue. This leads to compensatory cell division to replace lost cells. As the cells are proliferating, they suffer genetic damage following exposure to mutagenic chloroamines. Compounds produced by the nitric oxide pathway are also mutagenic. The N-nitroso compounds are both carcinogenic and immunosuppressive (Bartsch and Montesano, 1984; Haggerty and Holsapple, 1990). Increased cell division combined with DNA lesions result in higher mutation rates. Prolonged proliferation expands the mutated cells and increases the probability of cancer (Weitzman and Gordon, 1990).

FREE RADICAL SCAVENGING

Tissue destruction is normally prevented by antioxidant defense mechanisms. Several proteins and vitamins act as free radical scavengers. As examples, vitamin E inhibits peroxy radicals, glucose scavenges the hydroxyl radicals, and ascorbic acid reduces carbon-centered radicals. Large iron binding proteins can also act as scavengers. Ceruloplasmin inactivates lipid alkoxy1 radicals and transferrin binds hypochlorous acids.

MICROBIAL EVASION OF PHAGOCYTOSIS

Several species of bacteria have developed defense mechanisms that prevent phagocytosis or increase survival within the cell. Extracellular bacterial pathogens such as *S. pneumoniae* have developed a thick polysaccharide capsule that inhibits complement activation and phagocytosis. Other species (e.g., staphylococci) produce a thick fibrin coat of coagulase that retards phagocytosis.

Intracellular pathogens have developed more sophisticated methods to prevent killing by phagocytes. Members of the *Mycobacterium* family, *Legionella pneumoniae,* and *Chlamydia* species interfere with lysosome movement, inhibit fusion of lysosomes, or secrete a phenolic glycolipid that scavenges reactive oxygen species. Parasites such as *T. cruzi* are ingested normally, but lyse the phagosome membrane, enter the cytoplasm, and initiate a mutualistic relationship with the phagocytic cell.

IMMUNE DEFICI ENCIES ASSOCIATED WITH PHAGOCYTOSIS

There is an association between allotypic forms of the FcγR and disease. Individuals expressing the FcγRIIa-R131 receptor are more susceptible to infections with encapsulated bacteria. Encapsulated bacteria evoke a response consisting of IgG2 antibodies. The FcγRIIa-R131 receptors cannot bind IgG2-coated bacteria. The FcγRIIa-H131 allotype is the only functional FcγR capable of binding IgG2 antibodies (Deo *et al.,* 1997).

A normal function of tissue macrophages is the removal and destruction of aged red cells from circulating blood. In Gaucher's or Newmann–Pick's disease, there is a defect in the function of enzymes degrading sphingolipids. Because of the defect, red blood cell debris accumulates within the macrophage. The accumulated lipid inhibits the function of protein kinase C that is necessary for the membrane transduction of external stimuli that activate or regulate the macro-

phage. Because the macrophage cannot respond to functional stimuli, resistance to infection can be impaired (Burrell *et al.,* 1992).

A rarely reported disease is the lazy leukocyte syndrome or leukocyte adhesion deficiency (LAD). In these patients, adhesion of neutrophils to the endothelium or other cells and phagocytosis are impaired. The genetic defect is found in two forms. In LAD-1, the defect is associated with the complement receptor type 3 (CR3; Mac-1, CD1 1b/CD18) found on monocytes, neutrophils, NK cells, and dendritic cells. There is a mutational defect in transcription of the CD18 ß chain. In these patients, phagocytosis and killing are impaired (Fischer, 1990).

In subjects with LAD-2, the defect results from the absence or reduction of sialyl-Lewis X carbohydrate that is necessary for binding of E- and P-selectin (Fischer and Arnaiz-Villena, 1995). Thus, transendothelial migration of neutrophils is impaired.

MPO deficiencies are common autosomal recessive deficiencies that occur in 1 :4000 adults. Usually, the defect has no effect on host defense and there is no increased frequency of infections (Fischer and Arnaiz-Villena, 1995).

Recurrent infections are found in patients with chronic granulomatous disease (CGD). These individuals reveal an increased frequency of deep tissue infections and a failure to resolve inflammatory reactions mediated by neutrophils. Consequently, there is granuloma and scarring in tissue. Despite aggressive therapy, the disease is often fatal.

Phagocytes from CGD patients can ingest opsonized bacteria but fail to generate sufficient superoxide for production of hydrogen peroxide. Several different genetic defects are associated with the disease. In the X-linked form that accounts for 66% of all cases, a 91-kDa transmembrane protein is absent, mutated, or truncated. This protein is part of the complex with cytochrome b588 used to generate superoxide. In the autosomal recessive form of the disease, the cytochrome system is intact. The defect resides in the failure to phosphorylate a 47-kDa component of the NADPH system that activates neutrophils (Fischer, 1990; Dinauer and Orkin, 1992).

Subjects with CGD have recurrent infections with catalase-producing streptococci or *E. coli.* Bacterial catalase reduces the small amount of H_2O_2 produced by the macrophage, thus preventing intracellular killing. Catalase can act peroxidatively by oxidizing a hydrogen donor with H_2O_2 or catalytically by dismutation of H_2O_2 to H_2O and O_2. Other bacterial species that lack catalase (e.g., *S. pneumoniae)* are killed normally by phagocytes from CGD patients (Fischer, 1990).

CHEMICALS AND PHAGOCYTOSIS

Environmental chemicals and pharmaceuticals can alter phagocytic function (Table 3). Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used

Table 3. Chemicals that Alter Macrophage Activity in Animals

Chemical	Species	Effect
Lead	Mouse, rat	↑,↓
Cadmium	Mouse	↑,↓
Mercury	Mouse	N*
Nickel	Mouse, rat, rabbit	↓
Pentachlorophenol	Mouse, rat	↑
Toxaphene	Mouse	↓

*No effect.
Modified from *Immunology and Allergy* Procrice, Koller, 1985, 7:13–25.

in therapeutic reactions for treatment of inflammation. Besides inhibiting prostaglandin synthesis (Vane, 1971), NSAIDs inhibit many neutrophil pathways and function. Some inhibit the signaling pathway by uncoupling the G proteins in the cellular receptors for immunoglobulin or complement. Other NSAIDs such as salicylates inhibit the transcription of NF-κB (Kopp and Ghosh, 1994). The extravasation of neutrophils is also prevented by some NSAIDs. Indomethacin, diclofenac, and aspirin cleave L-selectin from the neutrophil surface. Without L-selectin, the cells are unable to attach to counter molecules on the capillary endothelium (Jutila *et al.,* 1989; (Humbria *et al.,* 1994).

Some antiarrhythmic drugs such as lidocaine, quinidine, or procainamide inhibit phagocytic function to varying degrees. Quinidine, which inhibits NADPH oxidase, can reduce the production of superoxide and hydrogen peroxide by 50%. Other drugs such as Cetiedil Citrate also inhibit NADPH oxidase.

In contrast, some drugs can increase phagocytic function. Zimet 3164 (a benzimidazole nitrogen mustard derivative) and levamisole (a commercially available broad-spectrum antihelmintic) increases the phagocytosis by PMNs.

Natural products isolated from microorganisms can also inhibit phagocytosis. Sulfatides isolated from *M. tuberculosis* block the activation of human monocytes. 17-Hydroxywortmannin, a fungal sterol metabolite, is a potent inhibitor of the respiratory burst. Slime glycolipoproteins isolated from *P. aeruginosa* inhibit phagocytosis by interfering with membrane invagination.

HAZARD IDENTIFICATION

Neutrophils play a critical role in defense against bacterial and fungal infections. The functional capacity of isolated or purified phagocytic cells can be determined by many different assays.

However, data from studies on cells from one source may not reflect changes in other phagocytic cell populations. For example, studies on peripheral blood cells may not reflect changes in the other components of the reticuloendothelial system (RES) such as Kupffer cell or alveolar macrophages.

The most common test used to study PMN function is the nitroblue tetrazolium test (NBT). Activated PMNs will phagocytize dye–protein complexes and incorporate them into phagosomes. In the presence of superoxide, the yellow dye is reduced to an insoluble, blue-colored formazan. Dye reduction may be determined by direct microscopy or spectrophotometrically after extraction of complexes by dioxan. The qualitative method is simple and inexpensive (Hudson and Hay, 1989). However, a positive NBT does not exclude the possibility of microbicidal defect. Patients with severe defects may produce enough superoxide to give a positive NBT, yet fail to kill ingested catalase-positive bacteria (Lew *et al.,* 1981)

A rapid, low-cost screening method for assessing phagocytosis has been developed using fluorescent covaspheres. Following immersion in methylene chloride vapor to dissolve extracellular beads, the number of ingested beads is determined by fluorescence microscopy and expressed as the percentage of macrophages ingesting beads (Burleson *et al.,* 1987). Although easy to perform, the assay is extremely labor intensive. From a scientific perspective, the assay measures only the initial phagocytic event. Normal ingestion does not preclude defects in bacterial killing (Burrell *et al.,* 1992).

A biologically relevant endpoint is the neutrophils' ability to kill ingested bacteria. In most assay systems, phagocytes are incubated with opsonized *S. aureus* for 60 min. After lysis of extracellular bacteria with lysostaphin, the number of surviving intracellular bacteria is determined. In normal peripheral blood phagocytic cells, the bacterial survival rate at 60 min is less than 5.0%. This assay measures both the inhibition and the stimulation of RES activity.

The most predictive for altered macrophage function (Anonymous, 1995a) is a holistic assay. Holistic assays test the function of the entire RES encompassing both fixed macrophages and circulating monocytes and neutrophils. In the assay, intravenous injection of ^{51}Cr-labeled SRBCs is used as the stimulus. At defined time points up to 60 min, a blood sample is obtained from the tail vein and the radioactivity determined (White *et al.,* 1985). Three endpoints are measured during the assay. Clearance of labeled cells from the blood is determined, and the data are expressed as the vascular half-life. The phagocytic index is derived from the slope of the clearance curve. Also determined is the organ distribution of radioactivity. These data are expressed as percent organ uptake and counts per minute per milligram of tissue.

Additional studies can be undertaken to define the defect within the respiratory burst. The higher energy level electrons from the superoxide molecule

can be used as a basis for quantitation. When the electron returns to the ground state, light is emitted. Luminol (a cyclic hydrazide) enhances the chemiluminescence determined in a liquid scintillation counter configured so that its photomultiplier tubes have been taken out of coincident setting (Burrell *et al.,* 1992).

Electron spin resonance using 5,5-dimethyl- 1-pyroline-N-oxide as the trap can be used to quantitate hydroxyl radicals produced during phagocytosis. Detection of H_2O_2 is usually determined following the indirect oxidation of hydrogen donors such as scopoletin (7-hydrocoumarin). In the first stage of the assay, H_2O_2 reacts with horseradish peroxidase. The reaction products oxidize scopoletin causing a reduction in the normal fluorescence (Rosen and Klebanoff, 1979).

There are several *ex vivo* or *in vitro* systems that are useful in measuring nitrite, the major reactive nitrogen intermediate produced in the nitric oxide pathway. Activated, peritoneal macrophages can be recovered from chickens inoculated with Sephadex or mice injected with thioglycollate (Dietert *et al.,* 1997). These cells can be stimulated with bacterial endotoxin (LPS) to produce NO. Cell lines can also be used to test for nitrite production. LPS-stimulated macrophage chicken (HD11) or mouse (Raw 264, WEHI-3, and J774.1) cell lines (Dietert *et al.,* 1997; Beug *et al.,* 1979) are commonly used for this purpose.

Using supernatant fluids from LPS-stimulated macrophage cultures, nitrite can be measured with a chomogenic reagent consisting of N-(1-naphthyl)-ethylenediamine dihydrochloride and sulfanilamide (Green *et al.,* 1982). Following interactions with nitrate, a red chromophore arises with peak absorbance of 546 nm (Dietert *et al.,* 1997).

In the design of experiments, careful attention must be given to technical factors and strain differences in the production of nitrite. Chickens cannot synthesize L-arginine because they lack select enzymes in the urea cycle (Tamir and Ratner, 1963). Therefore, the diet must be supplemented with L-arginine. Other genetic factors influence the amount of L-arginine available for the production of NO. Production depends, in part, on the renal arginase levels. Arginase levels are genetically determined and may differ between species and strains (Nesheim *et al.,* 1971).

Any disequilibrium between arginase and NOS influences the amount of available L-arginine. Arginase converts L-arginine to urea that is quickly excreted by the host.

In mammals, L-arginine is produced by enzymes in the urea cycle using ornithine, ammonia, and aspartate as building blocks. Supplemental dietary L-arginine may influence macrophage function in some species but not in others. Rats and mice may also differ in baseline levels of nitrite, the ability to produce nitrite, and the amount of nitrate produced following stimulation with LPS (Mills, 1991)

REGULATORY POSITION ON PHAGOCYTOSIS

Many macrophage function assays were evaluated by the EPA/NTP for inclusion in immunotoxicity screens. Because these assays yielded variable results and were very labor intensive, they were never validated. In an attempt to define effects of chemical on alveolar macrophages, the harmonized EPA guidelines may require a study of the phagocytic capacity (fluorescent bead assay) of lavaged alveolar macrophages (Burleson *et al.,* 1987). In addition, the Office of Research and Development within the EPA has suggested that macrophage/neutrophil microbicidal killing should be included as biomarkers in human immunotoxicity studies.

13

Immediate Hypersensitivity Reactions

INTRODUCTION

Immediate allergic reactions are mediated by the IgE class of antibodies and occur 15–20 min after exposure to antigen. The clinical signs and symptoms are related to the time course, the target organ, and the nature of the pharmacological mediators released from mast cells and basophils.

Allergic reactions may be systemic or localized in the nose, skin, lung, or gut. Systemic reactions often result in vascular collapse and death in a reaction called *anaphylaxis*. Less severe reactions occur in the nose (allergic rhinitis) causing nasal congestion, sneezing, and watery discharge. Allergic reactions in the lung result in an increase in airway caliber creating an asthmatic response. In the skin, leakage of fluid from the capillary bed into the skin results in urticaria (hives) in the area of the allergic reaction. Allergic reactions can also take place in the gut with associated cramping, diarrhea, and vomiting.

ATOPY

Clearly, immediate hypersensitivity runs in families and has a genetic basis. This genetic familial condition is called *atopy*. Kindreds within the same family may have allergic reactions such as asthma, urticaria, or rhinitis to the same or different antigens. In addition, all atopic members of a family will have high levels of IgE in the serum.

The genetic basis for asthmatic lung responses is unclear. Specific MHC

markers may be associated with response to ragweed. A recent study showed a linkage between bronchial hyperactivity and the ß subunit structure of the high-affinity IgE receptor complex (van Herwenden *et al.,* 1995). Other studies have implicated mutations in the *cc10* gene cluster as a cause for asthma. The *cc10* gene is an inhibitor of phospholipase and regulates prostaglandins, leukotrienes, and airway inflammation. Any dysfunction in the gene product results in skewed prostaglandin production and increased airway inflammation.

CHARACTERISTICS OF ALLERGIC PATIENTS

When compared with normals, asthmatics have a skewed profile of peripheral blood lymphocytes and clinical symptoms. The number of CD4 Th2 cells is greatly increased in allergic subjects. However, there is considerable debate about whether this change is a cause or an effect of asthma. Moreover, the surface of the Th2 cells has a higher density of a low-affinity receptor for IgE (CD23). The low-affinity receptor is a 30-kDa member of the C-type lectin family that includes the selectin adhesion molecules. Most asthmatics have hyperactive airways and respond to low concentrations of carbachol, a potent cholinergic stimulus, with bronchoconstriction. All have increased levels of IgE antibody in the serum.

INITIAL EVENTS IN AN ALLERGIC RESPONSE

Like all antibody responses, interactions between the MHC and the TCR initiate the signaling process. The major difference between normal and allergic responses is that the Th2 cell serves as the T effector cell. Following interactions between CD40 and its ligand (CD40L), the Th2 cells release IL-4, IL-5, and IL-6 in high concentrations (Fig. 1). Under the influence of IL-4, naive B expressing IgM or IgD undergo isotypic switching. The E heavy chain rearrangements result in the production of IgE (Coffman et al., 1986; Romagnani, 1989). In addition, soluble CD23 liberated from the Th2 cells interacts with the complement receptor 2 (CD21) on B cells to preferentially upregulate IgE synthesis (Delepresse *et al.,* 1992). Secreted IL-5 is also a major growth factor for eosinophils (Gulbenkian et al., 1992). Within 10–14 days, B-cell-produced antigen-specific IgE is found in the serum (Fig. 2). Normally, the concentration in serum is extremely low, ranging from 10 to 300 ng/ml.

IgE Fc RECEPTORS

Circulating IgE binds to receptors on basophils and mast cells. Two types of receptors (FcεRI and FcεRII), which differ in binding affinity, have been de-

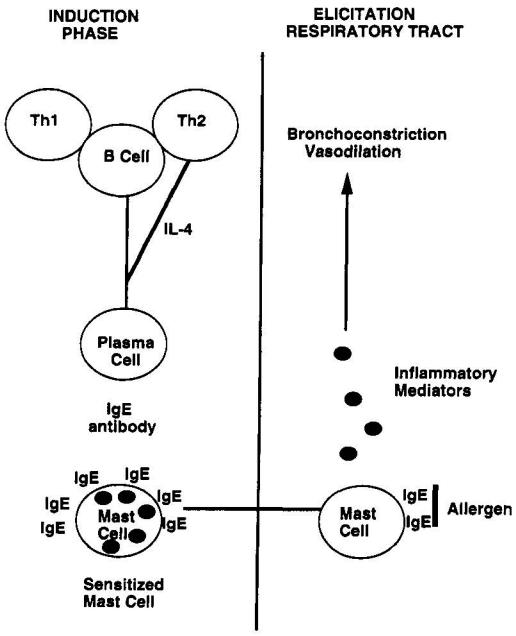

Figure 1. The induction and elicitation of an allergic reaction in the lung. Reprinted from *Respiratory Allergy,* European Center for Ecotoxicology of Chemicals, 1993, Monograph 19, p. 11.

scribed (Table 1). The high-affinity IgE receptors (FcεRI) are present on the surface of tissue fixed mast cells in the trachea and intestine, circulating basophils, Langerhans cells, monocytes from atopic individuals, and eosinophils from persons infected with parasites. IgE binds strongly to these receptors as evidenced by the 1×10^{-9} M dissociation constant.

Low-affinity receptors are present on T and B cells, monocytes, and eosinophils (Table 1). The binding affinity for IgE is distinctly lower (1×10^{-7} M) than the classical mast cell or basophil receptor (Capron *et al.,* 1986).

High-Affinity Fcε RI Receptor

The FcεRIconsists of α, ß, and two y chains (Fig. 3). IgE binds to the a chain (a 25-kDa transmembrane protein). Structural similarities to the FcγRII and FcγRIII receptors are found in the IgE-binding portion of the α chain. In addition, the α chain has the two loops characteristic of the Ig superfamily members and a short (20 amino acid) cytoplasmic domain.

In contrast to the α chain, the ß chain is a long (243 amino acid) polypeptide that crosses the membrane four times. Like the α chain, the ß chain has a short cytoplasmic domain (Liu, 1990).

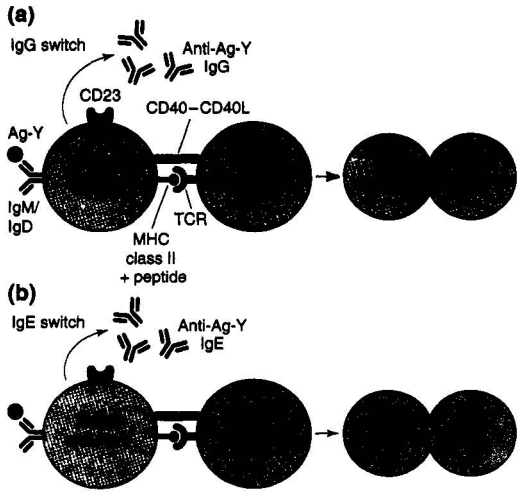

Figure 2. Cognate interactions between B and T cells. A naive B cell becomes activated via cross-linking of its membrane immunoglobulin (Ig). Following internalization and processing of antigen Y (Ag-Y), peptides are presented in a major histocompatibility (MHC) class 11-restricted way to responding Ag-Y-specific T cells. (a) If the Ag-Y-specific T cell is a Th1 cell, then the B cells will be induced to switch to IgG or IgA. (b) If the Ag-Y-specific T cell is a Th2 cell, then the B cell will be induced to switch to IgE production. Reprinted from *Immunology Today,* Mudde *et al.,* 1995, 16:380–2, with permission of Elsevier Science.

Table 1. Distribution and Affinity of FcεRI and FcεRII (CD23) Receptors

	receptors	receptors
Distribution	Mast cells Basophils	T and B lymphocytes Macrophage/monocytes Eosinophils Platelets
Number per cell	10^4 (basophils) 10^6 (mast cells)	10^3 (platelets) 106 (macrophages)
Affinity	10^9 M^{-1}	10^{-7}–10^{-8} M-1
Structure	Tetramer	Dimer

Reprinted from *Immunology Today,* Capron *et al.,* 1986, 7:15–8,with permission of Elsevier Science.

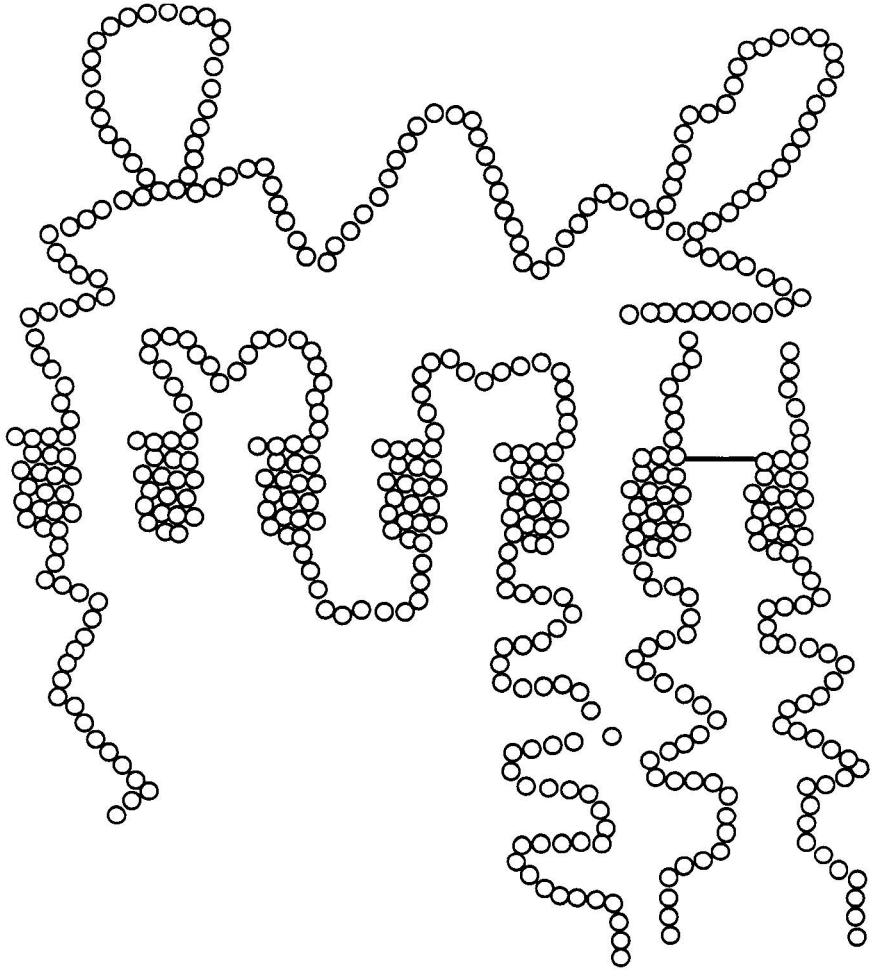

Figure 3. A schematic of the amino acid sequence of the Fcε receptor. Modified from *Immunological Reviews,* Metzger, 1992, 125:37–48, with permission from Munksgaard International Publishers.

The receptor also has two identical γ chains composed of 62 amino acids. Most of the amino acids in the chain are found in the cytoplasmic domain. Structural studies demonstrated that the y chains are homologous to the ζ chain of the CD3 molecule. Because antigen recognition motifs are found in the cytoplasmic domains of γ chains and the ß chain, these chains may be involved in signal transduction (Metzger, 1992; Liu, 1990).

Low-Affinity FcεRII (CD23) Receptor

Low-affinity receptors (CD23) for IgE are 30-kDa proteins found on B cells, monocytes, and eosinophils. Alternate splicing of messenger RNA can create two different allotypic proteins (FcεRIIa and FcεRIIb). FcεRII is constitutively expressed on B cells.

The CD23 is different from other receptors because it is a single chain with a C-terminus that is oriented outward. The molecule can coil about itself or form dimers and trimers. Because of the tight coiling, IgE can be held tightly for extended periods (Mudde *et al.,* 1995).

When antigen concentration is extremely low, the CD23 B-cell receptor may play a role in Th2 cell stimulation and expansion. IgE with a high affinity for an allergen can bind to the CD23 receptor and present the allergen to Th2 cells. Subsequent interactions between CD40 and CD40L in the presence of IL-4 induce isotypic switching to IgE and the expansion of allergen-specific B populations. The increased proliferation of Th2 cells by low allergen concentrations and the production of IgE may be responsible for expansion of allergic sensitivity from a single allergen sensitivity to multiple allergens (Mudde *et al.,* 1995).

Soluble CD23 and Inflammation. CD23 receptors solubilized during B-cell stimulation play a role in inflammation. Soluble CD23 (sCD23) reacts with the CD1 lb/CD1 1c (iC3B) complex on the surface of macrophages. Interactions between sCD23 and ligand cause an increase in the production of NO_2^-, H_2O_2, and cytokines (Kolb *et al.,* 1995). When the sCD23 is derived from the FcεRIIb allotype, interactions with the complement receptors generate IL- la, IL-1ß, IFN-γ, and TNF-α (Dugas *et al.,* 1995).

SURFACE INTERACTIONS DURING SECOND EXPOSURE

Mast cells and basophils from allergic subjects have increased numbers of high-affinity receptors for IgE. There can be from 5000 to 27,000 FcεRI receptor sites for IgE heavy chains per basophil and up to 40,000 in atopic individuals (Burrell *et al.,* 1992). At any time point, 20–40% of the total receptors are occupied by antibodies.

Cross-linkage of two cell-bound IgE initiates an energy-dependent, FcεRI signaling process that results in mediator release. Following receptor aggregation, the receptor y chain is phosphorylated by receptor-assisted protein kinase C. Aggregation also activates *lyn* (a member of the *src* family). Phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAMs) results in recruitment of *syk.* The latter binds to the phosphorylated ITAMs and is activated by autophosphorylation. After activation, *syk* moves to the cell membrane, binds to

the receptor ß chain, and regulates *c-myc* gene expression. The remaining portions of the activation pathways are similar to those described for T and B cells (Razin *et al.,* 1995).

ALLERGIC REACTIONS IN THE LUNG

Early Asthmatic Reactions in the Lung

Asthma is a disease characterized by partial airway obstruction that is reversible either spontaneously or with treatment. Up to 80% of patients with asthma have positive skin tests to common aeroallergens such as pollens or grasses. Over 75% of asthmatic patients have associated rhinitis (Nelson, 1985).

When exposed to the allergen for the second time, histamine, heparin, and trypase are extruded from the mast cells or basophils within 15–20 min. The preformed mediators have diverse biological functions. Histamine is a major pharmacological mediator released from mast cells and basophils. Interactions between histamine (H-1) receptors on smooth muscle induce contraction of smooth muscle. This causes a reduction in airway caliber and the characteristic "wheezing" associated with asthma. Histamine/H-2 receptor interactions increase mucus secretion and blood flow. Heparin is released as part of the histamine granule and functions as an anticoagulant. The most abundant protease in mast cells is trypase. This long-lived protease serves to activate other enzymes and functional molecules. For example, trypase activates many MMPs that are necessary for collagen degradation. In addition, trypase aids in the generation of the C3a anaphylatoxin.

The Arachidonic Acid Pathway. After secretion of the preformed granules, mast cells or basophils begin to synthesize mediators that augment the allergic response. Activation of the arachidonic acid pathway initiates a response resulting in activation of either the lipoxygenase or cyclooxygenase pathways.

Metabolism of arachidonic acid by the 5-lipoxygenase pathway produces leukotrienes (SRS-A) such as LTC4, LTD4, or LTE4. Leukotrienes bind specifically to receptors on smooth muscle and induce bronchoconstriction. Mast cells also synthesize and release prostaglandin D_2 (PGD_2) via activation of cyclooxygenase. PGD_2 interacts with smooth muscle receptors and acts as both a bronchoconstrictor and a vasodilator. Platelet-activating factor (PAF) is produced in interactions between membrane lipids or chemical reactions between arachidonic acid and phospholipase A_2. Bronchoconstriction, retraction of endothelial cells, and relaxation of smooth muscle are mediated by PAF.

Nitric Oxide Production. Production of nitric oxide from airway epithelial cells may play a critical role in amplification and perpetuation of the asthmatic response. Nitric oxide inhibits Th1 cell maturation and the production of IFN-γ. Production of IFN-γ is critical to the downregulation of IgE antibody production. The lack of IFN-γ allows the proliferation of Th2 cells and the production of IL-4 and IL-5. IL-5 plays a critical role in eosinophil recruitment into the airways. Eosinophils may play a major role in airway inflammation. The directed influx of eosinophils may be part of an innate defense mechanism against parasites that is accidentally activated during the asthmatic response (Barnes and Liew, 1995).

Late Asthmatic Reactions in the Lung

Over 50% of asthmatics have secondary reactions occurring 4 to 8 hr after the initial asthmatic episode. Late-phase reactions are characterized by prolonged contraction of smooth muscle, increased mucus secretion, and an inflammatory response.

Several different mechanisms are proposed to explain the clinical signs and symptoms of late phase reactions. One scenario implicates chemokines such as MCP-3, MCP-1, Rantes, and IL-3 as the cause of histamine release from basophils. This reaction does not require the presence of IgE or antigen.

Another proposed mechanism requires histamine releasing factor (HRF) and the presence of IgE on the mast cell surface. It is proposed that HRF reacts with cell-bound IgE in a way that cross-links or bridges two IgE molecules. Cross-linkage initiates the signaling process that releases mediators. However, HRF releases histamine from only 50% of asthmatic cells. It is possible that there are two IgE subtypes reflecting differences in glycosylation or alternative gene splicing. Only one subtype will react with the HRF.

A third mechanism implicates hypodense eosinophils as the effector cells in the late-phase reactions. PAF and LTB4 are chemoattractants for the eosinophils. Accumulation of the hypodense eosinophils enriches most inflammatory late-phase asthmatic reactions. Eosinophils express VLA-4 (CD49d, CD29) selectins that allow adherence to endothelial cells expressing VCAM-1. Release of major basic protein augments increased bronchial constriction induced by acetylcholine. Peroxidase and neurotoxin are also released from the hypodense cells and damage both endothelium and epithelium.

Occupational Asthma

Occupational asthma differs from classical aeroallergen atopic asthma in several respects. The disease often develops in people who are not atopic. Unlike classical asthma, the onset of asthma may require a latent period of several months to several years. When subjects are fully sensitized, they have demonstra-

ble pulmonary responses at atmospheric chemical concentrations far below the irritant concentrations causing symptoms in nonsensitized subjects (Newman Taylor, 1988).

Workplace asthma can be associated with substances such as high-molecular-mass proteins (usually 20–50 kDa) or small haptenic compounds. Workers in printing, fertilizer, enzyme producing, and soldering plants are at most risk for sensitization by high-molecular-mass compounds. In contrast, persons working with low-molecular-mass haptens such as anhydrides or isocyanates are at risk of sensitization (D. I. Bernstein *et al.,* 1982).

The role of the immune system and IgE production varies with the hapten. There is a strong association between anhydride-specific IgE and symptoms of immediate hypersensitivity (Zeiss *et al.,* 1980). In contrast, there is no clear association between hapten-specific IgE levels and isocyanate-induced asthma. Only 15% of workers with isocyanate-induced asthma have demonstrable anhydride-specific IgE (Keskinen *et al.,* 1978).

ALLERGIC REACTIONS IN THE SKIN

Urticaria is a short-lived, red, edematous lesion of the skin associated with itching. Nonimmunological and immunological mechanisms cause skin lesions. IgE-mediated immunological urticaria is more frequent in atopic subjects and is usually induced by penicillin or consumption of shellfish, soft fruits, and nuts (Anonymous, 1997). Nonimmunological urticaria can be elicited in healthy subjects by low-molecular-mass substances such as ammonium persulfate (Mahzoon *et al.,* 1977), dimethyl sulfoxide (Odom and Maibach, 1976), and cinnamaldehyde (Kirton, 1978).

Introduction of allergens into the skin induces the immediate release of histamine from tissue-bound mast cells. Histamine activates venule endothelial cells by interacting with surface receptors. Activated endothelial cells initiate synthesis of PAF and nitric oxide that induce relaxation of the vasculature. Red cells accumulate in the area creating a characteristic redness (erythema) in the skin. Concurrently, the endothelial cells retract creating openings in the venules. Plasma leaks from the vessel into the skin inducing edema or swelling in the skin (wheal). Blood vessels at the periphery of the wheal often dilate creating a flare (Mathews, 1990).

ALLERGIC REACTIONS IN THE GUT

Within minutes after ingesting allergenic foodstuffs, IgE-mediated reactions occur in the intestine and patients exhibit vomiting, diarrhea, malabsorption, and

blood and protein loss through the intestine. Food allergy is uncommon, affecting approximately 5% of infants and 2% of the adult population.

The major food allergens in the United States and Europe are peanuts, soybeans, tree nuts, milk, eggs, crustacea, fish, and wheat. The allergens may be major (80%) or minor (1%) components of the total plant protein (Table 2). Most food allergens are proteins with a molecular mass between 10 and 70 kDa (Hefle *et al.,* 1996).

Food allergens have unique chemical characteristics that prevent inactivation in the gut. In general, food allergens are stable at temperatures of 100°C for 3 hr over a pH range of 2.8 to 10 (Barnett and Howden, 1986). Depending on the nature of enzymatic treatment and the choice of methods for assessing the immunogenicity, food allergens are resistant to proteolysis and acid hydrolysis (Taylor, 1995).

In simulated gastric and intestinal models of mammalian digestion, many food allergens are stable for as long as 60 min. The resistance to digestion allows intact antigen to transit from the gut into the mucosa. One exception to this rule is ß-lactoglobin from cow's milk. This molecule becomes more antigenic following proteolytic digestion (Haddad *et al.,* 1979).

ORAL ALLERGY SYNDROME

Individuals with allergies to tree, grasses, weeds, and ragweed pollens may develop itching of the lips, tongue, and buccal mucosa after eating fruits (Enberg,

Table 2. Percent Allergenicity of Common Food Allergens

Protein	Total percent protein	Percent allergenicity
Egg allergens		
Ovalbumin	54	100
Milk allergens		
ß-Lactoglobin	9	72
Casein	80	56
a-Lactoglobin	4	14
Soybean allergens		
ß-Conglycinin (ß subunit)	18.5	75
Kuntiz trypsin inhibitor	2–4	25
ß-Conglutinin (α subunit)	18.5	20
Soy lectin	1–2	10

Modified from *Critical Reviews in Food Science and Nutrition,* Metcalfe *et al.,* 1996, 36:S177.

1991). Systemic effects are not engendered because the fruit allergens are easily degraded in the stomach. Oral allergy occurs because aeropollens, rubber products, and food allergens share similar antigens. Birch pollen, apples, carrots, potatoes, celery, hazelnut, and kiwi fruit have similar or identical allergenic epitopes. Ragweed pollen shares common epitopes with melons, bananas, zucchini squash and cucumbers. Latex antigens cross-react with bananas, chestnuts, and avocados (Anderson, 1996).

NON-IgE-MEDIATED ANAPHYLACTOID REACTIONS

NSAIDs

Anaphylactoid reactions are not mediated by IgE, but they have symptoms and time courses that mimic allergic reactions. NSAIDs are a major cause of anaphylactoid reactions. NSAIDs such as aspirin alter the arachidonic acid pathway and increase asthmatic response. Pulmonary hyperactivity induced by aspirin and other NSAIDs is not mediated by IgE reactivity. These patients manifest a cyclooxygenase blockade of arachidonic acid metabolism leading to preferential production of leukotrienes from the lipoxygenase pathway (Burrell *et al.,* 1992).

Sulfites

Asthma can also be induced by sulfite gases released when food is chewed or when gases are liberated by other means. Although the mechanism of action is unclear, the response may be associated with a sulfite oxidase deficiency in some asthmatics.

Radiocontrast Media

During the radiologic evaluation of patients, various types of low-molecular-mass, water-soluble, radiocontrast media are administered to patients via the intravenous route. Rashes, urticaria, angioneurotic edema, and smooth muscle spasms occur with some frequency. Occasionally, patients develop hypotension, pulmonary edema, and cardiac arrest as a result of an anaphylaxis-type reaction. Mortality from radiocontrast media administration is 1–5/100,000 (Moreau *et al.,* 1988).

Radiocontrast media may act directly on mast cells and basophils to release mediators. Salts of ioxaglic acid, ioxithalamic acid, and ioversol release preformed mediators such as histamine and trypase. But they do not initiate the *de novo* synthesis of prostaglandins and leukotrienes (A. Genovese *et al.,* 1996). The effect differs with the nature of the target cell (mast cells or basophils) and

the anatomic site. Hyperosmolality may play an important role in the activation of basophils but not mast cells (Stellato *et al.,* 1996).

Toluene Diisocyanate (TDI)

TDI is widely used in polyurethane production and in the manufacture of plastics, foam insulation, and synthetic coatings. The number of workers potentially exposed to TDI is large. Although the clinical signs and symptoms resemble classical asthma, TDI asthma may not be immunologically mediated. Less than 15% of TDI asthmatics have IgE directed toward the hapten (Butcher ***et*** *al.,* 1980). Moreover, the presence of isocyanate-specific IgE does not correlate with bronchial reactivity (Butcher *et al.,* 1977).

Studies suggest that TDI induces obstructive airways disease through pharmacologic, not allergic, mechanisms. TDI may be acting as a partial ß receptor blocker. Normally, stimulation of the ß receptor causes an increase in intracellular cAMP. High levels of cAMP relax smooth muscle and restore airway caliber. Blockade of the ß receptor prevents a rise in intracellular cAMP levels. Failure to produce sufficient quantities of cAMP from adenylate cyclase stimulation leads to unopposed α adrenergic stimulation resulting in bronchial constriction and mucus hypersecretion (Davies *et al.,* 1977).

Non-IgE-Mediated Food Reactions

Food-allergy-like symptoms can be induced by contaminated foods or foods containing high concentrations of allergic mediators. In scromboid fish poisoning, the normally high level of histidine in tuna or mackerel is decarboxylated to histamine by proteus or klebsiella contamination during food processing.

Histamine or HRF is found in high concentrations in Parmesan or Roquefort cheese, red wine, and strawberries. Other active substances that are implicated in allergic reactions are also found in foods. Serotonin, a small molecule that dilates capillaries, increases capillary permeability, and contracts smooth muscle, is found in high concentrations in bananas, pineapples, plantain, and avocados.

IgE AND PARASITIC INFECTIONS

Th2 responses are the major defense mechanism against helminths. Infections with *Trichuris, Nippostrongylus,* or *Heligmosomoides* can be terminated by a Th2 response that accelerates death and expulsion of the worms. In other infestations, a Th2 response against *Brugia* and *Schistosoma* protects the host against infection. For *Schistosoma,* the Th2 response is directed toward the schistosome eggs and is associated with granuloma formation.

Using trichinella infections as a model system, it is possible to define the sequence of events associated with a Th2 response to worms. Trichinella stimulates the Th2 cells to produce IL-4, IL-5, and IL-10. Increased levels of IL-4 stimulate B cells to produce antigen-specific IgE in quantities ranging from 100 to 10,000 ng/ml. After IgE reacts with the trichinella, immunocompetent effector cells react with the Fc portion of IgE. A number of different immunocompetent cells can kill the parasite via antibody-dependent cellular cytotoxicity (ADCC). The ADCC effector cells include effector macrophages, eosinophils, and platelets (Capron *et al.,* 1986).

PHARMACEUTICALS, CHEMICALS, AND ALLERGiC REACTIONS

Common IgE-Mediated Allergic Reactions

Reactions to penicillin are common in 1 to 10% of patients taking the drug. The Th2 response is directed toward metabolic products of the drug. The most common antigen is the penicilloyl derivative reacted with ε amino groups in lysine residues of proteins. A second antigen is created when the thiazilidine ring of penicillic acid is opened to create penamaldic acid. The acid can form disulfide linkages with proteins

Symptoms, occurring 10–14 days following treatment, can be distinctly different. Urticaria and angioedema characteristic of an IgE-mediated reaction have been reported. Serum sickness and immune complex vasculitis are a common occurrence. In rare instances, penicillin induces pulmonary fibrosis associated with an autoimmune response.

HAZARD IDENTIFICATION

Because IgE and non-IgE mechanisms (e.g., irritant reactions) can elicit symptoms of allergic reactions, it is necessary to define the mechanism. By definition, IgE-mediated immediate allergic reaction implies that (1) the biology of the host has been irreversibly altered, (2) the host will respond to lower concentrations of the sensitizing agent when compared with normal subjects, and (3) subsequent reactions in sensitized subjects may be injurious and even fatal. Conversely, non-IgE-mediated, irritant or nonspecific reactions do not alter the host with long-term untoward health effects. Thus, in the strictest sense, non-IgE-mediated reactions are industrial hygiene problems that can be resolved by remedial action. Following the remedial action, the problem will abate and the subject will return to a normal state. IgE-mediated allergic reactions are a much more

serious problem and can only be resolved by reducing the allergenic material to levels that do not evoke symptoms. Highly sensitized subjects may be forced to change lifestyles or occupations.

Standard toxicity testing is not designed to detect immediate hypersensitivity reactions. Other tests and endpoints may give some insights into the test material's ability to induce allergic reactions. An initial assessment of allergenicity can be made by searching the literature to determine whether the test compound belongs to a family of chemicals known to induce allergic reactions in humans.

Examination of structure–activity relationship is another important first step in the assessment process. From the structure and nature of chemically reactive groups, it is possible to determine whether the small-molecular-weight chemical (SMWC) can bind to proteins.

To elicit an immune or allergenic antibody response, the SMWC must covalently bind to protein creating a SMWC–protein complex. Characteristically, SMWCs form multiple covalent or hydrogen bonds with proteins. SMWC–protein binding can be measured in *in vitro* assays. Protein derivatization can be measured by high-performance liquid chromatography and monitoring shifts in retention time of native and modified proteins (Gauggel *et al.,* 1993).

Immunization of animals with the protein–haptencomplexes can be used to determine whether antigen-specific IgE antibodies are formed. The presence of antigen-specific IgE can be determined by ELISAs or radioallergosorbent tests (RASTs).

Often it is helpful to compare amino acid sequences of putative allergenic proteins with the known sequence of allergenic T-cell epitopes. The epitopes reacting with T cells are usually 8–10 adjoining amino acids in length and conserved in homologous allergens of disparate species (Rothbard and Gefter, 1991). Therefore, it is possible to define a sequence test that compares the amino acid sequence of the protein with those of known allergens (Metcalfe *et al.,* 1996).

The object of the epitope mapping exercise is to find matching sets of eight amino acids in the introduced protein and known allergens. Amino acid sequence homologies between the putative protein and known allergens can be determined by searching computer data bases. Several public domain data bases such as Gen Bank, EMBL, PIR, and Swiss Prot can be searched using the FASTA computer program (Pearson and Lipman, 1988).

The utility of allergen epitope mapping may be limited. A match of eight amino acids in the putative protein and the known allergen does not confirm allergenic potential. The matched amino acids may be unrelated to the allergenic epitope within the protein (Metcalfe *et al.,* 1996). More sophisticated approaches are needed to define the epitopes. Moreover, mapping only identifies linear epitopes and cannot identify discontinuous conformational epitopes created by the tertiary structure of the allergen. Although the number of conformational

epitopes in allergenic proteins is unclear, the discontinuous epitopes are major allergen components of birch pollen (Seiberler *et al.,* 1994).

The primary clinical means of detecting IgE-mediated hypersensitivity in humans is the skin test (scratch test). The skin is superficially abraded with a small, sharp instrument and small doses of suspected allergen are applied to the abraded areas. The appearance and size of the wheal and flare responses occurring within minutes, as compared with saline and histamine controls, are considered positive.

Skin test reactivity may or may not correlate with symptoms. Patients may exhibit positive skin sensitivity without showing clinical signs and symptoms of allergy on natural exposure to the same agent.

Laboratory Testing

Radioallergosorbent Test (RAST). Laboratory tests can be used to support a tentative medical diagnosis of immediate hypersensitivity. The RAST is an *in vitro,* radioisotopic method used to document the presence of allergen-specific IgE (Adkinson, 1974). In the assay, antigen is coupled to a solid-phase support (e.g., cellulose acetate disks) and incubated with test serum. Following washing, radiolabeled anti-human IgE is added. After further washing, the disk is placed in a suitable isotope counter and the radioactivity counted. The presence of antigen-specific IgE correlates with symptoms in only about 70% of patients (Berg and Johansson, 1974).

Histamine Release Assay. This assay measures histamine release from sensitized basophils following incubation with putative allergens. The correlation between *in vitro* histamine release is above 90% (Siraganian, 1975).

REGULATORY POSITION ON IgE ALLERGIC REACTIONS

There are no formal testing guidelines for testing the allergenic potential of test chemicals. Moreover, there are no validated animal models for assessing immediate hypersensitivity. Industrial and academic scientists have developed animal models used to screen for potential allergenicity. These animal models will be discussed in later chapters.

Manufacturers of biotechnology-derived plants and the FDA have devised a strategy assessing the allergic potential of transgenic plants and foodstuffs derived from these plant types (Metcalfe *et al.,* 1996).

There are two courses of action depending on whether the source gene introduced into foods originated in a plant known to be a source of allergens.

Proteins from Source Plants with Known Allergic Potential

If the gene originates in a plant with known allergens, the FDA, as part of its policy on foods derived from new plant varieties, has mandated a series of tests designed to assess the allergic potential of the gene product. To test for the presence of known source allergens in the new transgenic plant (e.g., peanut allergens transfected into soybeans), ELISAs can be performed using pooled IgE from allergic patients with a documented sensitivity to source plant allergens. A positive reaction in the assay gives strong evidence that a source allergen has been transfected into the new plant variety. Consequently, the FDA requires labeling of the product to indicate the presence of the allergen from the source plant.

Additional tests must be undertaken to ascertain whether normal food processing would eliminate the allergenic potential of the protein. For example, if derived oils are the final consumer products and the allergen is a protein, there is little possibility of exposure to the protein allergen. Similarly, normal food processing (e.g., heating or freezing) may destroy the allergenicity.

If the gene product is negative in the *in vitro* assays, additional tests would be undertaken to confirm the lack of allergenic potential. Subjects sensitive to the source protein would be skin tested with an extract from the new transgenic plant variety using an *in vivo* prick or scratch test. If positive skin tests are demonstrable, the FDA requires labeling as described previously. If the gene product is negative in both the *in vitro* and *in vivo* skin test assays, an *in vivo* challenge test is used to confirm nonallergenicity. Sensitive and nonsensitive patients would be challenged with the food in tightly controlled, double-blind, placebo-controlled food challenges (DBPCFCs) (M. Bernstein *et al.,* 1982). When no adverse reactions are observed in the testing protocols, it is assumed that the new plant variety does not express endogenous allergens present in the source plant.

The DBPCFCs are necessary to confirm food allergic reactions but they are not without risk to the patient. Double-blind testing is necessary because of the known discrepancy between subjective observations (e.g., migraine headaches and intestinal symptoms) and the challenge tests (Anonymous, 1997). In addition, the risk of anaphylactic reactions is an inherent danger in testing skin-test-positive patients.

To properly interpret the data from challenge tests, careful consideration should be given to technical and confounding factors (Bindslev-Jenson *et al.,* 1994). Obviously, the form (e.g., raw, cooked, or freeze-dried) and the amount of food necessary to evoke symptoms are critical. The identification and delineation of additive or synergistic factors that influence the data are also important. Select trigger reactions induced by drugs, alcohol, exercise, heat or cold temperatures may be necessary to elicit food allergic reactions in challenge tests.

Proteins from Plants with No Known Allergic Potential

If the protein is from a nonallergenic source plant, a different approach is undertaken. The basic premise is that there is no single predictive test for determining the allergenic potential of food proteins. Also, it is assumed that food allergens cross the intestinal membranes and are resistant to heat and acid digestion. Therefore, structure–activity relationships, T-cell epitope mapping, heat and acid stability yield critical information on the allergenic potential of proteins. Acid stability can be tested in gastric and intestinal models of mammalian digestion described in the *United States Pharmacopeia.* Typically, food allergens are stable for more than 1 hr in the acid environment.

14

Autoimmunity

INTRODUCTION

Autoimmune diseases occur in 5–7% of the U.S. population. The incidence of autoimmune diseases is expected to increase as the average age of the population increases. Autoimmunity is closely tied to the aging process.

Autoimmune diseases may be restricted to a target organ or result in a systemic, multiorgan response (Table 1). Specific target organs are the kidney, spleen, and lung. Multiorgan responses may involve all of the organs, joints, and the skin.

It is necessary to differentiate between an autoimmune disease and autoimmune responses. In autoimmune diseases, the immune system has generated memory cells and the response continues long after the initial stimulus has been removed. In contrast, select drugs can evoke an autoimmune response. The response has the same pathophysiology of an autoimmune disease, but the symptoms abate when the drug is withdrawn. Moreover, immunological memory cells are not generated and there is no long-lasting immune response.

ANTIBODY MEDIATED AUTOIMMUNITY

Goodpasture's Syndrome

Goodpasture's syndrome is an autoimmune disease causing rapid progressive pulmonary hemorrhage and/or glomerulonephritis. The autoantigen is part

Table1. Autoantigens Associated with Autoimmune Disease

Disease	Organ cell	Target antigens
IDDM	Islet cells	GAD65, GAD67 Tyrosine phosphatases
Addison's disease	Adrenal cortex	21-Hydroxylase
Pernicious anemia	Parietal cells	H+/K+-ATPase Intrinsic factor
Thyroid disease	Thyroid	Thyroid peroxidase, thyroglobulin, T_3,T_4, TSH, TSH receptor

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of the type IV collagen matrix within the basement membrane (Kalluri *et al.,* 1995). When both the lung and kidney are disease targets, the autoantigen is part of the α3(IV)NC1 collagen chain. The epitope resides in the C-terminus and consists of a discontinuous association of the last 36 amino acid residues. Over 85% of patients with lung and kidney disease form antibodies directed toward the α3(IV)NC1 collagen chain. The kidney is the sole target organ in the remaining 15% of patients with Goodpasture's. These subjects produce antibodies directed toward the α1(IV)NC1 or α4(IV)NC1 collagen chains (Hudson *et al.,* 1993; Kalluri et al., 1995). This collagen form is antigenically and structurally distinct from the α3(IV)NC1 chain.

Systemic Lupus Erythematosus (SLE)

In SLE, subjects form autoantibodies to a multitude of tissue antigens including DNA, mitochondria, red blood cells, and clotting factors. As a consequence of reactions between antibodies and target tissue, hemolytic anemia, leukopenia, and thrombocytopenic purpura are common observations. In addition, deposition of antibody–DNA complexes in the capillary bed induces an extensive vasculitis.

Graves' Disease

Graves' disease, which is an autoimmune response in the thyroid, results in hyperthyroidism. Antibodies react with the thyroid-stimulating hormone receptor (TSHR) stimulating the production of thyroid hormones. The antibodies are unique in that they avidly bind to specific regions of the TSHR including amino acid sequences 32–56 and 309–357 (Miyashita and Mori, 1994).

It is unclear whether antibodies or Th2 cells are the primary cause of the disease. There is a poor correlation between receptor-specific antibody levels and

the disease course. In contrast, sensitized Th1 cells that recognize thyroid antigens and other tissue epitopes are often associated with Graves' disease.

Th1 cells are also associated with an ophthalmic autoimmune response associated with Graves' disease. These cells can infiltrate the eye orbit via adhesion factor interactions and attack fibroblasts in the eye. In turn, the fibroblasts become effector cells in the ophthalmopathy (Heufelder, 1995). The mechanism by which the T cells escape the normal tolerance barriers is unknown.

Myasthenia Gravis

In this disease, patients form antibodies to the acetylcholine receptor that is responsible for transduction of nerve impulses (Yagi and Uono, 1979). Interactions between the antibody and the acetylcholine receptor induce receptor endocytosis and degradation (Lisak *et al.,* 1985). The resulting failure to respond to neural impulses creates the flaccid paralysis characteristic of myasthenia gravis.

Pernicious Anemia

Autoimmune gastritis, leading to pernicious anemia, is a chronic disease characterized by atrophic gastritis and circulating parietal cell antibodies. Autoantigens have been identified as the α and ß subunits of the gastric proton pump protein (Gleeson and Toh, 1991). Most patients with the disease form antibodies to the parietal cell components and intrinsic factor produced by parietal cells (Kaye, 1987). Complement lysis of parietal cells causes atrophic gastritis. In addition, antibody neutralization of the intrinsic factor activity prevents intestinal adsorption of vitamin B_{12} that is necessary for hematopoiesis. Abnormal hematopoiesis and megaloblastic anemia occur because of the vitamin deficiency.

THE Th1/Th2 PARADOX IN AUTOIMMUNITY

Because most autoimmune diseases are mediated by antibodies, it was assumed that CD4 Th2 cells were the primary effector cells. Recent data show that Th1 cells are involved in the induction of autoimmune diseases. Th1 cells are found in most experimental autoimmune animal models of experimental allergic encephalomyelitis, insulin-dependent diabetes mellitus (IDDM), and collagen-induced arthritis. Th1 cells are also involved in several human organ-specific autoimmune diseases. Cells with a Th1 phenotype have been isolated from infiltrates from Hashimoto's thyroiditis, Graves' disease, and multiple sclerosis (Trembleau *et al.,* 1995).

ETIOLOGY OF AUTOIMMUNE REACTIONS

IL-12-Dependent Activation of Autoreactive T Cells

The normal T-cell population in humans and animals contains quiescent autoreactive T cells. Microbial products such as endotoxins, bacterial DNA, and oligonucleotides containing unmethylated cytosine-guanine nucleotides can activate autoreactive T cells when antigen-presenting cells are producing IL-12.

Activation of autoreactive T cells is nonspecific in nature. IL-12 enhances the differentiation and proliferation of Th1 cells both *in vivo* and *in vitro.* Moreover, IL-12 induces IFN-γ production in T cells and increases activated T-cell adhesion to epithelial cells (Segal *et al.,* 1997). The ability to activate the autoreactive T cells is solely dependent on the production of IL-12 (Segal *et al.,* 1997).

In animal models of autoimmunity, administration of IL-12 accelerates the disease onset or increases the severity of disease processes. In models of IDDM, IL-12 administration increases the frequency of disease to 100% of female NOD mice compared with 60% in controls. Moreover, Th1 cytokines are produced by cells infiltrating the pancreas. In experimental allergic encephalomyelitis, mice given IL-12 develop a more severe disease with a protracted clinical course and develop Th1 cell infiltrates. In contrast, in the collagen arthritis model, IL-12 enhances the production of IFN-γ by collagen-specific Th1 cells. The IFN-γ upregulates production of IgG2a and IgG2b antibody isotypes. These collagen-specific, complement-fixing antibodies cause more severe, destructive arthritis when compared with control animals (Segal *et al.,* 1997).

Loss of Suppression and an Increase in Autoimmune B Cells

There is evidence in humans and animals that autoimmune thyroid disorders are related to dysregulation of antigen-specific suppressor T cells. The dysregulation is the result of a depletion of Ts cells or an inadequate activation of suppression (Volpe, 1994). The dysregulation may allow the production of CD5+ B cells. These "autoimmune B cells" bind many self antigens and can be autostimulated to produce antibodies directed toward self antigens. There is a significant increase in CD5+ B cells in patients with Graves' disease (Afeltra *et al.,* 1995).

Mutations in HLA Class II Antigens

IDDM, also known as juvenile or type I diabetes, occurs in only 0.2% of all diabetes. Usually, the disease begins early in life with a peak age of onset of 11 to 12 years. Destruction of insulin-producing ß cells is related to both cytotoxic T cells and an antibody response.

There is a strong association between the presence of cis-dimers of HLA-DQ and IDDM in all ethnic groups studied (Todd, 1990). Evidence suggests that a single amino acid substitution at position 57 in the ß-chain is responsible for the autoimmune disease. In normal subjects, aspartic acid is normally found at position 57 and the ß chain is minimally expressed on cells. IDDM patients commonly express high numbers of the ß chain and have alanine, valine, or serine at position 57 (She, 1996).

There are two hypotheses to explain the diversity of the autoimmune responses in IDDM. Both hypotheses assume that nonspecific inflammation or viral infections increase apoptosis with the release of autoantigens (Akerblom, 1992).

Epstein–Barrvirus (EBV) may be involved in the initiation of the IDDM immune response. A five-amino-acid sequence (GPPAA) in the 57th amino acid region of HLA-DQß1 is found in the BERF4-encoded ABNA3C protein of EBV. Patents with acute EBV infections and valine, serine, or alanine at position 57 develop acute diabetes. Antibodies against the EBV peptide may persist for several years (Parkkonen *et al.,* 1994). Complement-induced lysis of cross-reactive antibodies bound to ß cells may initiate the disease.

In the first scenario, glutamic acid decarboxylase (GAD65) is released from dying cells and reacts tightly with the mutated HLA-DQß1. The latter has a permissive site for amino acids and four anchor proteins suitable for binding GAD. Presentation of the GAD65 with the altered MHC generates cytotoxic T cells specific for insulin-producing ß cells (Figure 1). Cytotoxic T cells and activated Th1 cells secrete IFN-γ that is toxic to the islet cells. Secretion of IL-10 and IL-14 by other immunocompetent lymphocytes activates macrophages, and increases the activity of the cytotoxic T cells.

Killer T-cell lysis of virus-infected or apoptotic cells releases antigenic peptides such as insulin, heat shock protein 60 (Tishler and Shoenfeld, 1996) , carboxypeptidase H, islet cell surface antigen (ICA-512) (Dobersen, 1982) and islet cell mitochondrial antigen (Yeo, 1985). These protein antigens are presented to Th2 cells and B cells culminating in the production of autoantibodies.

In the alternate hypothesis, there is assumed to a “single hit” effect. More specifically, viral infection, nonspecific inflammation, or apoptosis induce the release of GAD and all of the antigenic proteins at same point in time. Therefore, the diversity of response is generated in a single step.

A neurological condition, stiff-man syndrome, may be associated with IDDM and Graves disease. The condition may be produced by autoantibodies directed toward GAD65. Under normal conditions, GAD converts glutamic acid to γ-aminobutyric acid (GABA). GABA is the major membrane-stabilizing neurotransmitter of the central nervous system. Destruction of GAD65 causes a severe neurological deficit.

IDDM may be exacerbated by the fact that many peptide antigens share

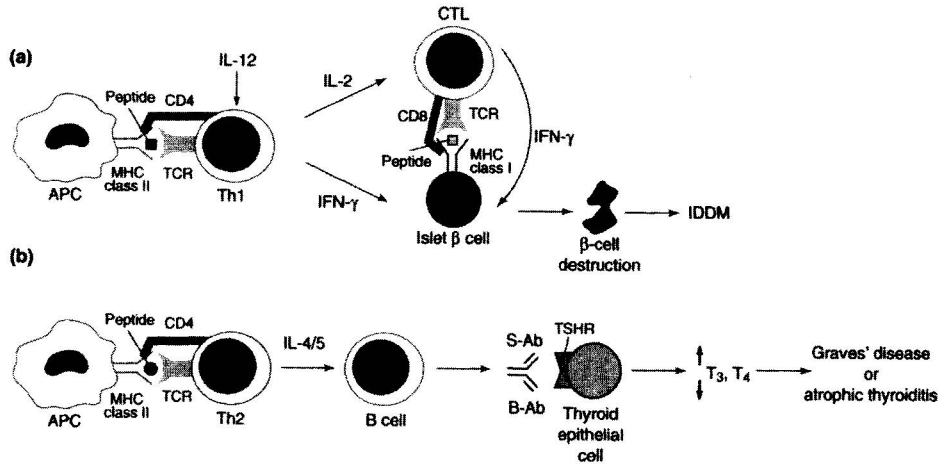

Figure 1. Model of pathogenic mechanisms involved in autoimmunity. Model of two mechanisms involved in autoimmune syndromes. (a) B cell antigen is processed by APCs and presented to CD4+ Th1 cells. Activated Th1 cells and CTLs secrete IFN-γ that is toxic to β cells. CTLs can also secrete cytotoxic agents. Th1 cells promote the activation and proliferation of CTLs by secreting IL-2. This mechanism leads to the destruction of β cells and the pathology of IDDM. (b) Viral antigen that mimics a cell molecule such as TSHR is processed and presented by APCs to Th2 cells that secrete both IL-4 and IL-5. These cytokines stimulate B cells to produce either thyroid stimulating or blocking antibodies. This results in decreased levels of T_3 or T_4 in Graves' disease or atrophic thyroiditis. Reprinted from *Immunology Today,* Song *et al.,* 1996, 17:232–8, with permission of Elsevier Science.

antigenic epitopes with proteins and microorganisms. Infections with these xenobiotics may trigger an autoimmune response to the pancreatic cells. The two GAD65 peptides p17 and p18 share a sequence homology with coxsackievirus P2-C (sequence identity PEVKEK) capsid protein and are the major epitopes recognized by T cells (Schloot *et al.,* 1997). Heat shock proteins share epitopes with many *E. coli* and *Lactobacillus* species. Carboxypeptidase H is structurally similar to both the coxsackie coat protein and nucleoprotein of influenza A.

Modified "Self" Antigens

In theory, it is possible to mount an immune response against modified self-determinants (neodeterminants). Although there are little data supporting the existence of neoantigens and their role in induction of autoimmune responses, two examples are usually cited. Changes in the Fc portion of IgG following reaction with antigen expose a neoantigen. This neoantigen elicits the transient production of rheumatoid factors (RFs) that are anti-IgG antibodies. Another example is the nephritic factor reported in glomerulonephritis. This autoantibody reacts with complement factor C3 neoantigens exposed during C3 activation (Theofilopoulos, 1995).

Binding of drugs or drug metabolites may create neoantigens. When the host tissue is modified by the interaction with the drug, antibody responses to "self proteins" would result. In an autoimmune response, the antibody would be directed toward the drug-altered self protein but not the native self protein (Theofilopoulos, 1995).

Molecular Mimicry

Bacteria are constantly evolving and devising mechanisms to evade the immune system. Often, bacteria express linear amino acid sequences that are homologous to "self proteins" in the host. Therefore, a response to the bacteria causes an autoimmune reaction in the host.

Streptococcus Infections and Heart Valves. Group A ß hemolytic streptococci possess antigens that are similar to those found on cardiac muscle. During a streptococcal infection, antibodies directed toward the bacteria also react with cardiac tissue, creating an autoimmune response. This autoimmune response may be the stimulus for heart valve damage in rheumatic fever (Theofilopoulos, 1995).

In the dental community, there is a concern that dental procedures can cause systemic infections with *Streprococcus mutans,* a common oral commensal responsible for dental caries. Further, it is assumed that the immune response to *S.*

mutans would produce antibodies that cross-react with heart tissue. It is unlikely that this organism could initiate or exacerbate heart valve damage. There is no antigenic similarity between heart valve antigen and high-molecular-mass surface protein antigen of *S. mutans* (Russell and Wu, 1990).

Ankylosing Spondylitis. Ankylosing spondylitis is a form of reactive arthritis following *Klebsiella* infections. Over 90% of patients express the MHC B27 marker on nucleated cells (Creamer *et al.,* 1992). Early studies indicated that there is molecular mimicry between MHC B27 and *Klebsiella pneumoniae* nitrogenase. The nitrogenase peptide KAKAQTDR fragment binds to the MHC B27 groove region that contains DRED sequence. Other sequences of *Klebsiella* such as pulD secretion protein (DRDE) also bind to the B27 molecule. Similarly, pulA (pullulanase) enzyme (Gly-X-Pro), and HLA B27 have substantial homology with collagen types I, III, and IV (Fielder *et al.,* 1995). Effector cell interactions between the modified MHC B27 initiate immune responses and the destruction of tissue.

Other antibody-mediated mechanisms may also augment the autoimmune response. The LPS endotoxin from *Klebsiella* can stimulate polyclonal activation of the B cells resulting in the generation of low-affinity IgM antibodies. Complement-mediated lysis of antibody-coated cells liberates antigenic enzymes that trigger the autoimmune reaction to additional tissue antigens.

Reactive Arthritis. Reactive arthritis is an autoimmune response in the major joints in the body. It is triggered by urethral infections with *Chlamydia trachomatis* (Larsen *et al.,* 1994) or gastroenteritis caused by *Yersinia* (Probst *et al.,* 1993), *Salmonella, Shigella,* or *Campylobacter* (Maki-Ikola *et al.,* 1991). There is extensive molecular mimicry *between* surface proteins on these bacteria and MHC B27. YadA *(Yersinia* adhesion molecule) and OmpH (an outer surface protein on both *Yersinia* and *Salmonella)* have sequences homologous to B27. Identical positional homologies also exist between B27 and a pentapeptide from *Shigella flexneri.* Therefore, reactions directed toward these bacteria create antibodies directed toward cells expressing the B27 marker.

Other Arthritic Conditions. In other forms of rheumatoid arthritis (RA), there is an association with MHC DR4/Dw4. In most of the DR4, there is a common five-amino-acid sequence Q(K/R)RAA that occurs in the ß1 chain (Auger *et al.,* 1997). Individuals who are homozygous for this "shared epitope" gene are at high risk for developing RA. Moreover, this gene is predictive of destructive bone disease. Peptides with the "shared epitope" can be processed and presented to T cells. This Q(K/R)RAA sequence may provide a means to select low-affinity, autoreactive T cells during maturation in the thymus.

Some human pathogens express the Q(K/R)RAA sequence in immunogenic proteins. The five-amino-acid sequence has been reported in DnaJ heat shock proteins from *E. coli, Lactobacillus lactus, Brucella ovis,* and Epstein–Barr gp110 protein (Albani and Roudier, 1992). These heat shock proteins bind avidly to the DR4 molecule and the complex is recognized by T cells.

A multistep process is responsible for the elicitation of RA. Neither the DR4 nor the DnaJ by themselves can initiate an autoimmune response. In the first step, immunogenic foreign material containing the "shared epitope" is localized in the inflammatory site by synovial type A macrophages. T cells that bind the Q(K/R)RAA are selected via high-affinity TCR interactions and expanded following T-cell stimulation. The reaction is perpetuated by the interaction of T cells with MHC-DR4, MHC-DR4-complexed foreign proteins containing the Q(K/R)RAA sequence or the Q(K/R)RAA sequence in soluble proteins. Destruction of cells carrying the MHC-DR4 marker may release other immunogenic self proteins or expose cryptic markers that contribute to "epitope spreading" (Elson *et al.,* 1995).

T. cruzi, the etiological agent of Chagas' disease (CD), initiates the polyclonal activation of B cells and production of antibodies. These antibodies are directed toward multiple tissue targets including heart muscle, DNA, and collagen (Heipe *et al.,* 1996). Antibodies directed toward cardiomyocytes recognize the C-terminus of the trypanosome ribosomal PO protein, the second extracellular loop of the human adrenergic receptors (ß1 and ß2), and the M2 muscarinic receptor (Elies *et al.,* 1996). Sequence comparisons between the ß1 receptor and the ribosomal protein (Ferrari et al., 1995) show a five-amino-acid sequence homology (AESEE in the ribosomal protein and AESDE in the receptor). Interactions between antibodies and cardiomyocyte adrenergic receptors modulate CAMP-activated calcium channels (Mjares *et al.,* 1996).

Other antigens on the surface of *T. cruzi* are structurally similar to the invariant chain CLIP protein fragment involved in class II antigen processing and recognition. Reactions between the anti-*T. cruzi* antibody and the complexed CLIP fragment can initiate lysis of the host cell. Often the class II markers with attached invariant chain are transported to the cell surface via the trans-Golgi network.

Cryptic "Self" Activation

In this hypothetical scenario, it is assumed that each self protein has several dominant epitopes that serve as self markers. During T-cell maturation, T cells reacting with these markers are negatively selected and destroyed or become tolerant of "self" markers. Conversely, there are many minor (subdominant) epitopes constituting "cryptic self" that can induce an immune response. This is possible because tolerance directed toward the "cryptic self" markers was not

established during development of the immune system. An immune response to these markers is usually not undertaken because of ineffective antigen processing or hindered access to the MHC that prevents the initiation of an immune response.

Chemical exposure or viral infections may provide the initiating event for presentation of "cryptic" self antigens. Viral infections increase the presentation of the subdominant maker by molecular mimicry (Theofilopoulos, 1995). Chemical exposure may also result in presentation to T cells by creating altered "cryptic" antigens with high affinity for MHC molecules. Antinucleolar antibodies induced by exposure to $HgCl_2$ are the result of presentation of "cryptic" self peptides to Th2 and B cells.

Sequestered Antigens

Tissue antigens, sequestered behind anatomic barriers, do not contact T cells when the repertoire of "self" antigens are determined. Sequestered antigens are found in tissues such as the eye and testis. T cells are not rendered tolerant or anergic to the sequestered antigens. When antigens from these immunologically privileged sites contact immunocompetent cells, induction of an autoimmune disease results.

Sequestered antigens come into contact with the immune system following trauma to an organ. Autoimmune uveitis or sympathetic ophthalmia following an eye injury or orchitis following a vasectomy are examples of responses to sequestered antigens (Theofilopoulos, 1995). However, trauma alone does not always stimulate an immune response. Tissuetropic pathogens may be needed for the production of costimulatory molecules necessary for elicitation of the immune response.

Protection against autoimmune disease is facilitated by the expression of FasL. High levels of FasL are expressed on cells in immunologically privileged sites such as the eye and testis. These same cells lack the costimulatory B7 molecule. Activated T cells entering a target organ upregulate the expression of MHC II molecules via the secretion of IFN-γ Endogenous antigens are presented to CD4 cells via the MHC II molecules. Without B7 costimulation, the Fas is upregulated and expressed on T cells. Linkage of Fas and FasL induces apoptosis in the autoreactive T cells (Dayan *et al.,* 1997), preventing stimulation of T cells.

Immune Deviation

The intraocular portion of the eye is not sequestered from the immune system. It is equipped with antigen-presenting cells, efferent and afferent immunological communication routes, and draining lymph nodes. Yet, CD8 cell medi-

ated autoimmune reactions in the eye are rare. This may be the result of many different factors. The anterior of the eye is lined by corneal epithelium lacking MHC markers and containing iris/ciliary bodies. The former cannot present antigen to T cells while the latter actually suppress T-cell activation. In addition, the aqueous humor contains inhibitors of antigen-driven T-cell proliferation (Streilein *et al.,* 1990b). As described previously, the epithelial cells express high levels of FasL, which downregulates immune responses (Dayan *et al.,* 1997). FasL deletes activated CD8 cells in the eye before they can initiate tissue destruction (Griffith and Ferguson, 1997).

The unique combination of immune privilege and immunosuppression is called anterior-chamber-associated immune deviation (ACAID). The specific features of the response are (1) suppressed delayed hypersensitivity, (2) normal antibody-mediated immunity, and (3) primed but not active cytotoxic T-cell responses. ACAID may have evolved to protect the "nonself" eye molecules such as retinal S proteins from delayed hypersensitivity or cytotoxic immunological reactions (Streilein, 1990). Because the eye cannot respond with delayed hypersensitivity reactions, it is extremely susceptible to infection with intracellular microorganisms (Gery and Streilein, 1994).

Polyclonal B-Cell Activation

Nonspecific proliferation of B cells has been implicated as a cause of autoimmune diseases. Although it is accepted that autoreactive B cells or anergic B cells can be activated by mitogenic stimulation by endotoxins or superantigens, it is unlikely that polyclonal B-cell activation plays a role in autoimmune diseases (Friedman *et al.,* 1993). Multiple data sets have shown that (1) tissue damage induced in autoimmune diseases is related to high-affinity IgG and polyclonal activation produces low-affinity IgM, (2) most animal models for autoimmune diseases involve T-cell-dependent production of antibody, (3) a genetic predisposition is necessary to evoke the pathology of the disease in the presence of high amounts of antibody, and (4) endotoxins may exacerbate or result in disease processes but they cannot in themselves initiate the disease process (Theofilopoulos, 1995).

Immune Dysregulation

Disturbances in the Th1/Th2 ratio have been considered a primary cause of autoimmune disease. Increased numbers of Th1 cells (CD4, CD45RO) were reported in Hashimoto's thyroiditis, Graves' disease, and certain forms of RA and diabetes. In mice, Th1 cells produce IFN-γ and TFN-α that activate cytotoxic T cells. In normal subjects, Th2 cells provide cytokines such as IL-4 and IL-10 that downregulate interferon production and inhibit autoimmune responses.

DRUGS, CHEMICALS, AND AUTOIMMUNE RESPONSES

Chemicals and drugs cause autoimmune responses in humans. The autoimmune response abates when the drug is removed. However, some responses can persist for several years.

Hemolytic Anemias

Anemias are induced by a number of different specific and nonspecific mechanisms. α-Methyldopa causes a true autoimmune hemolytic anemia in 16–18% of patients. Antibodies directed toward Rh antigens on red blood cells have been reported in patients receiving α-methyldopa. In less than 1% of patients, the drug caused complement-mediated autoimmune hemolytic anemia. The drug may initiate nonspecific B-cell proliferation and antibody production over extended time peroids. High levels of antibodies were found 2 years after discontinuation of the drug therapy.

Other anemias are the result of nonspecific mechanisms. Drugs such as phenacetin and quinidine form a complex with the antidrug antibody in the circulation. Complexes bind to red blood cells by nonimmunologic mechanisms. Red cells are lysed by complement activation.

Drugs can also react directly with the red cell membrane. When given in large doses, penicillins coat red cells. Drug-specific IgG reacts with the cell-bound penicillin. Cephalosporin can cause anemia by a mechanism similar to penicillin or modification of the red cell membrane that allows binding of serum proteins (Garratty and Petz, 1975).

NSAIDs can also cause hemolytic anemia. Ibuprofen, sulindac, naproxen, tolmetin, and feprazone cause hemolytic anemia. The specific mechanisms involved in hemolysis are not clearly defined (Sanford-Driscoll and Knodel, 1986).

Drug-Induced SLE

The lupus syndrome can be induced by procainamide, hydralazine, and isoniazid. Antiarthythmics such as amiodarone also cause an SLE-type response in 40–93% of the patients.

The mechanism of action is unknown. But the drugs can bind to nuclear antigens *in vitro* and possibly *in vivo* creating new antigens.

Only some patients taking the drug develop musculoskeletal symptoms (Stratton, 1985). The clinical symptoms are dependent on several factors including cumulative drug dose and acetylator phenotype in procainamide sensitivity (Weinstein, 1980). From 30 to 60% of the drug is excreted as N-acetylprocainamide and the rate of metabolism is determined genetically (Giardina, 1984). Slow acetylation of the drug is associated with the autoimmune response. As a

compensatory mechanism, these patients may also use an alternate metabolic pathway that oxidizes the drug, creating highly reactive metabolites capable of interacting with DNA.

Modification of Self Antigens

Pharmaceuticals or chemicals that induce antibodies to altered self are penicillin, halothane, tienilic acid, and quinidine. Two theories have been put forward to explain the induction of autoimmune diseases by modified self determinants.

Binding of the parental drug or metabolites to self molecules may induce new antigenic determinants recognized by T cells. Expansion of T cells provides help to B cells that recognize self antigens near the drug–self protein complex. In a mechanism analogous to a graft rejection, the drug or metabolite may interact with and modify self MHC markers. An expanded population of T cells provides non-antigen-specific help for B cells also carrying the modified self marker. Polyclonal activation of the B cells would produce antibodies with multiple specificities, including antiself antibodies.

Drugs and the Creation of "Neoantigens"

Drugs often create "neoantigens" that induce the formation of autoantibodies. These antibodies may be directed toward cells, tissue components, and red or white blood cells.

Because of chemical interaction with tissue, some drugs can create "neoantigens" by direct modification of self proteins. D-Penicillamine has free sulfhydryl and amino groups. These reactive groups can interact with free aldehyde groups on collagen using a mixed sulfide reaction or reduction of cysteine. Chemical modification of self molecules creates "neoantigens" (Jaffe, 1986). Because of the high incidence of autoimmune responses, D-penicillamine has limited use in the treatment of arthritis in humans (Hill, 1979).

HAZARD IDENTIFICATION

The presence of autoantibodies directed toward host tissue is a hallmark of an autoimmune response. Immunofluorescence assays can be used to detect the presence of autoantibodies. Data from these assays must be interpreted with caution, as antibody titers may decrease with time (Rose and Bhatia, 1995). Tissue-specific, autoreactive T cells should also be demonstrable in an autoimmune response. Proliferation of T cells following exposure to tissue antigens can be used as an *in vitro* correlate of Th1 autoimmunity.

Animals model can also be used to assess the biological significance of the preliminary studies. These models will be discussed in a later chapter.

REGULATORY POSITION ON AUTOIMMUNITY

Endpoints for autoimmunity are not normally part of standard immunotoxicity testing. Tests such as the popliteal lymph node assay can be used to screen for autoimmnity. This assay and other useful tests will be explored in later chapters.

15

Delayed Hypersensitivity Reactions

INTRODUCTION

Delayed-type hypersensitivity (DTH) reactions differ from immediate allergic reactions in clinical signs, chronology, histological features, and the nature of immune effector cells. Unlike immediate hypersensitivity, DTH involves lymphocytes and macrophages as effector cells. Antibodies are not involved in the reaction. Moreover, reactions occur 48–72 hr rather than 15–20 min following exposure to the sensitizing agent.

The nature of the response depends on (1) the anatomic location of the response (e.g., skin or lung) and (2) whether the sensitizing antigen is a bacterium, virus, or chemical. Skin reactions to tuberculosis result in redness and induration reflecting the influx of inflammatory cells. Poison ivy and low-molecular-weight chemicals elicit a different skin reaction characterized by cell lysis and edematous lesions. Chemicals such as beryllium or microorganisms like tuberculosis induce DTH reactions in the lung. In the lung, immunocompetent cells attempt to wall off the offending agent, creating granulomas and impairment of lung function.

DELAYED HYPERSENSITIVITY SKIN REACTIONS

Contact Dermatitis

Reactivity is usually directed toward low-molecular-weight chemicals that penetrate the dermis. Some chemicals bind directly to skin proteins while others

require metabolic or photoconversion prior to protein binding (Anderson *et al.,* 1995).

During the initial exposure, antigen is captured by Langerhans cells (LCs) or veiled cells that express high numbers of HLA-DR, DQ, and DP. Most of the chemical–protein adducts are processed by the LCs and immunogenic fragments are presented on the cell surface in context with MHC markers. Metal salts of nickel stimulate the immune system by different mechanisms. Nickel can react with the MHC markers directly or indirectly as nickel–protein complexes (Moulon *et al.,* 1995).

Soon after antigen processing begins, immunocompetent cells begin to produce and secrete a pattern of cytokines that are only produced during DTH reactions. IL-1ß is produced by the LCs and keratinocytes that also secrete IL-1α and chemokines IP-10 and MIP-2 (Enk and Katz, 1995; G. Muller *et al.,* 1996). Keratinocytes also secrete other cytokines such as GM-CSF and TNF-α that stimulate the maturation of LCs.

Because T cells are not present in large numbers in the skin, there is no way to generate an immune response. APCs must leave the skin and travel to a lymph node. The cytokines IL-1 and TNF-α produced by keratinocytes downregulate the expression of E-cadherin, a molecule that binds keratinocytes and LCs together in the skin (Blauvelt *et al.,* 1995). When the LCs are freed from the skin, they begin a migration to the paracortical regions of the lymph node. During the migration, the LCs undergo a maturation process to become mature LCs expressing MHC 11, ICAM, and B7 molecules (Steinman *et al.,* 1995).

Depending on the nature of the antigen, the LCs react with CD8 or CD4 (Th1) cells. Cytotoxic T cells are activated if the antigen is a viral peptide or a low-molecular-weight chemical. If the antigen is a bacterial fragment, CD4 (Th1) inflammatory, long-lived memory cells are generated. Rapid proliferation of T cells increases the frequency of antigen-specific, "sensitized T cells" from 1:100,000 to 1:1000–1:10,000 (Anonymous, 1997).

Two distinct skin reactions can be elicited on a second exposure to the sensitizing antigen. The response depends solely on the nature of the antigen. In the initial stage of the secondary response, memory T cells interact with endothelial cells expressing E-selectin and MHC 11–antigencomplexes (Picker *et al.,* 1993). If the antigen is a viral protein or low-molecular weight antigen, cytotoxic CD8 cells interact with the antigen on the surface of all cells expressing the MHC I marker. Lysis of these cells results in the characteristic weeping form of dermatitis or mummular eczema. Oils from poison ivy create these weeping skin lesions.

In contrast, bacterial antigens evoke a massive inflammatory response in the skin. Skin redness and induration are hallmarks of reactions in the skin. Skin testing for mumps or tuberculosis causes this characteristic reaction.

Although the skin response usually abates within 7 days, antigen-specific T cells persist in the skin for several months. Therefore, additional contact with the sensitizing agent results in accelerated (6–8 hr) skin reactions at the site of previous reactions (Yamashita *et al.,* 1989).

DELAYED HYPERSENSITIVITY REACTIONS IN THE LUNG

The model system for the study of delayed hypersensitivity reactions in the lung is the tuberculosis model. Inhaled *Mycobacterium tuberculosis* is ingested by alveolar macrophages but not killed. Rather, the organism lives within the phagocyte because it inhibits the fusion of primary or secondary granules with the phagosome. After 6–8 weeks, some antigen is released and presented in the lymph node to CD4 Th1 cells. Consequently, memory cells are generated (Abbas *et al.,* 1994).

Leukotactic cytokines (e.g., IL-1, TNF, and IL-6) and interferon released from T cells alter the trafficking and metabolic activity of lymphocytes, fibroblasts, and macrophages. Proteins synthesized by macrophages activate thromboplastin and plasminogen that trap and localize escaping bacteria.

A second exposure to sensitizing antigen elicits a massive inflammatory response. Like the skin response, antigen is presented on vascular endothelial cells that express MHC markers and E-selectin. These APCs present antigen to CD4 cells that secrete cytokines. Secretion of IL-2 induces the proliferation of both antigen-activated cells and innocent bystander T cells. Other cytokines such as IFN-γ, TNF, lymphotoxin, IL-8, and monocyte chemotactic factor (MCP-1) are also released by endothelial cells. Bystander T cells are an important component of a DTH reaction. Early in the process, over 90% of the T cells at the inflammatory site are activated innocent bystander cells.

Cytokines serve to activate cells in the area and/or increase the adherence of circulating immunocompetent cells to the vascular endothelium. These interactions are important as a preliminary event to leukocyte extravascularization.

Interferon activation of cells is critical for initiation of DTH responses. Interferon upregulates MHC II marker expression on cells localized in the area and increases antigen presentation. In addition, interferon activates and increases the cytotoxic potential of macrophages.

When the sensitizing antigen is present in the tissue, activated cells transmigrate from the vasculature to the tissue. Within 1–4 hr of T-cell activation, ICAM-1 (CD54) is upregulated on the vascular epithelium. This adhesion molecule localizes neutrophils via Mac-1–1CAM-1 interactions. Another adhesion factor, VCAM-1, is slowly upregulated over a period of 6 hr. Memory T cells attach to the endothelium following interactions between VCAM-1 on the endo-

thelium and VLA-4 on T cells. Other T cells attach to endothelium via interactions between ICAM-1 and LFA-1 on T cells.

In preparation for extravascularization, the shape of immunocompetent cells must be altered. The combined effects of IL-8 and MCP-1 are responsible for the shape change. These cytokines bind to heparin sulfate glycosaminoglycans on the endothelial cells' surface and increase the affinity between endothelial cells and the leukocytes. In addition, the cytokines induce a leukocyte shape change from a round immobile form to a flat migrating form while increasing the locomotion (Kuby, 1994).

Cells cannot extravasate unless the basement membrane is also remodeled. TNF and IFN-γ are necessary to initiate shape changes in endothelial cells that allow vascular egress of cells. In addition, deposition of fibrinogen and/or activated fibrin forms a latticework or scaffolding that assists migration of lymphocytes into the tissue. Leakage of protein into the tissue also reduces the shear forces in the blood and increases the attachment of leukocytes to the endothelium.

Activated lymphocytes, monocytes, and neutrophils from the peripheral blood have different fates in the tissues. Neutrophils often die within 2–3 days. T cells and monocytes become activated and remain in the tissue by upregulating receptors for extracellular matrix molecules. Some cells bind to fibronectin via VLA-4 and VLA-5 receptors. Other cells bind to laminin by upregulating receptors for VLA-6.

Activated macrophages have a multitude of roles in DTH reactions. First, interferon-stimulated macrophages attempt to kill microorganisms by phagocytosis and the release of reactive oxygen species. Second, macrophage synthesized factors such as prostaglandins, leukotrienes, and platelet-activating factors (PAF) induce short-lived, neutrophil-mediated inflammatory responses in tissues. The purpose of the reaction is to destroy bacteria. Finally, because of interferon upregulation of MHC markers, activated macrophages become upregulated APCs.

Other mediators also play a role in the termination of mycobacterial infections. Effector cell activity is upregulated by IFN-γ nitric oxide, and TNF-α

Lymphotoxin and TNF-ß are Th1-produced cytokines that kill mycobacterium-infected macrophages. Released microorganisms are destroyed by activated macrophages.

When the mycobacteria cannot be killed by macrophages or cytolytic proteins produced by T cells, the immune system attempts to encapsulate the bacteria in a collagen capsule (alternatively termed *granuloma, tubercles).* The macrophages fuse to form multinucleated giant cells that surround the bacteria. Nearby, endothelial cells produce chemokines such as Rantes that attract CD4 cells to the area. The CD4 cells form a cell layer around the giant cells. Secreted cytokines

activate the macrophages and giant cells. At the same time, TNF and IL-1 stimulate fibroblast proliferation and collagen production. Other macrophage-derived factors (platelet growth factor) augment fibroblast proliferation or induce endothelial cell migration (transforming growth factor) leading to new blood vessel formation. An influx of fibroblasts producing collagen leads to formation of the dense granuloma in the lung.

It is important to remember that the mycobacteria are never actually killed. Rather, the bacteria are simply "walled off" from the body and the granuloma are constantly maintained by Th1 cells. If cellular immunity is impaired by age or disease, the granuloma may rupture, spreading mycobacteria throughout the body.

BACTERIAL EVASION OF THE IMMUNE SYSTEM

Some strains of mycobacteria can evade a Th1-mediated inflammatory response. These strains inhibit the expression of the B7 molecule on the cells' surface and prevent effective triggering of an immunological response. Additionally, mycobacterium-infected macrophages produce prostaglandins and TGF-ß that downregulates the activity of the Th1 cells in the area of the inflammatory reaction (Saha *et al.,* 1994).

Other bacterial species such as *Trypanosoma cruzi,* listeria, and salmonella have developed alternate methods to evade the immune system. These microorganisms escape from the phagosomes and live within the cytoplasm of cells. Ultimately, some antigenic fragments are produced and the host responds with the production of CD8 cells that lyse the infected cells.

DELAYED HYPERSENSITIVITY REACTIONS IN ALLOGRAFTS

There are two DTH-like reactions associated with organ transplantation. In acute cellular graft rejection, alloreactive CD8 are generated. Parenchymal cells expressing MHC I markers are lysed following interactions between the CD8 cells and the MHC markers.

Graft-versus-host reaction (GVH) is another form of DTH reaction. Immunocompetent cells introduced into an immunocompromised host reject the host. An example of GVH is bone marrow transplantation where donor cells attack the host tissue. In the acute form of GVH, sensitized T cells and NK cells attack epithelial cells in the liver, skin, and gastrointestinal tract.

Weeping skin rashes, liver jaundice, and diarrhea are a consequence of the acute GVH immunological reaction. Chronic GVH is mediated solely by T cells and macrophages. Fibrosis of target organs often results in a loss of function.

PSEUDO-DELAYED HYPERSENSITIVITY REACTIONS

Beryllium Reactivity

Beryllium is a low-molecular weight industrial chemical widely used in the manufacture of light bulbs, metal alloys, and ceramics. It exists in several pathogenic forms including beryllium alloys and beryllium oxide fumes (IARC, 1993). Several different acute and chronic reactions have been described in the lung and skin. Acute pneumonitis, tracheobronchitis, and chronic beryllium disease have been reported in the lung. In the skin, beryllium may undergo hydrolysis to the free acid form causing eczematous or itchy, weeping skin lesions. These reactions are believed to be nonspecific irritant reactions. Granulomatous lesions occur when beryllium splinters enter the skin or following chronic low-level inhalation of beryllium.

Several lines of evidence suggest that the granulomatous skin and lung responses are mediated by T cells. In the lung, many CD4 T cells are found in the granulomas (Williams, 1989). Moreover, these cells respond, *in vitro,* to beryllium with proliferation and the release of IL-2 (Saltini *et al.,* 1989). The response is not a classical DTH response based on the release of mediators. In the beryllium lesions, CD4 cells release TNF-ß, a cytotoxic cytokine. The TNF-ß-induced cell lysis is independent of cell contact and represents a nonspecific inflammatory response.

Cutaneous Basophil Hypersensitivity

Cutaneous basophil hypersensitivity is a unique skin reaction in guinea pigs (Jones Mote). The reaction is elicited when high concentrations of antigen are used for sensitization (Askenase, 1973). When the animals are challenged with antigen, a poorly circumscribed, indurated skin lesion occurs at 24 hr and disappears by 48 hr (Mahapatro and Mahapatro, 1984). Histological analyses demonstrated that the lesion contains over 90% basophils. Because the reaction can be transferred to other animals with serum or cells, the mechanism is unclear. Evidently, two factors are necessary to elicit a response. A chemotactic peptide is necessary for basophil influx and basophil degranulation is necessary for induction of inflammation. Degranulation of basophils could occur because of leukocyte-derived releasing factors.

Increased vascular permeability and induration are caused by activation of serum enzymes. Fibrinogen activation increases vascular permeability, allowing cells to enter the tissue. Collagen activation of the Hageman factor induces the induration observed in the reaction (Askenase, 1977).

DRUGS, CHEMICALS, AND DELAYED HYPERSENSITIVITY

A variety of drugs have been reported to alter or cause delayed hypersensitivity reactions (Table 1). Benzocaine, neomycin, and sulfonamides are known sensitizers. In fact, sulfonamides have limited usage in the general population because of the high sensitization rate.

Sulfonamides are unique in that they require metabolism to become sensitizers. Sulfonamides are metabolized by phase II conjugation creating water-soluble molecules. N-hydroxylation creates reactive metabolites that readily complex with proteins to form immunogenic sensitizing complexes.

Metals are known sensitizers. Nickel is a frequent sensitizer in women. Clothing fasteners and costume jewelry (e.g., ear studs) are high in nickel content. Ear studs are usually gold coated over a nickel base. Normal abrasion of the outer coating exposes the nickel base and initiates sensitization.

Workers in paper, cement, and paint factories often develop contact dermatitis as a result of chromium used in the manufacting process. Chromium is also used as a catalyst in electroplating processes (Burrell *et al.,* 1992).

Table 1. Chemicals that Alter Delayed-Type -Hypersensitivity Reactions

Chemical	Species	Effect
Lead	Mouse, rat	↓
Selenium	Rat	↓
Tetrachlorodibenzofuran	Guinea pig	↓
Polychlorinated biphenyl	Rabbit	↓
Methylcholanthrene	Mouse	↕
Carbofuran	Rabbit	↓
2,4-Dichlorophenol	Rat	↕
Sodium hypochloride	Rat	↓
Diethylnitrosamine	Mouse	↑
Triphenyltin	Rat	↓
Dialkyltin	Mouse	↓

Modified from *Immunology and Allergy Practice,* Koller, 1985,1:13-25.

HAZARD IDENTIFICATION

Structure–Activity Relationships

Several computer models have been developed to predict the sensitization potential of chemicals (Table 2). Most models assume that a putative contact allergen reacts with a protein either directly or following biotransformation (Anonymous, 1997).

The models propose structural alerts associated with parental electrophilic fragments or potential electrophiles (proelectrophiles) produced as a result of metabolism. A model such as DEREK (Deductive Estimation of Risk from Existing Knowledge) skin sensitization rule base has been useful in defining contact allergens. The rule base uses 50 structure–activity endpoints that correlate with toxicity endpoints (Barratt *et al.,* 1994). In addition, some physiological characteristics of the chemicals are described (Roberts and Basketter, 1990). Although it is widely accepted that most sensitizers are electrophilic, the ability of a compound to penetrate the stratum corneum and partition into the skin tissue is important.

Skin penetration depends on the log of the octanol/water coefficient (log *P)* (Flynn, 1990). Compounds with high log *P* have greater lipophilicity and increased skin penetration. Permeability also decreases with increasing molecular weight or molecular volume. It follows that these endpoints are included in the DEREK decision making process.

DEREK's performance has been tested in two studies. In one study of 37 chemicals proposed by the European Centre for Ecotoxicology and Toxicology of Chemicals, DEREK was able to correctly identify 24 of 25 as sensitizers. Only two compounds, hydroxypropyl methacrylate and p-aminobenzoic acid, yielded false-positive reactions in the model. In another study of 84 contact allergens, the use of structural alerts showed an accuracy of 85% (Kayser and Schlede, 1995). Minor modification of existing rules giving structural alerts for phenols, thiols, and hydroxylamines may increase the accuracy rate to 95% (Barratt and Lan-

Table 2. A Comparison of the QSAR Models for Contact Allergens

Model	Chemicals	Source	Sensitivity	Specificity
DEREK	294	GPMT[a]	98%	82%
DEREK	93	Animal tests	58%	92%
MultiCASE	1034	GPMT human patch	78%	96%
ACD (Magee et al., 1994)	72	Ziegler data base (1989)	79%	88%

Modified from *Quantitative Structure Activity Relationship,* Graham *et al.,* 1996, 151–7,with permission of Wiley VCH. John Wiley and Sons Ltd.
[a]Guinea pig maximization test

gowski, 1997). The DEREK model has been useful in predicting moderate and strong sensitizers. Identification of weak sensitizers is still a problem.

A new model based on the MultiCASE system uses no preconceived mechanisms of sensitization (Klopman *et al.,* 1992). MultiCASE fragments chemicals into subsets containing two or more nonhydrogen atoms. Structural alerts are generated by identification of fragments statistically associated with active sensitizers or biological activity.

Compounds containing the active fragments and other modulating descriptors are determined. These descriptors may be log *P,* highest occupied molecular orbital (HOMO), lowest occupied molecular orbital (LOMO), molecular weight, or water solubility (Anonymous, 1997). The concordance between model predictions and *in vivo* evidence of sensitization was 90% (Anonymous, 1997).

Using a large data base of over 1000 animal and human sensitizers, the model could identify four major structural alerts: (1) a nitrogen atom double bonded to a carbon or another nitrogen, (2) substituted aromatic structures, (3) electrophilic moieties, and (4) thiol or disulfide structures.

Lymphocyte Transformation Assay

In addition to subjectively measured human skin tests, *in vitro* assays utilizing human donor cells can be used to assess DTH. The extent of antigen-specific lymphocyte proliferation can be quantitated by measuring tritiated thymidine uptake in stimulated cultures after exposure to appropriate antigen. The lymphocyte transformation assay is an excellent correlate of DTH reactions (Rockland *et al.,* 1970). The assay has been used to provide *in vitro* evidence of hypersensitivity drug reactions. In a serum matrix, drugs or free haptens can stimulate the proliferation of T cells without the necessity of prior hapten–protein binding (Stejskal *et al.,* 1986).

The lymphocyte transformation test is a popular test among cellular immunologists because the endpoint is objective and quantifiable. It is comparatively easy to carry out, and the data obtained can be semiautomated (in radioisotope counters).

REGULATORY POSITION ON DELAYED HYPERSENSITIVITY

Regulatory agencies base the hazard identification and risk assessment on guinea pig models for delayed hypersensitivity. The use of these models will be described in later chapters. European regulatory agencies are reluctant to accept data from human studies. Objections to human data are ethical in nature rather than scientific. This has limited the use of human data on DTH in the regulatory process.

16

Killer Cells

INTRODUCTION

The host must defend itself against intracellular viruses, microbes, autoreactive lymphocytes, and the proliferation of neoplastic cells. Evolutionary pressures have resulted in the development of efficient cell-based methods that kill infected or abnormal cells. Many different immunocompetent cells can participate in the killing process. Besides T cells, NK cells, eosinophils, macrophages, and polymorphonuclear leukocytes can lyse infected or abnormal target cells. Some cytolytic mechanisms are antigen specific and genetically restricted. Other cytolytic reactions are nonspecific and non-MHC restricted.

CLASSICAL CYTOTOXIC T CELLS

CD8 cytotoxic T cells circulate in the blood and lymph and react with tumor cells, virus-infected cells, or allogeneic cells expressing the MHC I markey. Initially, these cells are oligoclonal. But following interactions between the MHC I, the CD3 complex, and antigen, CD8 cells proliferate rapidly creating a population of antigen-specific cytotoxic T cells.

The generation of antigen-specific T cells requires many different signals. Activation of resting T cells occurs when adhesion factors LFA-1 and CD2 on the antigen-specific CD8 cells interact with ICAM and LFA-3 molecules on the target cells or endothelial cells. Membrane perturbation allows an influx of

calcium that is necessary for cellular activation. Intracellular signaling is initiated when the TCR interacts with the MHC–antigen complex on the target cell. Cytolytic T cells can only recognize antigen presented in association with MHC I A, B, and C molecules. Secondary interactions between B7 and CD28 initiate proliferation and production of cytokines.

Based on the cytokine production and response patterns, there are two subpopulations of T killer cells. Tc1 cells are activated and stimulated by IL-12 and secrete IL-2 and IFN-γ. A second subset (Tc2) is stimulated by IL-4, secretes IL-4, 5, 10, and downregulates Tc1.

The role of these subsets in disease is unclear, Generally, the Tc1 population is cytolytic and protective. Conversely, Tc2 is only weakly active in classical cytotoxicity assays but may protect the host by secretion of small cytolytic molecules.

The nature of the virus and the expression of MHC I markers determine the involvement of CD8 (Tc1) cytotoxic cells in the infection. T killer cell response is crucial for the resolution of noncytopathic viral infections. Therefore, infections with lymphocytic choriomeningitis virus evoke a cytotoxic response. In contrast, cytopathic viruses (e.g., vesicular stomatitis, vaccinia, or influenza) are controlled by soluble mediators such as interferon and antibodies (Kagi and Hengartner, 1996).

Cytotoxic T cells can terminate viral infections without killing the infected cell. In a transgenic mouse model of hepatitis B virus infection, adoptively transferred cytotoxic T cells can abolish hepatitis gene expression without killing the infected cells. The downregulation of viral genes is mediated by IFN-γ and TNF-α (Guidotti and Chisari, 1996).

Termination of viral infections without destroying cells may be critical to the host's survival during overwhelming viral infections of multiple tissues. A cytotoxic T-cell response would destroy most, if not all, function of multiple organs.

Cytotoxic T cells have direct antifungal activity against *Cryptococcus neoformans* and *Candida albicans.* This direct cytotoxicity effect often parallels the development of DTH responses. In some experimental systems, generation of cytotoxic lymphocytes requires the production of IL-2. Other lymphocyte subsets (CD4+ and CD56+ cells) are also cytotoxic in some forms of the DTH reaction (Levitz e? al., 1995).

Human peripheral blood T cells can also directly kill parasites and bacteria. Mitogen- or antigen-activated CD8 cells can kill multicellular schistosomula of *Schistosoma mansoni,* trophozoites of *Entamoeba histolytica* or *Toxoplasma gondii.* Occasionally, direct contact with the microorganism is not required: CD8 T cells activated by *Pseudomonas aeruginosa* polysaccharide antigen secrete a "lymphokine" that can kill several different species and strain of bacteria (Levitz *et al.,* 1995).

TUMOR-INFILTRATING LYMPHOCYTES (TILS)

TILs are often found in solid tumors such as melanomas and gliomas. TILs are the result of antigen-driven recruitment and proliferation of oligoclonal T cells within the tumor. As a consequence of activation, TILs overexpress the TCR ß-chain variable regions. Moreover, the variable regions have recurrent transcripts in all patients with the same tumor type. For example, seven patients with melanoma had TCR ß variable region 13SlJ2S2. Another recurrent, variable ß chain is found in patients with glioma. Most patients have V14 sequences.

Phenotypically, the TILs in solid tumors are both CD4 and CD8 cells (Whiteside and Parmiani, 1994). Each population can be divided into subsets. A small population of CD4 cells can lyse tumor cells when antigen is presented on either MHC I or MHC II molecules. Most of the CD4 cells recognize Hsp 70 associated with MHC II markers on the surface of APCs (Goedegebuure and Eberlein, 1995). Evidently, CD4 killer cells can be produced following interactions with superantigens such as staphylococcal enterotoxin (Nishimura *et al.,* 1994). Both CD8 Tc1 and Tc2 killer cells are found in solid tumors. The production of IL-12 greatly enhances the cytolytic activity of CD8 cells (Whiteside and Parmiani, 1994).

NATURAL KILLER CELLS

In most species, NK cells are neither T nor B cells, but rather represent a distinct third lineage of large granular lymphocytes. The only exception is the mouse where activated T cells can sometimes act as NK cells. Human NK cells express CD2, LFA-1, CD1 la/CD28, CD16, CD49/CD29, CD56 (a heterodimer adhesion molecule), NKG2, and CD94. CD94 may be part of a unique HLA specific inhibitory receptor complex. The function of NKG2 is unknown (Levitz *et al.,* 1995). NK cells can lyse target cells from many different tissues in a spontaneous and non-MHC-restricted manner.

NK cells perform several important roles in immune responses. Because most NK cells are found in the peripheral blood, they play a critical role in destruction of cancer cells found in the bloodstream (Hanna and Fidler, 1980). In addition, NK cells terminate viral infections in cells that have downregulated MHC I markers. In this context, NKs are the major defense mechanism against herpes, vesicular stomatitis, and adenovirus infections (Biron *et al.,* 1989).

Activation of NK Cells

Activation of NK cells requires a series of interactions between the NK and target cells. The rat NKR-P1A and mouse NKR-P1C are receptors on NK cells

that activate natural killing. The NKRs are type II integral membrane proteins whose extracellular domains have the structure of C-type (calcium-dependent) lectins. The NKR superfamily encompasses several gene families including Ly-49 (mice and rats), NKR-P (mice, rats, and humans), NKG2 (humans and rats), and CD94 (humans) (Giorda and Trucco, 1991).

The signaling mechanism activating NK cells is unclear. A tyrosine motif in the N-terminus of the NKR-P1A has a sequence (YxxL) consistent with a phospholipase C (PLC) binding motif (Larose *et al.,* 1993). However, several experiments have failed to document binding of PLC-γ1 to the NKP-P1A (Larose et al., 1993; Ryan and Seaman, 1997). NKR signaling increases phosphoinositol turnover, mobilizes intracellular calcium (Ca^{2+}), initiates phosphorylation of protein tyrosine kinases, and releases cytokines (Ryan and Seaman, 1997).

Inhibition of NK Cell Function

There is an inverse relationship between the expression of MHC class I molecules and NK cell killing. Engagement of NK receptors and the MHC I results in a negative signal to the NK cell that prevents lysis of target cells (Table 1).

In mice and humans, different NK receptors interact with the MHC I molecules to send the negative signal. The mouse inhibitory receptor (Ly-49) is a member of the C-lectin, type II glycoprotein superfamily (Fig. 1). Because of alternative gene splicing and allelic polymorphism, Ly-49A, Ly-49C, and Ly-49G.2 proteins have been described. An individual NK cell may express one or more different alleles (Lanier and Phillips, 1996).

Gene products of the Ly-49 family react with different murine MHC (H-2) molecules: Ly-49A reacts only with $H\text{-}2D^d$ and $H\text{-}2D^k$; Ly-49C recognizes $H\text{-}2K^b$ in $H\text{-}2^b$ mice and, in $H\text{-}2^d$ mice, Ly-49C reacts with $H\text{-}2^b$ and $H\text{-}2^d$ (Lopez-Botet *et al.,* 1996).

The human killer cell inhibitory receptors (KIRs) are members of an immunoglobulin-related superfamily (Fig. 1). The KIRs have either two IgG or three IgG-like loops with sulfide bonds characteristic of the Ig superfamily (Lanier and

Table 1. NK Cell Coded Molecules that Regulate NK Cell Activity

Molecule	Distribution	Ligand	Effect
NKR-PI	NK	Carbohydrate	Activates
Ly-49 A, C, and G	NK	A-$H\text{-}2D^d$ D^k B-$H\text{-}2K^b$ G-$H\text{-}2D^dL^d$	Inhibits
CD69	Hematopoietic	Carbohydrate	Activates
CD94	NK, T cells	HLA, A, B, or C	Activates or inhibits

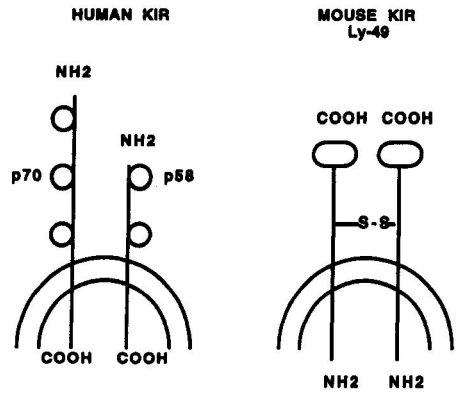

Figure 1. Structural features of human and mouse killer cell inhibitory factors.

Phillips, 1996). At this juncture, 13 cDNAs have been reported within the KIR family. KIR isoforms react with the MHC class I A, B, or C molecules on target cells.

Another member of the KIR immunoglobulin family can inhibit or activate NK cell function, depending on the nature of complexed proteins. The CD94 C-lectin complexes with two different proteins (43 and 39 kDa). A complex containing CD94/p43 dimers recognizes MHC class I A, B, or C alleles and inhibits NK cell function. In contrast, complexes of CD94/p39 activate the killer cells (Lopez-Botet *et al.,* 1997).

Despite structural differences, Ly-49 and the KIR superfamily share a common signaling mechanism. All receptors contain a tyrosine-based ITIM motif with two I/VxYXXL sequences. Following *lck*-dependent tyrosine phosphorylation, there is an association with the SH2-containing tyrosine phosphatase SHP-1. SHP-1 delivers a negative signal to the NK cells. The functional integrity of the two intracytoplasmic YXXL motifs is necessary for the KIR-mediated inhibition (Binstat *et al.,* 1997).

LYMPHOKINE ACTIVATED KILLER CELLS (LAKs)

The cytolytic activity of NK cells is enhanced following exposure to IFN-α IFN-ß, IFN-γ and IL-2. The NK cells become LAK cells that can lyse a wide range of allogeneic and autologous tumor cells.

Clinical trials demonstrated that when NK cells and IL-2 are infused into patients with end-stage metastatic cancers, there is a reduction in tumor regression. In some cases, the tumor is eradicated (Finklestein and Miller, 1990).

NK AND LAK CELLS IN DISEASE

The NK and LAK cells play a role in the termination of viral infections. During the early stages of a viral infection, NK cells are the primary effector cells until a CD8 T-cell response occurs. Also, NK cells may supplant the T-cell response during infections with adeno- and herpesviruses that downregulate the MHC I marker expression.

Bacterial infections may be terminated by NK and LAK cells. Direct interactions between NKR-P1 and bacterial cell surface carbohydrate determinants result in the lysis of the bacterium (Yokoyama, 1995).

Interleukin-activated LAK cells can lyse monocytes infected with mycobacteria, listeria, and leishmania that survive as intracellular parasites. The cytolysis of infected cells involves two different mechanisms. First, LAK cells may kill infected cells by cell contact using classical NK–target cell receptor interactions. Second, the NK cells can secrete IFN-γ and GM-CSF that increase the intracellular killing of bacteria thus preventing reinfection of nearby cells.

Indirectly, NK cells play a role in the termination of opportunistic fungal infections in immunocompromised individuals. When *Candida albicans* interacts with NK cells, cytokines such as TNF, IFN-γ and GM-CSF are released. These cytokines recruit and activate neutrophils that ingest and kill the fungi (Bancroft, 1993).

NK AND CTL TARGET CELL LYSIS

Following membrane fusion between the killer and target cells, target cell lysis is mediated by two independent mechanisms.

Perforins and Target Cell Lysis

In one cytolytic mechanism, soluble perforin molecules are secreted by NK cells and CTLs. Perforins have a sequence homology with the complement membrane attack complex (C5b, C6, C7, C8, and C9). The 20% sequence homology between C6 and C9 resides in a 270-amino-acid sequence (Liu *et al.,* 1995). Like the terminal complement components, perforin monomers perforate target membranes. Polymerization of the monomers creates a 10- to 20-nm pore in the membrane. Loss of membrane integrity causes the cells to die of osmotic lysis (Liu *et al.,* 1995).

Granzyme Lysis of Target Cells

When killer cells come in direct contact with target cell membranes, granzymes from the effector cells are injected into the target cell. There are

multiple granzyme isoforms with different chemical activities (Table 2). Granzyme A2 and trypase 2 cleave basic residues in proteins and initiate destruction of target cell DNA. Granzyme B, which is the most abundant granzyme species in killer cells, cleaves acidic proteins at the carboxy residues. In addition, the B isoform releases a highly active DNAase from actin. The DNAase mimics the activity of the calcium dependent endonuclease associated with apoptosis or programmed cell death. Both perforin and granzyme B cause the premature activation of $p34^{cdc2}$ kinase, a cell cycle control protein. For reasons that are unclear, inappropriate activation of this protein during the cell cycle leads to cell death that resembles apoptosis (Symth and Trapiani, 1995). The CD8 cells also secrete a number of cytotoxins that have detrimental effects on the target cell. These mediators include TIA-2, TNF-α, TNF-ß, leukalexin, and fragmentin (Symth and Trapiani, 1995).

CD4 LYMPHOCYTE LYSIS OF TARGET CELLS

Activated CD4 cytotoxic T cells kill target cells by a different mechanism. Target cells express a Fas (APO-1, CD95) 42-kDa glycoprotein. Interactions between Fas and the Fas ligand on CD4 T cells activate an endonuclease in the target cells (Fig. 2). Fragmentation of DNA drives the target cell into apoptosis. Defects in genes coding for either Fas or the Fas ligand are associated with lymphoproliferative diseases.

The Fas cytolytic pathway may serve two different functions. It may be a part of the normal homoestatic mechanism maintaining normal number of cells in organs with a high proliferation rate (Podack, 1995). Also, the CD4 cells may

Table 2. Properties of Killer Cell Granzymes

Granzyme	Species[a]	Activity	Expression
A	M, R, H	Trypase	CTL, NK
Trypase 2	R, H	Trypase	CTL, NK
B	M, R, H	Asp-ase	CTL, NK
C	M, R	?	CTL
D	M	?	CTL
E	M	?	CTL
F	M, R	?	CTL
G	M	?	CTL
H	H	?	CTL
Metase-l	R, H	Metase	NK

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[a]M, Mouse; R, Rat; H, Human.

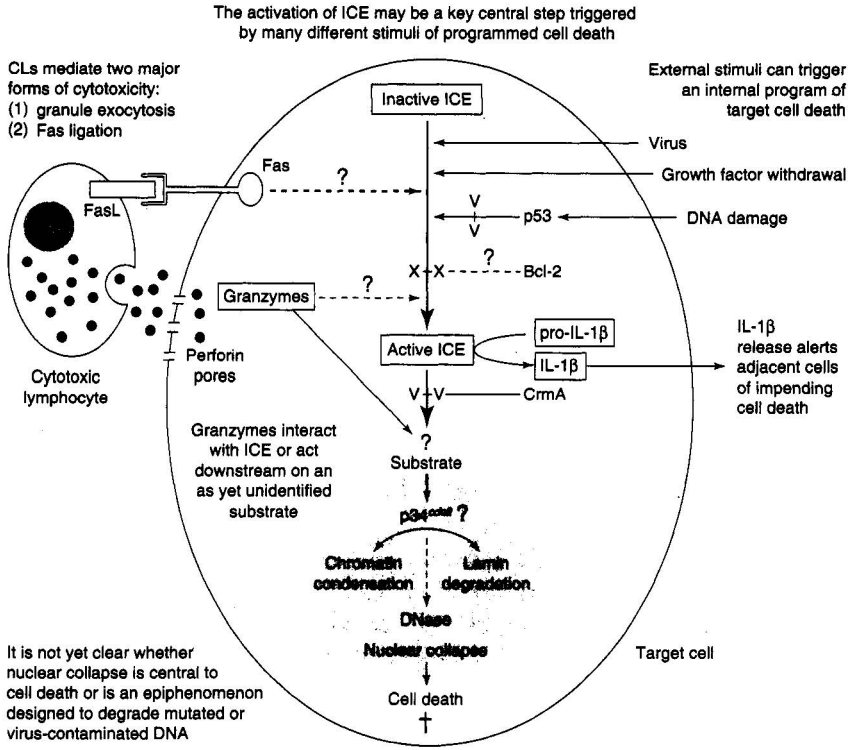

Figure 2. Speculative model of lymphocyte-mediated cytotoxicity. Endogenous proteases involved in cell death such as IL-1ß converting enzyme (ICE) may be activated by signals provided by infectious viruses, growth factor withdrawal, or DNA damage. Reprinted from *Immunology Today,* Symth and Trapiani, 1995, 16:202–6, with permission of Elsevier Science.

have an immunomodulatory role because they preferentially eliminate activated MHC 11-positive cells (e.g., monocytes/macrophages and B cells) preventing overreaction of the immune system (Hahn *et al.,* 1995).

MACROPHAGE KILLER CELLS

Depending on the stimuli, macrophages can become inflammatory, primed or activated macrophages. Inflammatory macrophages exposed to IFN-γ or bacterial endotoxin (LPS) become activated (Lewis, 1995). Activated macrophages can kill tumor cells *in vitro.* The response is selective in that normal cells are spared but nonspecific in that different types of tumor cells can be killed.

The macrophage cytolytic mechanism is poorly understood. Possible cytotoxic mediators secreted by macrophages are hydrogen peroxide, proteases, TNF-α, and nitric oxide. It is also possible that depletion of media by arginases plays a role in tumor cell killing (Lewis, 1995)

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)

Over 80% of NK cells have a low-affinity receptor (CD16) for the Fc portion of IgG (FcγRIIIa). CD16 is usually noncovalently associated with CD3ζ and FcεRIγ dimers (Anderson *et al.,* 1990). However, intracellular signal transduction is mediated by the CD16 receptor. The association of CD3ζ or FcεRIγ is dispensable for NK cell development and function but CD16 is required. When antibody is bound to target cells, the Fc portion binds to the NK cell receptor (Vivier *et al.,* 1992).

In HIV and other viral infections, ADCC can be readily demonstrated using antibody directed toward the envelope protein and effector cells from infected patients (Brenner *et al.,* 1991). The mechanism by which ADCC effector cells lyse target cells is unclear. However, CD16 stimulation leads to the release of intracytoplasmic NK cell granules (Vivier *et al.,* 1991).

IMMUNODEFICIENCIES ASSOCIATED WITH KILLER CELLS

Primary NK cell immunodeficiencies are rare. Perhaps the most studied of the NK deficiencies is the Chediak–Higashi syndrome that affects the nervous system, melanocytes, and the immune system. In these patients, NK cells bind to target cells but fail to deliver the lethal hit because of abnormal cytoplasmic granules. These patients have less than 10% of the normal NK cell function (Roder *et al.,* 1983). There is an association between NK defects and severe herpes or varicella infections. NK cell defects associated with other immunodeficiencies are shown in Table 3.

TUMOR CELL EVASION OF KILLER CELLS

Tumor cells may evade killer cell destruction by the production of FasL that induces apoptosis in adversarial TILs, CTLs, and NK cells expressing the Fas marker. Constitutive expression of FasL (CD95L) has been described in NK lymphoma, large granular lymphocytic leukemia, melanoma, hepatocellular he-

Table 3. Immunodeficiencies with NK Cell Defects

Severe combined immunodeficiency
Reticular dysgenesis
Common variable immunodeficiency
Chediak–Higashi syndrome
Leukocyte adhesion deficiency
Bloom's syndrome
Chronic fatigue syndrome

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patic carcinoma, and astrocytoma. To prevent autoreactions, the Fas (CD95) molecule is downregulated on tumor cells.

Cytotoxic T cells have different sensitivities to tumor-induced FasL apoptosis. TILs and cytotoxic CD4 cells are extremely sensitive to FasL-induced apoptosis. In contrast, a lower proportion of CD8 and NK cells can be killed by the Fas death-producing signal.

Tumor cells have developed mechanisms to counter the effects of perforins and granzymes. Many tumors secrete an active form of TGF-ß that inhibits both perforin and the granzyme serine esterase activity. Some tumors also express mutated receptors for perforin that result in binding without the formation of transmembrane pores.

CHEMICALS ALTERING KILLER CELL FUNCTION

Chemicals known to alter cytotoxic T cells and NK cells are shown in Table 4.

Table 4. Effect of Chemicals on Natural Killer Cell Function

Chemical	Species	Effect
Polychlorinated biphenyl	Rat	↓
Chlorine dioxide	Rat	↑
Selenium	Rat	↑
Ethylnitrosourea	Rat	↓
Diethylnitrosamine	Mouse	↑
Methylcholanthrene	Mouse	↓
Manganese	Mouse	↑

Modified from *Immunology and Allergy Practice,* Koller, 1985, **7**: 13-25.

HAZARD IDENTIFICATION

Proliferation of Cytotoxic T Cells

The most common test used to assess the function of cytotoxic T cells is the mixed lymphocyte reaction (MLR). The MLR occurs because of the incompatibility of MHC markers on the two lymphocyte populations. In the assay, stimulator lymphocytes are treated with mitomycin C to prevent cellular division. Allogeneic splenic lymphocytes from treated and control groups are mixed with the stimulator cells for several days. At the optimum time point, tritiated thymidine is added to the wells. The amount of tritiated thymidine incorporated into the cell is directly proportional to the cellular division. Usually, the data from an MLR are expressed in two different ways. The counts per minute (cpm) of the test versus control reaction can be used for comparison. However, the effect of spontaneous proliferation in the control reactions is not taken into consideration. A high proliferation rate in the control skews the data and increases the possibility of a false-negative reaction. To bypass this problem, the data can be expressed as the stimulation ratio (SR). The ratio is derived by dividing the test cpm by the autologous control. It is important to remember that the MLR probes only the ability to proliferate when confronted with allogeneic cells. Decrements in the CTL killing of target cells cannot be discerned in this assay.

Modifications of the MLR are necessary when rat lymphocytes are used in the system. Splenic lymphocytes from the rat do not proliferate in the MLR. Macrophages contaminating the lymphocyte preparation generate cytostatic metabolites via L-arginine metabolism (Mills, 1991). These cytostatic molecules inhibit T-cell proliferation. It is necessary to use lymphocytes from the lymph nodes as proliferators in the MLR.

The relationship between decrements in the MLR and biologically relevant immunosuppression is unclear. When statistically significant dose-related effects were used as the criteria for an immunotoxic effect, decrements in MLR predicted immunotoxicity only 50% of the time (Luster *et al.,* 1992a).

Lysis of Target Cells by Cytotoxic T Cells

Induction of cytotoxic lymphocytes and target cell lysis can be probed in a biphasic assay. In the induction phase, splenic lymphocytes or thymocytes are incubated with P815 murine mastocytoma cells in a T cell conditioned medium containing cytokines and interleukins for 5 days. In the effector phase, washed T cells from the induction phase are mixed with 51Cr-labeled P815 cells. Cellular lysis as determined by 51Cr release is used to assess function of the T cells (Burrell *et al.,* 1992). This assay has not been validated for use in immunotoxicology studies.

Lysis of Target Cells by NK and LAK Cells

Different target cells are used to study the function of NK cells in mice, rats, and humans. In the human system, the K562 blastocytoma cell line is used as target cells. The YAC-1 cell line is used as target cells for both rat and mouse NK cells. In the assay system, target cells are labeled with ^{51}Cr and reacted with splenic lymphocyte effector cells using several effector/target cell ratios. The assay is terminated after 4 hr and the amount of chromium in the supernatant fluid determined.

Before assaying for LAK function, splenic effector cells are incubated with IL-2 or IFN-γ for 24 hr. In murine or rat assays, activated cells are incubated with ^{51}Cr-labeled target cells at low effector/target ratios. Lower ratios are possible because of the heightened LAK cell cytolytic activity.

In humans, it is possible to use two different target cell populations to define LAK activity. Raji cells (NK and LAK sensitive) and K562 cells (NK insensitive and LAK sensitive) are used in the assay system.

The NK assay has been evaluated for sensitivity and predictability for immunotoxicity (Luster *et al.,* 1992a). Like the cytotoxic T-cell assay, decrements in NK function have only a moderate concordance (69%) with immunotoxicity when statistically significant, dose relationships are the criteria for immunotoxicity. Recent data, using the B16F10 tumor model in mice, showed that NK cell activity must be suppressed by 50% before significant effects on the host were observed (White, 1996).

Lysis of Target Cells by Activated Macrophages

P815 cells are the target of choice because they are easily killed by macrophages and they are not susceptible to cytolysis induced by TNF or hydrogen peroxide. In the assay, ^{51}Cr-labeled target cells are reacted with macrophages that have been (1) stimulated with LPS, (2) stimulated with LPS and IFN-γ, or (3) not stimulated. Mixtures are incubated for 16 to 24 hr and the amount of released chromium determined. Generally, tumor killing is observed in reactions containing macrophages, LPS, and IFN-γ. However, results are often difficult to interpret because of the high spontaneous release of chromium (e.g., 15–20%) during the long incubation period (Lewis, 1995).

Lysis of Target Cells by ADCC

Most cells that express Fc receptors for IgG can mediate ADCC. Included in this effector population are monocytes, eosinophils, NK cells, and activated murine CD3 cells (Lanier *et al.,* 1985).

The nature of the effector cell is dependent on both the nature of the chromium-labeled target cell and the source of the target-cell-specific IgG. Hu-

man Chang liver cells coated with rabbit IgG directed toward the liver cells are lysed by NK cells. Monocytes are the sole effector cells when human red blood cells coated with human anti-blood-group antigen are used in the assay (Poplack *et al.,* 1976). Finally, when chicken red blood cells coated with rabbit anti-red-cell IgG are used in the assay, both monocytes and NK cells can lyse the target cells (Burrell *et al.,* 1992).

REGULATORY POSITION ON CYTOTOXIC CELLS

The National Toxicology Program evaluated the MLR and the NK assay for inclusion in screens to detect immunotoxicity. Both the NK and MLR proved to be fairly sensitive in detecting immune events. The NK cell assay has been included in the new harmonized guidelines issued by the U.S. EPA. The assay is listed as an optional endpoint to be evaluated on a "case by case basis."

17

Killer Cells and Viral Infections

INTRODUCTION

Any general theories of immunology have to be seen in the context of viral diseases (Zinkernagel, 1997). In 1917, millions were killed by a unique influenza strain. Much later, it was observed that latent viruses such as Epstein–Barr virus (EBV) could cause debilitating mononucleosis in Caucasians and an often fatal lymphoma in black Africans. Other data from transplant patients undergoing cyclosporin A therapy showed that these patients have an increased incidence of EBV-associated lymphoproliferative disorders (Tanner and Alfieri, 1996). Recently, the HIV pandemic has further spurred interest in immune response to acute and chronic viral infections.

CYTOTOXIC T-CELL RESPONSE TO VIRUSES

In the initial stages of a viral infection, NK cells are activated by type 1 interferon and viral glycoproteins. Besides the cytolytic role, NK cells are necessary for the generation of cytotoxic T cells. Two signals are necessary for differentiation of T cytotoxic cells. Initially, there are interactions between APCs, MHC I, TCR, and costimulatory molecules. NK cells or IL-2 in high concentrations provide secondary accessory signals necessary for T-cell activation.

Early in the immune response (7–10 days), several different responses are occurring in the regional lymph nodes. Follicular dendritic cells present low affinity antigens to CD4+ Th2 cells that are suppressive and anti-inflammatory.

At the same time, CD8 cells in the node begin to proliferate vigorously. At defined time points, almost 20% of the CD8 cells are dividing in response to a viral infection.

The high number of CD8 cytotoxic cells dampens the NK/LAK response because they can more effectively compete for IL-2. High-affinity IL-2 receptors on the CD8 cells compete for IL-2 with moderate- or low-affinity receptors on NK cells. Because the CD8 cells can better compete for IL-2, the immune response favors the CD8 cells.

Cytotoxic T cells interact with the membrane of virus-infected cells and initiate lysis by a number of different mechanisms. Lysis of lymphocytic choriomeningitis virus involves perforin. Local secretion of granzymes is responsible for the destruction of vaccinia, vesicular stomatitis, and influenza virus. Other unique mechanism can also result in cell lysis. For example, interactions between CD8 cells and hepatitis B-infected cells can suppress gene expression, causing cell death. Conversely, select CD8 cells can trigger a regulatory cascade that inhibits viral replicaton without target cell lysis (Doherty, 1995).

LATENT VIRUSES

Some viruses can infect cells, incorporate their DNA into the host genome, and establish latent infections. Two examples of latent viruses are EBV and HIV.

EBV

EBV, a member of the herpesvirus family, is a double-stranded DNA virus. Infection of human B cells occurs by attachment of two major virion glycoproteins, gp 350 and gp 220, to the type 2 complement receptor (CD21). Because the initial infection is rapidly controlled by killer cells and antibodies, EBV infections are common in children and are usually asymptomatic in nature. However, some viral DNA is incorporated into the B cells and one in every million B cells is latently infected.

Reactivation of the viral infections in young Caucasian adults causes infectious mononucleosis with the characteristic generalized lymphadenopathy, sore throat, and muscle weakness. Again, killer T cells that recognize both the MHC I marker and EBV nuclear antigens resolve the infection over time.

In other parts of the world, infection with EBV is associated with cancers. Nasopharyngeal carcinomas in Chinese have been attributed to EBV infections. Burkitt's lymphoma in equatorial Africa and variable B-cell lymphomas in Caucasians are often the result of latent EBV infections.

CD8 Control of EBV Infections. Several lines of in vivo evidence show that impairment of cytotoxic T-cell function is required for malignant transfor-

mation of B cells. Impairment or deficiencies of T-cell subsets that control B-cell proliferation or cytotoxic T cells are frequently associated with recurrent EBV infections. Malaria infection is known to initiate a selective T-cell defect that impairs the function of cytotoxic T cells. Burkitt's lymphoma has an increased frequency in areas where malaria is endemic. In addition, B-cell lymphomas are often found in patients following immunosuppressive therapy or allograft maintenance.

Cytotoxic T cells normally inhibit the proliferation of transformed B cells. *In vitro,* infected B cells will not proliferate unless contaminating T cells are removed or inactivated by drugs such as cyclosporin-A. Also, EBV-specific cytotoxic T cells are present in the blood of acutely ill or recovered patients. These cells recognize and kill, in an MHC-restricted manner, B cells expressing EBV nuclear antigens. At least four different nuclear antigens are expressed on latently infected cells and early in viral infections.

Progression of EBV to Malignancy The continued proliferation of infected B cells is the result of several different signals. EBV-synthesized LMP-1 is a receptor for CD40. In addition, LMP-1 binds to the intracellular TRAF family (TRAF, 1, 2, 3, 5,6) through the cytoplasmic region of the protein (Inoue, 1997). Other signals are also necessary for B-cell proliferation. Induction of Cdk4 and Bcl-2-xL and suppression of p27kip-1 are necessary for entrance into the cell cycle and suppression of apoptosis. EBV does not induce or upregulate Bcl-2. Rather, the virus encodes a BHRF-1 protein that is homologous to the Bc1-2 protein and protects against apoptosis (Inoue, 1997).

A scenario for the progression from infection to malignancy can be modeled. Assuming that the T-cell response is impaired, B cells begin to proliferate in the dark areas of the germinal center. During the rapid and continued proliferation, errors are made by recombinases and isotypic switching enzymes, resulting in translocation of genes to the Ig loci. If the *myc* gene is associated with translocation to the Ig locus, the cells are prevented from leaving the cell cycling compartment and continually proliferate. Proliferating cells with the *myc* would have select growth advantages over other transformed cells, allowing the formation of monoclonal tumors. In addition, the combined translocation creates a "resting cell" phenotype that is neither rejected nor destroyed by the immune system. The transformed Ig locus downregulates HLA class I markers, adhesion molecules, and the EBV-coded surface proteins (Klein, 1996).

HIV

HIV infections are not latent infections. The virus is extremely active in the lymph nodes from the earliest stages of the disease process, but the clinical manifestations or susceptibility to opportunistic pathogens only occurs in the later stages of the disease.

Lymph Nodes and HIV. From a mechanisitic viewpoint, HIV infection is a disease of the immune system. Early in the disease process, viruses are localized in the lymph node follicular dendritic cells where they initiate a normal immune response. However, continued viral replication in the node destroys the follicular dendritic cells, reducing the antigen presentation and production of IL-12 (Miedema, 1995).

The production of IL-12 is necessary for a multitude of immunological functions (Fig. 1). Production of IL-12 by phagocytic cells is necessary for development and differentiation of Th1 cells, enhancing the NK cell response and the production of IFN-γ (Trinchieri, 1995a).

In AIDS patients, the normal levels of IL-12 are reduced by 90–95%. The lack of IL-12 results in a skewed lymphocyte subset population in the blood. The development of Th1 cells is downregulated and the Th2 lymphocyte population, the permissive site of HIV replication, becomes the predominant Th population in the blood and spleen (Dalgleish, 1995).

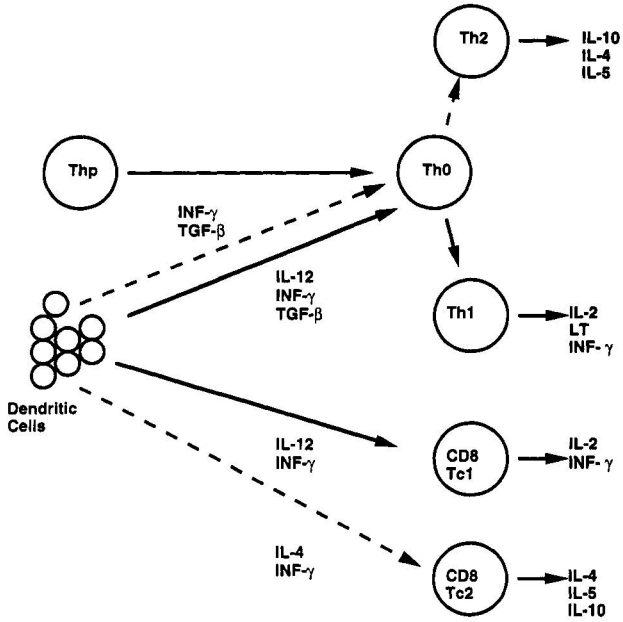

Figure 1. The effect of HIV on follicular dendritic and CD8 cells. Differentiation patterns of CD4 and CD8 cells. The differentiation of CD4 Th1 and CD8 Tc2 cells is under the control of IL-12. HIV destruction of the dendritic cells in the lymph node shifts the pattern of cytokines in favor of the development of CD4 Th2 and CD8 Tc2 cells. The CD4 Th2 cells subsequently become infected with HIV. Solid arrows denote normal cytokine pattern. Dashed arrows indicate HIV-induced cytokine pattern. Modified from *Immunology Today,* Mossman and Sad, 1996, 17:138–45.

HIV and CD4 Helper Cells. The mechanism by which HIV infects the cells has only recently been defined. Interaction of the virus with two receptors is necessary for cell binding and infection. The CD4 interaction is necessary for cell binding, but HIV binding to a chemokine receptor designated CCRS is necessary for infection of the cell. Persons in high-risk groups resistant to HIV have a mutation in the gene coding for CCRS. This mutation appears to confer resistance to the disease (Cohen, 1997).

During rapid viral reproduction, the Th2 cells in the immune system are undergoing HIV-induced apoptosis. Reactions between the HIV envelope glycoprotein gp120 and the Th2 cells induce lymphocyte apoptosis. High viral loads in the blood can induce apoptosis in 1 X 10^9 Th2 cells per day. The body attempts to maintain homeostasis by constantly repopulating the blood with Th2 cells. As the capacity to repopulate Th2 cells from stem cells appears to be finite, the system ultimately becomes exhausted and production of Th2 cells ceases (Miedema, 1995).

HIV and CD8 Cytotoxic T Cells. Cytotoxic T cells are involved in protection from HIV. Early during the disease, two types of CD8 cells are involved in the response. One CD28+ population is the classical MHC-restricted cytotoxic T cells capable of lysing infected cells. A second population consists of noncytotoxic CD8, CD28– cells that secrete the soluble protein CD8 T-cell antiviral factor (CAF). CAF has no homology or identity with known cytokines, interferons, growth factors, or chemokines. It appears to inhibit viral replication, preventing viral mutations leading to drug-resistant or highly pathogenic strains. CAF is active against HIV-1, HIV-2, and SIV strains including cytopathic and noncytopathic strains. In addition, CAF blocks the function of classical CD8 T killer cells (Levy *et al.,* 1996). From a clinical perspective, there is a strong correlation between the presence of noncytotoxic CD8+, CD28– T cells and the symptomatic status of HIV patients.

These noncytotoxic CD8 T cells may be part of a natural or innate defense mechanism against massive and overwhelming viral infections (Levy *et al.,* 1996) that must be eradicated without damage to the host. During HIV infections, approximately 250 million lymphocytes, macrophages, and other cell types are infected with the virus. Cytotoxic T-cell killing of infected lymphocytes and macrophages would further damage the immune system and compromise the host. Lysis of other infected tissue cells would cause massive tissue injury and release large amounts of virus into the blood. It is unlikely that the host would survive such an insult.

Later in the disease process, the CD8 cell population ceases to effectively function in defense of the host. In part, this may be reflect the shift from Th1 to Th2 cells described above. Cytokines from Th1 cells (e.g., IL-2) enhance the antiviral activity of CD8 cells. Conversely, IL-4 and IL-10 from Th2 cells de-

crease the antiviral CD8 response. The fate of CD8 cells exposed to high levels of IL-4 and IL-10 is unclear.

Loss of CD8 cell function may be associated with anergy induced by a loss of the CD28 marker and apoptosis. Downregulation of the CD28 is triggered by IL-4. Failure of CD28 to ligate with the CD80 on APCs or CD86 on memory cells leads to anergy, a loss of T-cell activation, and reduced proliferation in the CD8 population. In addition, apoptosis of CD8+, CD28−, and CD28+ cells is initiated by the induction of the Fas ligand causing endonuclease-mediated DNA fragmentation commonly observed in apoptosis.

Cytotoxic CD8 cells are important in HIV infections. Long-term survivors of AIDS have an intact cytotoxic T-cell function. CD8 cells are involved in the initial clearance of the virus and continual destruction of virus-infected cells. In children and adults, a loss of CD8 function correlates with increased viremia, spread of the virus within the host, and the onset of clinical symptoms.

VIRAL EVASION OF KILLER CELLS

Viruses have developed sophisticated mechanisms for evading the immune system. These mechanisms include short incubation times, antigenic shift and drift, and the production of virokines.

Antigenic Drift and Shift

Influenza strains are constantly expressing new epitopes resulting from antigenic shifting and drifting. Different mechanisms are at play during antigenic drifts and shifts. Viruses create new epitopes by minor changes in the amino acid or carbohydrate sequences of surface proteins. Thus, the antigens are constantly drifting. For example, immune responses to influenza are directed toward the hemagglutinin projected from the virion. Hemagglutinins and neuraminidase have a high degree of genetic instability and undergo spontaneous point mutations that create new epitopes.

Antigenic shifts occur following epitope exchanges between humans, animals, and the virus. In the swine flu influenza pandemic, commingling of genetic material from the Hong Kong influenza strain (H3N2) and swine influenza (H3N1) resulted in the new strain that was H3N1.

Virokines

Virokines are receptor molecules secreted or produced by viruses. In effect, they inhibit the elicitation of an immune response capable of destroying the virus or virus infected cells. Select viral species secrete interleukin recep-

tors (Doherty, 1995). Members of the poxvirus family produce and secrete a molecule having structural homologies to the ß chain of the IL-1 receptor. Binding of free IL-1 to the protein prevents the activity of the major proinflammatory cytokine and allows viral replication within the cells. Rabbit myxovirus and vaccinia (a member of the poxvirus family) secrete IFN receptors (Mossman *et al.,* 1995). Binding of IFN-α and -ß inhibits the normal activation of NK and LAK cells. Finally, some herpesvirus strains secrete receptors for IL-8 and IL-17 (Ahuja *et al.,* 1994). If these chemokines do not react with their normal targets, immunosuppression occurs within the localized area infected with the viruses.

Viruses and MHC Regulation and Function

Viruses can alter the function of MHC molecules by producing proteins that interact with the MHC during synthesis, translocation, or reactions with molecular chaperons (Table 1). The ICP47 protein produced by the herpesvirus interferes with the binding of the TAP protein to class I MHC molecules. Thus, MHC cannot be effectively transported across the membrane of the endoplasmic reticulum. Adenoviruses interfere with MHC I expression at a later step in the process. The adenovirus E19 protein binds to class I MHC and prevents egress from the endoplasmic reticulum. EBV produces the protein BZLF2 that binds to the ß chain of the MHC II molecule in the endoplasmic reticulum. On BZLF2–MHCII interaction, antigen presentation is inhibited (Ploegh, 1995).

Table 1. Viruses Capable ofAltering the Expression of MHC Markers

Virus	MHC I	MHC II
Adenovirus	D[a]	–
Cytomegalovirus	D	D
Electromelia	D	–
Hepatitis	D	–
Herpes	D	–
HIV	D	U
Human papillomavirus	D	U
Measles	U	U
Moloney leukemia	U	–
Rous sarcoma	D	D
Vaccinia	D	–
Vesicular stomatitis	D	–

[a]D, downregulated; U, upregulated.

CHEMICALS INCREASING SUSCEPTIBILITY TO VIRUSES

Many chemicals can decrease NK cell function and increase morbidity and mortality following viral infections (Table 1). Intraperitoneal administration of cyclophosphamide (Selgrade *et al.,* 1982a), cyclosporin, or 7.12-dimethylbenz-[a]anthracene (Selgrade *et al.,* 1988) inhibits NK cell function and increases mortalities following murine cytomegalovirus infections. In the influenza model, phosgene gas enhances influenza virus infections (Selgrade *et al.,* 1989) by suppressing the virus-specific CD8 cytotoxic and NK cell responses (Burleson and Keyes, 1989; Erlich and Burleson, 1991). The immunotoxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mice (House *et al.,* 1990) has also been studied in the influenza model. A single dose of 0.1 μg/kg resulted in significant mortality when animals were challenged with the virus.

HAZARD IDENTIFICATION

The effect of xenobiotics on the course of viral infections is usually studied in two murine models. Murine cytomegalovirus is a well-established model for the study of opportunistic herpesvirus infections common in AIDS or other types of chemically induced immunosuppression (Preiksaitis, 1989; Betts and Hanshaw, 1977). NK, LAK, and virus-specific cytotoxic T cells are required to eradicate the infection (Selgrade and Daniels, 1995). Intransal instillation of influenza virus is the second infectivity assay. This cytopathic virus replicates in the lungs of rats or mice. Resolution of the infection requires many different immunological mechanisms including interferon, alveolar macrophage function, cytotoxic T cells, NK cells, and antibody production (Burleson *et al.,* 1987). Both animal models will be discussed in detail in a later chapter.

REGULATORY POSITION ON VIRAL INFECTIVITY MODELS

The EPA requires animal models in Tier 1l studies to determine the biological significance of decrements in function of NK or cytotoxic T cells.

18

Transplantation

INTRODUCTION

In the 1970s, it was found that pregnant women often formed antibodies directed toward MHC markers on cells from the fetus. This suggested that heterologous MHC markers acted as isoantigens. More specifically, the immune system recognized as foreign MHC markers that were not part of the host. Later, it was recognized that MHC A, B, and C markers evoked a cytolytic T-cell response. As knowledge of the MHC polymorphism expanded, it was found that T helper cells react with MHC class II markers (e.g., *D, DR, DQ,* and *DP* alleles) and elicit the production of antibodies.

In early transplantation studies, allografts were accepted or rejected depending on the similarity between donor and recipient MHC markers. Other studies indicated that the MHC had multiple loci with many pleomorphic alleles. From these early studies evolved the concept that MHC molecules were isoantigens similar to red blood cell antigens. The two isoantigen families differed in that the MHC antigens were expressed on nucleated cells whereas the ABO blood group antigens were restricted to red blood cells.

HUMAN MHC COMPLEX

Several sets of genes involved in antigen processing and presentation are clustered together in the MHC. These sets of genes include class I genes *(HLA-A,*

B, and *C),* class II genes *(DR, DQ, DP, DN, DO, DM),* LMP-1 and 2, TAP-1 and 2, complement C2, and Hsp 70 (Beck et al., 1996).

In addition to the class I and II loci involved in antigen presentation, each sublocus has different isoforms or alleles. Within the class I locus, there are 24 *HLA-A* alleles, 27 *HLA-B* alleles, and 11 *HLA-C* alleles. Multiple alleles of class II *(DR, DQ, DP, DMN, DO,* and *ND)* have been described (Table 1).

Genetic polymorphism is not unique to class I and II loci. There are multiple allelic variants of TAP-I (1A to 1C), TAP-2 (2A to 2E), and LMP-2 (LMP-2d, LMP-2b. and LMP-2q) (Zhou *et al.,* 1993) within the MHC (Chevrier *et al.,* 1995). The transporter associated with antigen processing (TAP) delivers antigen to the lumen of the endoplasmic reticulum for association with class I molecules.

Table 1. MHC Loci and Alleles within Each Locus

Class I				Class II			
A	*B*		*C*	*Dw*	*DR*	*DQ*	*DP*
A1	*B5*	*B5(15)*	*Cwl*	*Dwl*	*DR1*	*DQw1*	*DPw1*
A2	*B7*	*Bw.52(5)*	*Cw2*	*Dw2*	*DR2*	*DQw2*	*DPw2*
A3	*B8*	*Bw53*	*Cw3*	*Dw3*	*DR3*	*Dqw3*	*DPw3*
A9	*B12*	*Bw54/22*	*CW4*	*DW4*	*DR4*	*DQ4*	*DPw4*
A10	*B13*	*Bw55/22*	*Cw5*	*DW5*	*DR5*	*DQw5*	*DPw5*
A11	*B14*	*Bw56/22*	*Cw6*	*Dw6*	*DRw6*	*DQw6*	*DPw6*
Aw19	*B15*	*Bw57/17*	*Cw7*	*Dw7*	*DR7*	*DQw7*	
A23	*B16*	*Bw58/17*	*Cw8*	*Dw8*	*DRw8*	*DQw8*	
A24	*B17*	*Bw59*	*Cw9*	*Dw9*	*DR9*	*DQw9*	
A25	*B18*	*Bw60*	*Cw10*	*Dw10*	*DRw10*		
A26	*B21*	*Bw61*	*Cw11*	*Dw11*	*DRw11*		
A28	*Bw22*	*Bw62*		*Dw12*	*DRw12*		
A29	*B27*	*Bw63*		*Dw13*	*DRw13*		
A30	*B35*	*Bw64*		*Dw14*	*DRw14*		
A31	*B37*	*Bw65*		*Dw15*	*DRw15*		
A32	*B38*	*Bw67*		*Dw16*	*DRw16*		
Aw33	*B39*	*Bw7*		*Dw17*	*DRw17*		
Aw34	*B40*	*Bw70*		*Dw18*	*DRw18*		
Aw36	*Bw41*	*Bw72*		*Dw19*			
Aw43	*Bw42*	*Bw73*		*Dw20*	*DRw52*		
Aw66	*B44*	*Bw75*		*Dw21*	*DRw53*		
Aw68	*B45*	*Bw76*		*Dw22*			
Aw69	*Bw46*	*Bw77*		*Dw23*			
Aw74	*Bw47*			*Dw24*			
	Bw48	*Bw4*		*Dw25*			
	Bw49	*Bw6*		*Dw28*			
	Bw50						

LMPs-2 and 7 are two subunits of the cytosolic proteasome involved in the generation of endogenous peptides.

In some species, the polymorphic TAP and LMP may contribute to the incidence or severity of autoimmune diseases. In the rat, TAP polymorphism governs the peptide sets presented to T cells. One TAP isoform presents peptides that are involved in autoimmune diseases (Armandola *et al.,* 1995). In humans and mice, there is no evidence to support the concept that TAP isoforms present different peptide sets (Zhou *et al.,* 1993; Chevrier *et al.,* 1995). In contrast, human data suggest that the LMP-2 ß allele in patients with juvenile rheumatoid arthritis increases susceptibility to the disease. Homozygosity also predisposes to a more progressive and articular disease form (Pryhuber *et al.,* 1996).

EVOLUTION OF THE MHC COMPLEX

Because the immune system is responsible for the survival of the host, it is not unexpected that the system has redundant or backup antigen presentation systems (e.g., multiple class I and II loci). The polymorphic system precludes the possibility that a dysfunction in a single class I or II molecule would prevent a response to an infectious agent.

A polymorphic HLA does not guarantee the survival of the host. Using molecular mimicry, select bacteria express molecules with extensive homology to MHC markers. Because these bacteria are regarded as "self," the immune system does not respond to the infection and death may result.

To dissipate the effects of molecular mimicry, and ensure the survival of, at least, a small proportion of the species, multiple alleles of each MHC locus have evolved. In the class I markers, the allelic variation in amino acids is located in the a-helical sides of the protein-binding cleft or the ß-strands forming the cleft floor. Although the allelic variation in class II markers is assumed to be identical to the class I markers, the structural details are unclear.

Allelic polymorphism influences the nature of the bound antigens. Antigens will bind specifically to alleles within a certain locus. For example, lambda repressor (LR) antigen, thyroid peroxidase (TPO), and polyalanine peptide (PAP) all react with DQ molecules. However, LR binds preferentially with DQ8, TPO binds to DQ2, and LR binds strongly with DQ1 (Kwok *et al.,* 1995).

NEW NOMENCLATURE FOR THE CLASS II ANTIGENS

Because more advanced molecular biology techniques have identified additional variants or subgroups within a single allele, a standardized nomenclature was proposed by the World Health Organization Nomenclature Committee for

the HLA system. All genes in the class II region are designated by the letter D. This is followed by the designation for a sublocus (e.g., *P, Q* or *R).* The letter *A* or *B* denotes an α or ß chain location. For example, *DRB1* denotes a gene that encodes a product expressed on the ß chain of the *DR1* molecule. Using a four-digit system, different alleles are numbered. The first two digits describe the closest associated serological specificity (e.g., DRB 1*03). The last two digits denote subgroups within a known serological specificity, such as DRB 1 *0301 (She, 1996).

HLA ASSOCIATION WITH DISEASES

Ankylosing Spondylitis

There are associations between select MHC alleles and diseases (Table 2). Ankylosing spondylitis is a form of reactive arthritis following klebsiella infections. Over 90% of patients express the MHC-B27 marker. Antibodies directed toward the klebsiella cross-react with B27 markers. Klebsiella nitrogenase reductase peptide KAKAQTDR binds to the B27 groove region that contains DRED. Also, there is molecular mimicry between *Klebsiella pneumoniae* pulD secretion protein, pulA (pullulanase) enzyme (Gly-X-Pro), HLA-B27, and collagen types I, 111, and IV.

Although the mechanism involved in the induction of ankylosing spondylitis is unclear, LPS endotoxin from the klebsiella may stimulate polyclonal acti-

Table 2. Association between Specific MHC Alleles and Diseases in European Caucasoids

Disease	HLA allelle	No. studies	% Association	Relative risk*
Ankylosing spondylitis	*B27*	8	80	87.8
Reiter's syndrome	*B27*	9	80	35.9
Rheumatoid arthritis	*DRw4*	31	64	4.0
Myasthenia gravis	*B8*	16	39	3.4
	DRw3	17	40	3.0
Addison's disease	*Dw3*	21	70	8.8
Grave's disease	*B8*	18	44	2.5
	Bw35	20	57	5.0
Celiac disease	*B8*	20	67	8.6
	DRw3	27	96	73.0
Chronic hepatitis	*B8*	16	36	9.2
	DRw3	7	79	4.6

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*Relative risk is the chance of individuals with a particular HLA allele of developing a disease.

vation of the B cells resulting in the generation of low-affinity IgM antibodies. Complement-mediated lysis of antibody-coated host cells liberates antigenic enzymes that trigger an autoimmune reaction.

MHC MARKERS AND PATERNITY

Two complete sets of MHC markers are present in each individual. Occasionally, there are homozygotes for a specific allele in a locus. More commonly, individuals are heterozygous at each locus. The complete set of MHC alleles is termed the MHC *haplotype.* Occasionally, different MHC alleles are inherited together. This phenomenon is called *linkage disequilibrium.*

Paternity testing using HLA markers is feasible because the HLA complex is polymorphic and markers are usually inherited as a block. Normal inheritance patterns show that one chromosome containing MHC *A, B, C, D, DR, DQ,* and *DP* alleles is inherited from the mother while another complete set is inherited from the father. Paternity can be excluded on the basis of differences in one or more alleles of loci *A, B,* or *C* (Fig. 1).

TRANSPLANTATION

Proper matching of tissue from the donor and recipient is essential to the acceptance of the graft or increased frequency of graft survival. Survival of

Mother	Father
A3. Bw35, Cwl, Dwl, DR1, DQw1, DPw1	A3, B7, CW4. DW3, DR2, DQw1, DPw3
A2,B8, Cw3, Dwl, DR2, DQw3, Dpw3	A9. B12, Cw2,Dw2, DR3, Dqw2, DPw4

Child 1	Child 2
A3, Bw35, Cwl, Dwl, DR1, DQw1, Dpw1	A2,B8, Cw3, Dwl, DR2, DQw3, DPw3
A9, B12, Cw2, Dw2, DR3, Dqw2, DPw4	A3, B7, CW4, DW3, DR2, DQw1, DPw3

Child 3	Child 4
A3, Bw35, Cwl, Dwl, DR1, DQw1, Dpw1	A3,B7, CW4,DW3,DR2,DQw1,DPw3
A2,B8, Cw3, Dwl. DR2, DQw3, DPW3	A9, B12, Cw2,Dw2, DR3, Dqw2, DPw4

Figure 1. The inheritance pattern of MHC markers used for the determination of paternity and putative transplant donors.

kidney transplants depends on proper matching of the *A, B,* and *DR* loci. Differences in survival as a reflection of mismatches in each locus are shown in Table 3.

In preparation for a transplant, a number of MHC typings are performed on the donor and the recipient. Because antibodies directed toward MHC markers can be induced by transfusions, pregnancy, or prior transplantations, the recipient's serum is tested for the presence of antibodies directed toward all known MHC alleles. This is done at different times and with different panels of cells so that genetic disequilibrium is taken into consideration. Preexisting anti-MHC antibodies are the cause of hyperacute rejection.

Next, CD19 B cells are isolated from both the donor and the recipient. These cells are rich in class I and class II markers. Panels of antibodies with known MHC allelic reactivity are reacted with the cells. Following lysis with complement, the number of dead cells is determined for each antibody cell combination.

Cell-based reactions such as the mixed lymphocyte reaction (MLR) have been used to determine matches in the class II loci. Other more sophisticated techniques such as PCR or PCR-SSP can also be used to probe patterns of MHC polymorphism between donor and recipient.

GRAFT REJECTION

Hyperacute Rejection

This form of rejection occurs in minutes following transplantation. Preexisting antibodies bind to the epithelium of the transplanted cells and activate the classical complement cascade. Complement fragments activate the coagulation pathway liberating the von Willebrand factor. In turn, the liberated factor activates platelets. Platelets are further activated by attachment to the basement

Table 3. Effect of MHC Class I Mismatches on Survival of Kidney Allografts

Mismatch			Percent survival
A locus	*B* locus	*C* locus	
0	0	0	93
1	0	0	87
0	1	0	84
0	0	1	83

membrane that is exposed following complement lysis of epithelial cells. From a physiological perspective, there is a thrombotic occlusion of the graft vasculature.

Antibodies directed toward MHC markers can be induced by several different mechanisms. Natural antibodies to foreign MHC markers can be stimulated by bacterial infections. Carbohydrate antigens in bacteria cross react with red blood cell antigens. Red blood cells and epithelial cells also share common carbohydrate antigens.

Often an immune response to a select bacterium will generate IgM antibodies to red cell antigens that are different from the host. In contrast, antibodies of the IgG class are formed in response to protein alloantigens.

Exposure to foreign alloantigens is common during blood transfusions, pregnancy and prior transplantations. Because of the presence of preformed antibodies, transplants from husband to wife or from child to mother are routinely unsuccessful.

Acute Vascular Rejection

In some instances, antibodies are formed in response to mismatched MHC class II products. Interactions between IgG antibodies and endothelial cells activate complement. Necrosis of the epithelial cells is observed 1–10 days following transplantation.

Cellular Graft Rejection

In the classical cellular responses to grafts, MHC antigens are shed into the lymphatic system and transported to a regional lymph node. Generation and activation of effector cells takes place in the node. The mechanism used in activation of effector cells has always been problematic. Soluble MHC antigens are extremely weak immunogens and they have close structural homology with the recipient MHC markers. Thus, it is usually very difficult for APCs to present soluble MHC markers to the immune system.

Recently, a unique APC in the lymph node has been described. This cell is called a *stimulator* or S+ APC. Interactions between the soluble MHC proteins and the S+ are the major mechanism by which soluble MHC antigens are presented to the immune system.

Activated lymphocytes cause necrosis of the parenchymal cells within the graft. The most common mechanism is the generation of CD8 cytotoxic T cells directed toward class I MHC molecules on vascular and parenchymal cells. Other cells are not believed to be involved because the expression of CD8 inhibited the activity of NK and macrophage killer cells.

Table 4. PCR Based HLA Typing Methods

Method	Multiple probes	Post-PCR manipulations	HLA marker applications
PCR-SSO dot blot	+	H, SW, AR/CD	A, B, DRB, DQA, DQB, DPB
PCR-SSO Reverse dot plot PCR	+	H, SW, CD	DRB, DQA, DQB, DPB
Oligocapture	+	H, SW, CD	DRBS
Dual phase	+	H, SW, CD	DRB
PCR-RFLP	+	RE, AGE	DRB, DRQA, DQB, DPA
PCR-SSP	–	AGE	A, C, DRB, DQA, DQB

Abbreviations: H, hybridization; SW, stringent washing; AR, autoradiography; CD, colorimetric detection; AGE, agarose gel electrophoresis: RE, restriction endonuclease digestion.

HAZARD IDENTIFICATION

Molecular Biology Typing of MHC Class I and II

Because of the extreme polymorphism of the class II antigens and their importance in graft rejection, molecular biology approaches have been used to type class II markers. Early methods used restriction fragment length polymorphism (RFLP) to type *HLA-DR/Dw, DQ,* and *DP* alleles. Because the RFLP was cumbersome and lacked a high degree of specificity, it was replaced by polymerase chain reaction (PCR)-based technology.

Two PCR-based techniques have emerged for typing class II markers. The first is sequence-specific oligonucleotide typing (PCR-SSO). In the technique, target sequences from polymorphic exons of HLA genes (e.g., exon 2 of HLA class II genes) are amplified by PCR. The SSO probes are hybridized to the amplified sequences (Table 4). Each probe is complementary to different motifs

Table 5. Scoring of MHC Class I Matches Using the Complement Lysis Assay

Rating score	Percent cells lysed
0	No cells killed
1	1–20% killed
2	21–40% killed
4	41–60% killed
6	61–80% killed
8	81–100% killed

within an allelic hypervariable region. Thus, the technique can identify single or allelic combinations.

There are two basic formats for the technique: membrane-based dot plots or reverse dot plots. To facilitate high throughput, nonradioactive reporter molecules, oligocapture sandwich, or dual-phase oligocapture assays have been developed.

The second technique involves the digestion of PCR products by a panel of restriction endonucleases. The fragments are then subjected to conventional RFLP analyses on agarose or acrylamide gels (PCR-RFLP). To increase the resolution of the PCR-RFLP, internal control restriction sites in PCR primers and single restriction sites are commonly included in the assay. A single-step PCR with sequence-specific primers (PCR-SSP) is commonly used to type cadaveric tissue for organ transplantation. The entire assay can be completed in less than 2 hr.

Typing of HLA class I markers by molecular biology techniques is difficult for three reasons: (1) There are many pseudogene sequences that can confuse the analyses of class I A, B, and C PCR amplification products; (2) amplification of linked sequences in exon 2, intron 2, and exon 3 is necessary because of the polymorphisms in exon 2; and (3) the presence of polymorphic sequence motifs in the class I genes confounds the analysis.

Serological Typing of MHC Class I Markers

Multiparous women produce antibodies to class I markers present on the fetus. The reactivity of these antibodies have been defined over the years. Antibody panels with known class I allelic specificity have been developed. In the assay, cells are reacted with a panel of antibodies in the presence of complement. Cells are then stained with ethidium bromide to differentiate between live and dead cells. Dead cells stain red whereas live cells stain green. The cells are scored on the basis of the percent cells killed (Table 5).

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The Kines

INTRODUCTION

Cytokines are low-molecular-weight peptides produced by multiple cell types during an immune response. They react with receptors on the cell surface of target cells. Like hormones, cytokines have a high dissociation constant K_d between 10^{-10} and 10^{-12}. By altering the function of many different cell types, they modulate and regulate the inflammatory response.

Cytokines have different target cells. Some cytokines can bind to the cell that has secreted the peptide (autocrine activity), a nearby cell (paracrine activity), or cells in other immunocompetent organs (endocrine activity). Besides the role in modulating the immune response, cytokines are involved in hematopoiesis, cell differentiation, cell growth, and innate immunity.

Chemokines are low-molecular-weight proteins secreted by T cells. These molecules are chemoattractants for macrophages, neutrophils, and eosinophils. In addition, chemokines often act as cellular activators and growth factors for other cells. Thus, they have both paracrine and endocrine functions.

CYTOKINES

The 17 or 18 cytokines described in the literature have multiple functions. However, they can be grouped into functional families. These functional groups are involved in (1) activation and maturation of lymphocytes, (2) inflammation, (3) innate immunity, and (4) hematopoiesis.

Activation and Maturation of Lymphocytes

Interleukin 2. IL-2 (or T-cell growth factor) is a 14- to 17-kDa glycosylated protein. Following antigen stimulation, IL-2 is synthesized in high concentrations by CD4 cells and in low concentrations by CD8 cells (Waldmann, 1993). Production is transient with peak activity occurring within 4 hr of activation. IL-2 acts on the same cells that synthesized the cytokine (autocrine effect) or on nearby T cells (paracrine effect). The IL-2 structure is common to a number of cytokines reacting with receptors having the WSXWS motif.

The IL-2 receptor is composed of two different proteins with differing affinities for IL-2. The IL-2Rα protein has a molecular mass of 75 kDa, reacts with the anti-TAC monoclonal antibody, has a low affinity (10^{-8} M) for IL-2, and cannot initiate signal transduction. When IL-2Rß protein is expressed alone, it does not bind IL-2 (Waldmann, 1993).

The addition of a third protein (IL-2Rγ) to the IL-2Rα-IL-2Rβ complex changes the conformation of the IL-2Rß and increases the affinity constant for IL-2. Stabilization of the high-affinity unit may be facilitated by an additional 95- to 105-kDa protein (Waldmann and Goldman, 1989).

The IL-2Rγ protein increases affinity constants in T and NK cells. Activated T cells expressing the α, ß, and γ proteins have a high affinity (10^{-11} M) for IL-2. Large granular lymphocytes (NK cells) expressing only IL-2Rß and IL-2Rγ proteins have intermediate binding affinity (10^{-9} M) for IL-2 (Greene and Leonard, 1986).

IL-2 is important in the generation of an immune response. The concentration of IL-2 produced by Th1 cells is directly related to the magnitude of the T-cell-mediated immune responses (Blazek and Mathe, 1983).

IL-2 also enhances the cytolytic activity of NK cells creating lymphokine-activated killer (LAK) cells (Damle and Bradley, 1988). With respect to B cells, IL-2 acts both as a growth factor and as a stimulus for antibody production.

Interleukin 4. IL-4 is a monomeric 20-kDa peptide produced by CD4 Th2 lymphocytes, activated FcεRI+ mast cells, basophils, and occasionally CD8 cells (Brown and Hural, 1997). It functions as a regulator of allergic reactions (Save-1koul and van Ommen, 1996). Following stimulation with antigen, IL-4 serves as an autocrine growth factor for activated Th2 cells and a paracrine factor for the growth and differentiation of naive, antigen-stimulated Th2 cells (Duschl *et al.,* 1995). At the same time, IL-4 induces isotypic switching in B cells that secrete only IgE antibodies.

There are effects on other cell targets. IL-4 increases the expression of adhesion molecules on vascular endothelial cells (VCAM-1) resulting in adhesion of eosinophils, lymphocytes, and monocytes (Zurawski and de Vries, 1994). Also, IL-4 is a growth factor for mast cells.

Transforming Growth Factor. TGF-ß is a small (28 kDa) cytokine produced in a latent form by T cells and monocytes. When acting as a cytokine, TGF-ß is a negative regulator that counteracts proinflammatory cytokines. It inhibits the proliferation of mitogen- or antigen-stimulated T cells. Also, TGF-ß inhibits the function and activation of macrophages, polymorphonuclear cells, and vascular endothelial cells.

Modulators of Inflammation

Multiple cytokines are involved in augmenting and regulating the inflammatory response.

Interleukin 5. IL-5 is a 45-kDa dimeric polypeptide produced by Th2 and mast cells. It acts as a growth and differentiation factor for eosinophils and governs eosinophilic inflammation of the airways (Anderson and Coyle, 1994). Also, IL-5 activates the helminth killing process in mature eosinophils (Tagboto, 1995). In mice, it serves as a T-cell replacement factor for B-cell activation and antibody synthesis, and it acts as a cofactor for activation of cytotoxic lymphocytes.

The IL-5 receptor is composed of two polypeptide chains (a and ß). The α chain is unique to IL-5 while the ß chain is common to the IL-5 receptor, the IL-13 receptor, and the GM-CSF receptor (Keegan *et al.,* 1994). The receptor contains five shared amino acid residues tryptophan–serine–x–tryptophan–serine (WSXWS) where X is variable. This sequence is distal to the transmembrane region.

Interleukin 10. IL-10 is a 16- to 20-kDa polypeptide with sequence homology to the Epstein–Barrvirus BCRFl gene (Moore *et al.,* 1993). IL-10 is produced and secreted by many different cells including Th2, activated B cells, activated macrophages, and skin keratinocytes (Spits and de Waal Malefyt, 1992). It functions as a macrophage inhibitor preventing the expression of MHC 11, the costimulatory marker B7, and the production of cytokines TNF, IL-1, and IL-12. In human cells, IL-10 acts as a T-cell replacement in B-cell activation and isotypic switching to IgG4 (Spits and de Waal Malefyt, 1992).

Downregulation of macrophage markers necessary for T-cell stimulation prevents T-cell proliferation and the induction of cytokines. Moreover, T cells cannot respond to cytokines induced by a variety of soluble antigens and alloantigens, or cytokine production by NK cells.

Interleukin 12. IL-12 is composed of 35-kDa (p35) and 40-kDa (p40) proteins that are covalently linked to form a heterodimer. Subunit p35 is pro-

duced by T, B, NK cells, and monocytes. Activated monocytes are the primary source of the p40 chain. The p40 protein is homologous to the IL-6 receptor and contains an Ig domain and the WSXWS motif.

IL-12 binding to T and NK cell receptors induces multiple effects. High levels of IL-12 favor the development of CD4 Th1 versus CD4 Th2 cells. In addition, IL-12 induces differentiation of CD8 cells to active cytotoxic T cells (Hiscox and Jiang, 1997). NK receptor binding increases the cytolytic activity and serves as a growth factor for NK cells (Trinchieri, 1995a). Often, IL-12 also induces the production of IFN-γ by NK cells (Biron and Orange, 1995).

Interleukin 16. IL-16 is a 56-kDa homotetrameric protein synthesized by CD8 and epithelial cells following stimulation with histamine. Target cells include T cells, monocytes, and eosinophils. IL-16 reacts with CD4 cells and downregulates proliferative responses initiated by class II MHC molecules. The T cell unresponsiveness may result from IL-16-mediated interruption in the IL-2R signaling pathway (Theodore *et al.,* 1996). In addition, IL-16 is a chemoattractant for T cells, monocytes, and eosinophils.

Interleukin 17. IL-17 (or CTLA-8) is produced by αβ TCR+, CD4–, CD8+ lymphocytes and memory T cells, a small but important subpopulation in immune regulation (Kennedy *et al.,* 1996). The secreted 17-kDa protein is homologous to the ORF13 gene of *Herpesvirus saimiri,* a known lymphotropic virus (Kennedy *et al.,* 1996). IL-17 is a proinflammatory cytokine that upregulates surface expression of ICAM-1 in human fibroblasts (Yao *et al.,* 1995b). Like other cytokines, IL-17 can stimulate epithelial, endothelial, and stromal cells to secrete IL-6, IL-8, G-CSF, and prostaglandin E_2 (Fossiez *et al.,* 1996). IL-17 can also augment nitric oxide production by activating NF-KB independently of IL-2ß interactions (Attur *et al.,* 1997).

Iterferon-γ. IFN-γ (immune or type II interferon) is a 21- to 24-kDa homodimeric, glycosylated polypeptide. It is produced by CD4 Th0, CD4 Th1, CD8, and NK lymphocytes following antigen stimulation.

Besides its biological function in preventing viral dissemination, IFN-γ has multiple functions on different target cells. It increases the antigen presentation and recognition of antigens by CD4 and CD8 cells by increasing class I and class 11 MHC marker expression on macrophages, neutrophils, and endothelial cells. IFN-γ orchestrates leukocyte–endothelium interactions allowing transit of phagocytic cells and CD4 lymphocytes from the blood into the tissue (Boehm *et al.,* 1997; Hilkens *et al.,* 1996). Also, it activates monocytes and macrophages and increases intracellular killing of ingested bacteria and extracellular killing of

tumor cells. Killing of tumor cells, however, requires a second signal provided by endotoxin or TNF.

IFN-γ influences the growth and differentiation of T and B cells. It promotes the maturation and differentiation of CD4 Th1 cells while inhibiting the proliferation of Th2 cells. The maturation of CD8 cells and stimulation of NK-cell cytolytic activity require IFN-γ. In B cells, IFN-γ prevents isotypic switching to IgG1 and IgE.

Lymphotoxin. Lymphotoxin (or TNF-ß) is a small (21–24 kDa) protein produced exclusively by activated T cells. Lymphotoxin has 35% homology with macrophage TNF and competes with TNF for the same receptor (Paul and Ruddle, 1988). Usually, lymphotoxin acts as a paracrine factor in local responses. Functionally, it (either alone or in concert with IFN-γ) is a potent activator of neutrophils and increases lymphocyte adhesion to vascular endothelial cells (Korner and Sedgwick, 1996). It contributes to defense against viruses and bacteria and against tumors (Paul and Ruddle, 1988). However, it may have other effects on target cells including killing, induction of differentiation, and growth stimulation.

Cytokines in Innate Immunity

Interferon-α and ß Families. Type I interferons are composed of two subgroups. The interferon-a family consists of approximately 20 different proteins with an average molecular mass of 20 kDa. Structurally dissimilar interferon-ß is a single protein.

Termination of viral infections is the major function of IFN-α and ß. In the context of host defense, IFN-α and ß serve four functions: (1) they initiate the production of 2'3'-oligoadenylate synthase that interferes with the replication of both DNA and RNA, (2) they prevent synthesis of essential amino acids such as tryptophan, (3) they augment and accelerate the autolytic activity of NK cells, and (4) they upregulate MHC I expression and enhance the CTL response.

Tumor Necrosis Factor-α. TNF-α is a 17-kDa protein produced by mononuclear phagocytes and T cells following exposure to bacterial endotoxins. It is unusual in that the molecular orientation is reversed. The amino acid terminus and the transmembrane fractions are within the membrane. The C-terminus is extracellular. Enzymatic cleavage of surface-bound TNF-α creates fragments that circulate in the blood. These fragments form a trimeric 51-kDa ß-strand "jelly roll" (Gruss and Dower, 1995). The homotrimers bind to two different receptors, p75 or p55, which are members of the TNF type III receptor family.

At low (10^{-9} M) concentrations, TNF acts in an autocrine and paracrine fashion. It helps to terminate viral infections by increasing the expression of MHC I molecules and the CTL response to infected cells. In bacterial infections, TNF increases the killing activity of neutrophils and the production of IL-1, IL-6, and TNF. To a lesser extent, TNF acts on other phagocytes (Havell, 1992).

TNF also upregulates the endothelial cell expression of adhesion molecules. The upregulation of the adhesion factors promotes localization of neutrophils, monocytes, and lymphocytes in areas of inflammation.

At moderate levels, TNF acts as endogenous pyrogen stimulating the hypothalamus to induce fever. The fever induction is often augmented by TNF-induced IL-1 and IL-6 production. In addition, TNF acts on the liver inducing the production of acute-phase proteins such serum amyloid A, C-reactive protein, and α_2-macroglobulin. The production of acute-phase proteins reduces the activity of proteases and tissue injury.

In high concentrations, TNF acts as endocrine hormone causing tissue injury, intravascular coagulation, and shock. At concentrations approaching 10^{-7} M, animals usually die of circulatory collapse.

Circulatory collapse is induced by several different mechanisms. Using the nitric oxide synthase enzyme, TNF induces the production of NO that decreases myocardial contractibility. Relaxation of smooth muscle and vascular tone further depresses blood pressure and tissue perfusion. Finally, TNF shifts the activity of procoagulants and the upregulation of endothelial adhesion factors. Both factors induce clotting and the occlusion of blood vessels by neutrophils attaching to the endothelium (Beutler and Grau, 1993).

Interleukin 1. IL-1 is comprised of two polypeptides each having a molecular mass of 17 kDa. IL-1α and IL-1 ß are the products of two genes and have less than 30% homology to each other (Conti, 1991). Most of the IL-1 is synthesized by macrophages and keratinocytes (Kupper, 1988) activated by endotoxin, TNF, or macrophage cytokines (Krakauer, 1986). Synthesis of IL-1 by keratinocytes provides a mechanism for inflammatory responses in local areas devoid of macrophages.

IL-1 precursors are 33-kDa proteins cleaved by an endogenous protease to the biologically active 17-kDa form. IL-1ß is often found in the circulation after gram-negative sepsis; thus, IL-1ß acts as an endocrine hormone. IL-1 binds to receptors that are members of the Ig superfamily. The type I receptor binds IL-1ß, whereas the type II receptor binds IL-1α (Conti, 1991).

The effects of IL-1 are concentration dependent. At low concentrations, IL-1 mediates inflammatory responses similar to TNF. It causes upregulation of MHC I, initiation of coagulation, and increased leukocyte adhesion in the vasculature (O'Garra, 1989a,b). At high concentrations, IL-1 acts as an endocrine

hormone that causes fever, synthesis of acute-phase proteins, and metabolic wasting.

Interleukin 6. IL-6 is produced by mononuclear phagocytes, endothelial cells, fibroblasts, and IL-1-stimulated T cells. The IL-6 homodimer is a single gene product of 26 kDa.

Normally, this cytokine binds to a dimeric receptor consisting of a 60-kDa binding protein with the WSXWS motif and a 130-kDa signal transduction protein. The gp130 receptor binding protein is shared by IL-6, IL-11, leukemia inhibitory factor, oncostatin M, ciliary neurotropic factor, and cardiotropin-1. Stimulation of cells with IL-6 causes dimerization of the gp130 protein, activation of intracellular kinases, and modification of transcription factors (Taga and Kishimoto, 1997). A nuclear factor controlling IL-6 gene expression (NF-IL-6) is also involved in the transcriptional regulation of acute-phase proteins (Kishimoto, 1992).

IL-6 has several biological functions. It causes hepatocytes to synthesize fibrinogen, an important acute-phase protein. In addition, IL-6 may serve as an autocrine growth factor for normal and malignant B cells (e.g., plasmacytomas and myelomas) and as a costimulator for T cells and thymocytes. Some data also suggest that IL-6 serves as a cofactor for the growth of hematopoietic stem cells (Kishimoto, 1992).

Cytokines and Hematopoiesis

Interleukin 3. IL-3 (or multilineage colony-stimulating factor) is a 20- to 25-kDa protein synthesized by CD4, mast cells, and eosinophils (Frendl, 1992). Like 1L-6, IL-3 binds to a receptor containing the WSXWS motif and a 130-kDa signaling unit. The receptor is shared with IL-5 and other colony-stimulating factors such as GM-CSF.

Functionally, IL-3 promotes growth and maturation of mast cells in the bone marrow. It also controls antigen presentation of macrophages via regulation of class I antigens, ß2 integrins (e.g., CD1 1a/CD18), and macrophage cytokines. Administration of IL-3 leads to enhancement of T-cell-dependent immune responses (Frendl, 1992).

Interleukin 7. IL-7 is synthesized by bone marrow stromal cells and fibroblasts. The 25-kDa monomeric protein acts on immature B and T cells. IL-7 acts on B-cell progenitors and stimulates proliferation and differentiation (Valenzona *et al.,* 1996). It also induces proliferation in CD3−, CD4−, CD8− thymocytes (Su *et al.,* 1997).

Although the mechanism of action is not fully defined, IL-7 may activate the CdK4/cyclin D3 complex and initiate the transition into the G_1/S phase of cell proliferation (Itoh *et al.,* 1996).

In addition to effects on immature cells, IL-7 has effects on mature cells within secondary lymphoid organs. Administration of IL-7 increases the numbers of CD4+, CD8+, NK cells, and LAK cells in the spleen (Leong *et al.,* 1997). Moreover, IL-7 prevents programed cell death or apoptosis in natural NK by enhancing bcl gene expression (Armant *et al.,* 1995).

Interleukin 9. IL-9 is a 20-kDa cytokine produced by bone marrow stromal cells. *In vivo,* IL-9 may support the growth of mast cell progenitors. Its function on mature lymphocytes is unclear. However, evidence suggests that IL-9 potentiates IL-4-induced IgE and IgG1 synthesis and release (Petit-Frere *et al.,* 1993).

Interleukin 11. IL-11 is also produced by bone marrow stromal cells. It stimulates the production and growth of stem/progenitor cells such as megakaryocytes and macrophages in the intestinal crypts of the bowel. In short bowel syndrome, IL-11 has a trophic effect and accelerates the recovery of the bowel after resection (Liu *et al.,* 1996).

Colony-Stimulating Factors

C-kit Ligand. The c-kit ligand (or stem cell factor) is a 24- or 27-kDa protein monomer produced by adipocytes, fibroblasts, endothelial cells, and other bone marrow stromal cells. The 27-kDa transmembrane protein form interacts with a tyrosine kinase protein receptor (c-kit) that is an oncogene product. Interaction between c-kit and the ligand stimulates the pleuropotential stem cell to produce mast cells and eosinophils (Ryan *et al.,* 1997).

Granulocyte–Macrophage Colony-Stimulating Factor. Activated T cells, macrophages, endothelial cells, and fibroblasts produce GM-CSF. This factor promotes the growth of platelets and red blood cell progenitors (Fabian *etal.,* 1992). Committed white blood cell progenitors are also stimulated to differentiate into granulocytes and mononuclear phagocytes. In peripheral tissues, GM-CSF activates mature leukocytes localized in inflammatory lesions. GM-CSF binds to a receptor with the characteristic WSXWS motif and a 150-kDa signaling unit.

Monocyte–Macrophage Colony-Stimulating Factor. M-CSF (or CSF-1) is a dimeric 40-kDa protein produced by macrophages, endothelial cells, and fibroblasts. Within the bone marrow, precursors committed to monocyte devel-

opment are targets for M-CSF. These committed cells are at a later developmental stage than targets for GM-CSF. The receptor for M-CSF is a tyrosine kinase that has structural homology to c-kit. In mature macrophages/granulocytes, M-CSF can enhance the killing of ingested bacteria and fungi (Brummer and Stevens, 1994).

Granulocyte Colony-Stimulating Factor. G-CSF is produced by macrophages, endothelial cells, and fibroblasts. It acts on progenitor cells committed to differentiation into granulocytes. Also, G-CSF has effects on mature neutrophil function. Administration of G-CSF significantly enhances neutrophil function during infections with *K. pneumoniae* (Gagnadoux *et al.,* 1997). Although the mechanism is unclear, G-CSF upregulates the expression of CD1 1b/c on the neutrophil surface (Zhang *et al.,* 1997).

CHEMOKINES

Chemokines are families of small (8–10 kDa) proteins that stimulate leukocyte movement (chemokinesis) and serve as chemoattractants (chemokines) for neutrophils, basophils, eosinophils, and lymphocytes (Kaplan *et al.,* 1995; Graves and Jiang, 1995). In addition, chemokines modulate many different cellular functions including respiratory burst, expression of adhesion molecules, intracellular calcium levels, and activation of the contractile elements within the cytoskeleton.

In the last 10 years, over 30 cytokines have been described. The superfamily is structurally differentiated based on amino-terminal cysteine spacing at the N-terminus. The CY chemokine family has an amino acid between the two cysteines (CXC) while the ß family has sequential cysteines (CC) (Wells *et al.,* 1996b).

The a Chemokine family

The CXC or α chemokines are produced by activated monocytes, vascular endothelium, and fibroblasts (Table 1). Chemokines have effects on different cells. They act primarily on neutrophils during inflammatory processes but do not attract monocytes (Graves and Jiang, 1995). The best characterized CXC chemokine is IL-8.

The B Chemokine Family

In contrast, CC or ß cytokines are synthesized by activated T cells (Table 2). The ß family acts on basophils, monocytes, and eosinophils. Some select members of this family are also chemoattractants for T cells (Graves and Jiang, 1995).

Table1. The α Chemokine Family

α family	Target cells
Neutrophil activating protein-I (NAP-1); IL-8	Neutrophils
GRO αβγ	Neutrophils
Platelet factor (PF-4)	Platelets and resting cells
IL-10	NK cells

Mechanism of Action

Chemokines activate leukocytes by two different mechanisms. Some chemokines bind to the heparin sulfate proteoglycans on the surface of endothelial cells. In a complex series of interactions between the chemokines and the proteoglycans, adhesion molecules are upregulated. Leukocyte chemokinesis is stimulated by interactions with leukocytes and the adhesion factors.

Other chemokines interact specifically with a cell surface receptor. Most receptors are G-coupled proteins with seven transmembrane domains similar to that described for IL-8. Chemokines bind to the α subunit of the heterotrimeric GTP-binding protein that exchanges GTP for GDP. In the process, PI-PLC ß1 and ß2 are activated. Dissociation of the complex allows isomers to activate cellular enzymes such as adenyl cyclase (Bohm *et al.,* 1997).

The chemokine G-protein-coupled receptors are neither unique nor specific to chemokines. These receptors are a functionally diverse group utilized by peptide and nonpeptide hormones, chemokines, neurotransmitters, prostanoids, and proteinases (Bohm *et al.,* 1997).

Chemokine receptors can be functionally segregated into (1) leukocyte chemokine receptors, (2) herpesvirus homologues of the α or ß receptors, and (3) the Duffy erythrocyte antigen that binds both α and ß chemokines.

Table 2. The ß Family of Chemokines

ß family	Target cells
Macrophage inflammatory protein-a	Basophils, eosinophils, CD8CD45RO+
Macrophage inflammatory protein-ß	CD45RA, CD8, TIL
Macrophage chemoattractant proteins	T cells (MCP-1), monocytes (MCP-1/2/3)
Rantes	CD4, monocytes, eosinophils

ROLE OF KINES IN DISEASE

Cytokines

The roles of cytokines in inflammatory reactions are well understood. Initially, macrophages release TNF-α and IL-1. These cytokines act on other cells to initiate the release of IL-8. In addition, they upregulate the expression of adhesion factors such as ELAM-1 and VCAM-1. IL-8 serves as a chemoattractant for neutrophils that are localized by the upregulated neutrophil adhesion factors. Early in the inflammatory response, IL-1, IL-6, and TNF-α act on the hypothalamus to increase body temperature and release ACTH. The same three cytokines release acute-phase proteins from the liver.

Because bacteria have a narrow temperature growth range, fever prevents bacterial replication until other immune mechanisms can be implemented to fight the infections. ACTH acts on the adrenal cortex to release corticosteroids. Some steroids activate immune effector cells.

In the *Leishmania major* murine model, the cytokine profile plays a role in resistance or susceptibility. Protection is dependent on successful expansion of CD4+ Th1 effector cells (Wakil *et al.,* 1996). Expansion of Th1 cells is promoted by the presence of IL-12 (Heinzel *et al.,* 1995). Neutrophils, macrophages, keratinocytes, and endothelial cells produce IL-12, but macrophage populations cannot produce IL-12 without IFN-γ priming. Mice with defects in production of IL-12 are extremely sensitive to leishmania infections (Mattner *et al.,* 1996).

If the host does not respond to leishmania with the production of IL-12, compensatory cytokine production increases the susceptibility to infection. Production of IL-4 and IL-10 decreases macrophage intracellular killing and the organism survives in the host (Vouldoukis *et al.,* 1997). Both cytokines also downregulate the NO synthase and prevent the production of NO necessary to kill leishmania (Vouldoukis *et al.,* 1995). Several cell types can produce IL-4. These cells include a CD4+ NK 1.1 population, a small subset of T cells with a limited $V_\beta 4/V_\alpha 8$ ′TCR repertoire; mast cells and eosinophils.

Chemokines

There are defined temporal relationships between the production of chemokines and onset of disease. For example, in murine experimental collagen arthritis models, there is a strong relationship between the production of MIP-2 and MIP-α in the joint and the magnitude of arthritic inflammation. Also, in humans there is a relationship between the production of Rantes, MIP-α, and MCP-3 and eosinophil-mediated allergic airway inflammation (Strieter *et al.,* 1996).

Chemokines play unique roles in the pathophysiology of HIV infections. CD4 is known as the HIV receptor, but HIV attachment to CD4 is not sufficient

to initiate entrance into the cell. Recently, it has been shown that simultaneous attachment of HIV to the CD4 and p chemokine receptors allows entrance into the cell (Wells *et al.,* 1996a; Paxton *et al.,* 1996). Thus, the chemokine receptor acts as an obligatory coreceptor for infection.

During the infective process, chemokines play an important role in host defense. Rantes, MIP-α, and MIP-ß stimulate CD8 cells that control the HIV infection. MIP-α is a CC subfamily chemokine that has a wide range of pro-inflammatory activities including leukocyte chemotaxis (Cook, 1996).

Rantes activates CD4 cells in an antigen independent manner by initiating the release of IL-2 and IL-5 and upregulating the IL-2 receptor (Schall, 1991). In other cell types such as eosinophils, Rantes upregulates adhesion factors, enhances transendothelial migration, and alters cell density.

Rantes, MIP-α, and MIP-ß augment the cytotoxic T and NK cell function. In the case of T cells, these chemokines upregulate the expression of B7–1 costimulatory molecule (Taub *et al.,* 1996).

Destruction of T cells producing these cytokines during HIV infection results in an inability to stimulate CD8 cytotoxic and NK cells. The decline in CD8 cells is usually associated with the onset of active disease.

Termination of select bacterial and fungal infections requires the production of chemokines. Chemokines are part of the normal response to bacterial infections in the lung. The CXC chemokine IL-8, macrophage inflammatory protein 2 (MIP-2), and the CC chemokine increase the microbicidal capacity of polymorphonuclear cells and alveolar macrophages. In the absence of IL-10 production in the lung, these chemokines enhance proinflammatory responses during *Klebsiella pneumoniae* infections and increase short- and long-term survival (Standiford et al., 1996). Fungal infections with *Cryptococcus neoformans* are terminated by MCP-a-recruited macrophages (Strieter *et al.,* 1996).

Chemokines are also involved in tumor growth and metastasis. Initial tumor growth is under the control of growth factors supplied by the vascular epithelium or infiltrating cells. The influx of these cells is controlled by cytokines. Together with autocrine motility factors, chemokines regulate proteases that break down the extracellular matrix and basement membranes allowing the dissemination of cells from the primary tumor (Negus, 1996).

Chemokines are the driving force in the leukocyte emigration from the vasculature into the tissue. Leukocyte adhesion in the postcapillary venules requires the presence of specific chemokine receptors. Receptors for IL-8 are present in the small veins and postcapillary venules of human skin. Receptor-bound IL-8 is continuously internalized by epithelial cells and transported to the luminal surface. This provides a continuous gradient of IL-8 from the vasculature into the tissue (Rot *et al.,* 1996).

MICROBIAL AND VIRAL EVASION OF THE KINES

Cytokines

Several bacterial pathogens have devised mechanisms to counteract the effects of cytokines. Leishmania species produce glycoinositol-phospholipid and lipophosphoglycan. The former downregulates NO production while the latter inhibits IL-12 production. *T. cruzi* and *Brucella* species also produce a surface protein similar to "mucin." This protein inhibits the function, and possibly the production of TNF-α.

Some viral species have copied the gene for a particular cytokine and use the gene product to inhibit the immune response. The poxvirus receptor homologues of host defense proteins are the most studied (Alcami and Smith, 1995). These homologues include vaccinia IL-1 receptor homologue, Shope fibroma virus and myxoma virus expressing a TNF receptor, and the myxoma virus expressing IFN-γ receptors (Ahuja *et al.,* 1994).

Other viruses such as EBV produce proteins that are homologous to IL-10 (Moore *et al.,* 1991). Production of the IL-10 homologue activates the T-cell IL-10R and inhibits the production of cytokines. Also, IL-10 functions as a macrophage inhibitor preventing the expression of MHC 11, the costimulatory marker B7, and the production of cytokines TNF, IL-1, and IL-12.

Chemokines

Some viruses have acquired copies of genes for chemokine receptors (Smith, 1996). Human cytomegalovirus infects epithelial cells, myeloid cells, and lymphocytes. The viral genome has several (US27, US28, and US29) open reading frames producing proteins with moderate G-protein receptor homology and a high homology with the amino acid sequence of the Rantes/MIP-1α receptor. When the US28 gene product was expressed, it bound MIP-1α, MIP-1ß, MCP-1, and Rantes (Ahuja *et al.,* 1994).

Herpesvirus saimiri (HVS) is a close relative of EBV. It does not cause disease in the squirrel monkey (the natural host), but does cause lymphomas and leukemia in other monkey species. One open reading frame in the DNA (ORF ECRF3) has 30% homology with the receptor for IL-8 and suggests that the OW ECRF3 and IL-8R are functionally related. The ORF ECRF3 product expressed in frog oocytes can bind the α chemokines IL-8, GRO-α, and NAP-2 (Ahuja *et al.,* 1994). Because the herpesvirus establishes a long-term relationship with the host, the expression of a nonfunctional IL-8R contributes to the infection by neutralizing IL-8.

In contrast to the human cytomegalovirus IL-8 receptor, the ORF ECRF3 is

functional and transmits signals to the cytosol. This mechanism may allow activation of HVS-infected cells by α chemokines. The biochemical changes resulting from the chemokine signals may produce viral proliferation or induction of viral latency (Ahuja *et al.,* 1994).

THE KINES AND IMMUNODEFICIENCY

Because IL-2 is a signal-transducing cytokine, it is involved in the growth, differentiation, and activation of a variety of lymphocyte subsets. The functional receptor consists of α, ß, and y subunits. The ß and y chains are necessary for signal transduction. In addition, the y chain is essential for the binding of IL-4, IL-7, IL-9, IL-15, and IL-17. Mutations in the y chain cause human X-linked severe combined immunodeficiency (SCID) (Sugamura *et al.,* 1996).

Deficiencies in IL-2 have been reported in patients with partial T-cell deficiencies and SCID. One subject had only an IL-2 deficiency with no changes in numbers of T and B cells. Bone marrow transplants or IL-2 administration has successfully treated the disease.

In antibody deficiencies, cytokines may be absent or produced in excess. Multiple interleukin deficiencies have been reported in cases of X-linked agammaglobulinemia or common variable immunodeficiency. In contrast, excessive production of IL-6 was reported in patients with hyper-IgE syndrome and Castleman's disease (Stiehm, 1993).

Cytokine receptor defects have been demonstated in select immunodeficiencies. Defects in the IFN-γ receptor were reported in four children with severe atypical mycobacteria (e.g., *M. avis)* or salmonella infections. Molecular biology studies showed that these patients had a point mutation (nucleotide 395) in the receptor gene that introduced a stop codon. The mutation resulted in a truncated protein lacking the transmembrane and intracellular domains required for signal transduction. Another patient had a deletion in nucleotide 131 resulting in a frame shift and a premature stop codon at nucleotide 187. This child died as a consequence of immunization with attenuated bacillus Calmette–Guérin tuberculosis organism.

DRUGS AND CYTOKINES

Cyclosporin A (CYA) is a neutrophilic peptide isolated from *Tolypocladium inflatum.* It is used as an immunosuppressant in organ transplants and is especially useful as a therapy for controlling graft-versus-host reaction following marrow transplants. CYA blocks transcription of IL-1, IL-2, IL-2R, IFN-γ, and TNF.

Mechanistically, CYA binds to an intracellular receptor on the cyclophilin–calcineurin complex. CYA blockage of a receptor inhibits the normal serine/threonine phosphatase activity of the complex. Consequently, NF-AT cannot be translocated to the nucleus (Liu, 1993).

The long-term administration of CYA often has serious side effect. In many patients, there is an increased incidence of cancers. Because of the immunosuppressive effects of CYA, lymphomas arise from reactivation and uncontrolled proliferation of EBV-infected cells (Kamel *et al.,* 1993).

The time of tumor onset is rapid in patients treated with CYA. When conventional immunosuppressive therapy is used, tumors begin to arise in 1–15% of patients after 5 years (Anonymous, 1995a). CYA treatment reduces the time for tumor onset to 14 months for lymphomas and 26 months for other tumors (Penn, 1988). Cessation of immunosuppressive therapy often reverses the clinical symptoms of the lymphoma.

FK506 is an 822-kDa macrolide isolated from *Streptomycetes tsukubaensis.* It acts in a manner similar to CYA. FK506 binds to a peptidyl-propyl isomerase (fujiphilin) that is a cytostolic binding protein. A complex of FK506 and fujiphilin inactivates the enzymatic activity of calcineurin necessary for the activation and translocation of NF-AT (Liu, 1993).

FK506 has become the drug of choice for management of organ rejection. It is well tolerated in humans and has no side effects. In contrast to CYA, there is no hepatotoxicity or lymphoproliferative disorders associated with the drug. Moreover, FK506 is active at concentrations 10–100 times lower than CYA.

HAZARD IDENTIFICATION

Cytokine profiles may be altered after exposure to an immunotoxicant. Therefore, measuring the levels of cytokines may be a promising new tool for assessing immunotoxicity. Cytokine patterns can be defined by ELISAs, bioassys, or cDNA probes.

Commercial ELISAs for all mouse cytokines are currently available, but rat reagents are severely limited. Usually, rat cytokine determinations require molecular biology probes, while ELISA reagents can be used in mouse studies.

ELISAs are rapid, monospecific, and sensitive at low cytokine levels. The major disadvantage of the ELISA is that it cannot differentiate between functional and nonfunctional cytokines.

cDNA probes for rat and mouse IL-1 to IL-6, IL-10, IL-13, and IFN-γ are available and the availability of reagents is expanding rapidly. Reverse transcriptase-PCR (RT-PCR) primers have been designed around a small intron spanning sequence allowing competitive PCR or competitive RT-PCR.

Bioassays can also be used to detect cytokines or chemokines. Each assay

Table 3. Cell lines Used in Bioassays to Detect Cytokines

Cytokine	Cell line	Species specific
IL-1	D10S	No
IL-2	CTLL-2 or HT-2	No
IL-3	FDC-P1 (mouse)	Yes
	TF-1 (human)	
IL-4	CT.4S (mouse)	Yes
	TF-1 (human)	
IL-5	BCL_1 (mouse)	Yes
	TF-1 (human)	Yes
IL-6	7TD1 (mouse)	No
IL-7	IXN/2B (mouse)	No

has its advantages and disadvantages. Bioassays rely on specific cytokine-induced changes in cell lines. The bioassays are sensitive in the picogram range and are easy to perform (Table 3). However, cell line maintenance is expensive and recombinant cytokine standards are often unavailable.

It may not be easy to interpret the data from cytokine profiles in mechanistic terms. The nature of the sample and the temporal relationships between stimulation and expression are important factors in data interpretation. Cytokines are present for only short time periods and act over a short distance between cells.

Cytokines can only be measured in body fluids such as blood or urine. Although ELISAs and bioassays are exquisitively sensitive, cytokines may be diluted below the level of detection in the blood volume.

Although it is possible to ascertain the effects of chemicals on cytokine production *in vitro,* the data may not reflect *in vivo* effects. For example, *in vitro* treatment of murine splenocytes with CYA and the mitogen Con A did not decrease the levels of IL-4, TNF-α or IFN-γ Exactly opposite results have been observed *in vivo* in humans (Vandebriel et al., 1996).

In other cases, alterations in cytokine profiles did not correspond to the known characteristics of the chemical. Treatment with $HgCl_2$ induces a Th2 response in $H\text{-}2^s$ mice. An increase in IL-4 was observed, but other cytokines involved in a Th2 response (IL-5, IL-6, TGF-ß, and IFN-γ) remained within normal limits (Vandebriel *et al.,* 1996).

In contrast, cytokine profiles in lymph nodes may be useful in differentiating between allergic sensitization and irritation. Mice exposed to trimellitic anhydride (TMA), a known allergenic chemical, had high levels of Th2 cells and IL-4 and IL-10 but only low levels of IFN-γ. Conversely, mice treated with oxazolone, a known contact sensitizer, had low levels of IL-4 and E-10 and high

levels of IFN-γ. Similar cytokine profiles have been reported after exposure to other allergens and contact sensitizers (Dearman *et al.,* 1995).

REGULATORY POSITION ON CYTOKINES

Measurement of cytokines may be a sensitive indicator of immunosuppression or dysregulation. Altered cytokine prolifes have been described following treatment with immunosuppressive agents; however, the data may not be interpretable in mechanistic terms (Vandebriel *et al.,* 1996). Clearly, more research is needed to define utility of cytokine measurements in hazard identification. Cytokine measurements may be useful in mechanistic studies designed to define the mode of action of a chemical or pharmaceutical. Several centers within the FDA require cytokine measurement in Tier II studies.

20

Assessment of Biological Significance

Host Defense and Tumor Models

INTRODUCTION

Standard toxicology studies are screens that detect gross insults to immunocompetent organs. Using *ex vivo* assays, it is possible to identify potential immunosuppressive hazards. The biological significance, in terms of decrements in host defense, is usually unknown. Additional investigations are necessary to determine the biological significance because the immune system has redundant effector mechanisms and a functional reserve.

The host has increased susceptibility to infection or cancer when the different effector mechanisms are reduced and the functional reserve of the immune system is exceeded. In mice, immune effector mechanisms must be decreased by 40–50% before the host has increased susceptibility to microbial insults or tumor development.

Regulatory agencies are reluctant to accept the functional reserve concept because it has only been documented in adult mice. There is no information on the functional reserve of the immune system in neonates, adolescents, and the elderly.

Other potential effects on the immune system cannot be discerned in standard studies. Autoimmune reactions and immediate or delayed hypersensitivity reactions can only be demonstrated in specialized tests. Usually, these tests define both the hazard and the biological significance.

IMMUNE ACTIVATION/AUTOIMMUNITY

Popliteal Lymph Node Assay

Drug reactions bear little similarities to known spontaneous autoimmune diseases in humans. Based on the similarities between graft-versus-host (GVH) reactions and drug reactions, it was suggested that a GVH response was the mechanism for drug-induced autoimmune reactions (Gleichmann and Gleichmann, 1976).

A GVH is typically associated with B-lymphocyte proliferation and increased production of autoantibodies. It is assumed that the drug interacts with or modifies class II markers on the macrophage surface (Hurtenbach *et al.,* 1987).

Studies in mice showed that an enlarged popliteal lymph node (PLN), characteristic of a GVH reaction, can be induced in F_1 hybrids by injecting parental cells into the footpad. The lymph node weights and cellularity index in the treated footpad differ from those of control footpads in the same animal (Gleichmann *et al.,* 1984). In addition, there marked similarities between the immunological and histological features of the PLN response and GVH (Krystyniak *et al.,* 1992). Recent studies have demonstrated that footpad inoculation of drugs known to cause autoimmune or GVH reactions in humans gave positive reactions in the PLN assay (PLNA). Generally, positive PLNA responses were associated with drugs causing generalized lymphadenopathy, serum sickness, lupus, or scleroderma-like responses (Descotes and Verdier, 1995).

Although only a limited number of chemicals *(N* = 17) have been tested in the assay, it is agreed that the direct PLNA (subcutaneous injection of test chemical, metabolite, or test chemical plus S9 mix) can detect T-cell-activating compounds, including autoimmunogenic compounds (Table 1).

Table 1. Drugs and Chemicals Tested in the Popliteal Lymph Node Assay

Positive human GVH	PLN	Negative human GVH	PLN
Carbamazepine	+	Deoxycycline	–
Chlorpromazine	+	DMSO	–
Hydrazaline	+	Indomethacin	–
Isoniazid	+	Levamisole	–
Nitrofurantoin	+	Penicillin	–
Phenazone	+	Phenobarbital	–
Phenytoin	+	Promethazine	–
Procainamide	+		
Streptozotocin	+		
Zimelidine	+		

A significant response in the direct PLNA suggests, but does not prove, specific T-cell sensitization. The adoptive transfer PLNA must be used to assess specific sensitization. Following test chemical exposure, splenic and lymph node T cells are transferred to the hind paw of syngeneic recipients. Control animals receive cells from unexposed or solvent-treated donors. One day later, recipient animals are challenged at the transfer site with a nonstimulatory dose of the test chemical, metabolite, or test chemical plus S9. When the syngeneic animals develop a positive PLN in 3–5 days, sensitization is proven.

The PLNA does have some limitations that will require additional investigation. The rate of false-positive and -negative reactions is unknown. False-negative reactions were obtained with compounds such as procainamide and isoniazid. Similarly, some known irritants give false-positive reactions in the assay. Therefore, additional validation studies must be undertaken to determine the rate of false-positive and -negative reactions before either assay can be used in routine studies.

In the PLNA design, careful consideration should be given to the technical aspects of the study. The PLN response is strain specific. In mice, only the BALB/c and C57BL/10 strains respond in the assay system. Similarly, in rats only the Brown Norway rat gives a positive PLN result with chemicals known to induce GVH reactions in humans.

Great care must be taken in selection of the carrier or vehicle used in the assay. Many vehicles including ethanol, acetone, corn oil, and paraffin oil cause false positive reactions in the assay system (Descotes and Verdier, 1995). Moreover, the response time must be carefully monitored. Most PLN responses are measured 7 days after test compound exposure. With some compounds (e.g., zimelidine or streptozotocin), the response may be delayed as long as 21 days (Thomas *et al.,* 1989; Krystyniak *et al.,* 1992).

The sensitivity of the assay depends on the endpoint measured. Usually, lymph node weights are the sole endpoint measured in the standard PLNA. The addition of cellularity determinations and conventional histopathological measurements increases the sensitivity and the accuracy (Descotes, 1990).

Flow cytometry measurements also increase the accuracy of the PLNA. Flow cytometry can be used to differentiate between irritant and specific cellular activation (Gerberick *et al.,* 1997).

Animal Models of Autoimmunity

Brown Norway Rat Model. Norway rats immunized with nontoxic concentrations of mercuric chloride ($HgCl_2$) develop a disease characterized by Th2-cell-dependent B-cell stimulation. Stimulation culminates in the production of autoantibodies directed toward DNA, glomerular basement membrane, collagen IV, thyroglobulin, and myeloperoxidase.

The autoimmune disease is characterized by an autoimmune glomerulonephritis and immune complex vasculitis. Humans exposed to mercury develop the same kidney lesions. It is unclear whether this model can be used in the study of other chemicals.

Systemic Lupus Erythematosus. Three different models of spontaneous and two experimental SLE models have been described. The cross between New Zealand Black (NZB) and New Zealand White (NZW) yields hybrids that spontaneously develop a disease similar to human SLE. Hybrid BxSB mice also develop a lupuslike disease that occurs primarily in males. Mice carrying the *lpr* gene on an MRL strain background also spontaneously develop SLE (Dixon, 1982).

SLE can also be experimentally induced in select mouse strains by the administration of anti-idiotypic antibodies or chronic GVH reactions (Gleichmann *et al.,* 1984; Mendlovic *et al.,* 1989).

Scleroderma. Two models, one spontaneous and one induced, of scleroderma have been reported in the literature. The spontaneous disease is found in inbred White Leghorn chickens (Gershwin *et al.,* 1981). Scleroderma can also be induced in the rat following cyclosporin treatment or a GVH reaction (Bos *et al.,* 1989; Furst *et al.,* 1979).

Hemolytic Anemia. NZB mice spontaneously develop hemolytic anemia (Furst *et al.,* 1979). Hemolytic anemia can also be induced in mice after treatment with levadopa (Sharon and Naor, 1989).

Thyroiditis. Spontaneous thyroiditis occurs in the OS chicken, Buffalo (BUFF) rat, and BB/W rat. Thyroiditis can be experimentally induced in mice, rats, guinea pigs, and rabbits (Kuppers *et al.,* 1988).

IgE-MEDIATED IMMEDIATE ALLERGIC REACTIONS

Data from standard toxicology studies yield little information on the ability of a test compound to induce immediate hypersensitivity in the skin or lung. Two types of molecules can initiate hypersensitivity reactions. High-molecular-weight proteins are common allergens. Low-molecular-weight chemicals (LMWC) are haptens and must bind to protein to elicit an immune response.

It is possible to define the allergic potential of LMWC using a multilevel or tier approach to testing. In Tier 1 studies, structure–activity relationships in

known classes of allergens are ascertained. In addition, the theoretical covalent protein-binding capacity of the chemical is determined. In Tier 2, the actual chemical–protein binding is defined *in vitro* assays and the results expressed as the moles of hapten bound per mole of protein. If the chemical binds to protein and creates a putative antigen, guinea pigs are immunized to determine the immunogenicity of the class of antibody produced to the chemical (Tier 3).

Finally, the allergenicity is determined in a guinea pig inhalation model (Sarlo and Clark, 1992). Tests are conducted that determine (1) whether the chemical has the potential to cause allergic reactions via the inhalation route, (2) the no observable adverse effect level (NOAEL) with respect to allergic symptoms, and (3) the dose response relative to the reference chemicals known to cause allergic reactions by the inhalation route. If the responses of the two materials are the same, then consumer and occupational exposures to the new material are set at the same level as those for the historical ones. If the responses are different, then adjustments in exposure are made accordingly.

Guinea Pig Inhalation Tests

The guinea pig model reproduces the characteristics of pulmonary allergic hypersensitivity and airway inflammation observed in humans. Inhalation is the exposure route for the sensitization and the elicitation phases. Like the human response, the guinea pig smooth muscle responds to histamine and other pharmaceutical mediators. In addition, both immediate and late-onset responses can be demonstrated (Griffiths-Johnson and Karol, 1991). Therefore, results of inhalation studies in guinea pigs can be considered as a basis for evaluating allergenicity.

Several parameters are usually monitored during the challenge phase of the study. An increase in the breathing rate or body temperature is often used as an index of an immediate allergic response. Immediate or late allergic reactions induce increases in the breathing rate of 35% or greater from controls. Temperature increases of 0.6°C from baseline values are also associated with immediate allergic reactions (Karol, 1995).

It is also possible to differentiate between immediate allergy and irritation by combining measurement of breathing frequency and expiratory times. Other respiratory parameters have proven useful in the determination of allergic reactions in the guinea pig. The measurements include flow volume loops, respiratory minute volumes, peak expiratory flows, tidal volumes, and plethysmographic pressure (Thorne and Karol, 1988).

Antibodies directed toward chemicals provide additional immunological evidence of an immediate allergic reaction. Sometimes, the presence of allergic antibodies may negate the need for the costly and time-consuming inhalation studies. Antibody levels can be assessed by ELISA or the passive cutaneous

anaphylaxis (PCA) assay. The latter assay is an *in vivo* skin test technique that takes advantage of the ability of guinea pig antibodies to passively fix to mast cells/basophils allowing release of mediators. Serum from putatively sensitized animals is injected into naive animals. Several hours later, dye is injected into the bloodstream and antigen is placed into the skin. A positive allergic reaction causes the seepage of dye into the skin.

Although the guinea pig is generally accepted as the animal of choice for inhalation studies, there are differences between the animal response and the human lung response. In humans, preexisting airway hypersensitivity is important in the elicitation of asthma (Bleecker, 1985). Yet, the guinea pig inhalation assay does not incorporate this parameter. Different antibody classes mediate the response in humans and guinea pigs. Guinea pigs produce IgGl and there is often no consistent association between IgGl production and the onset of allergic symptoms (Ritz *et al.,* 1993). In contrast, humans produce IgE as the allergic antibody and there is an association between elevated IgE levels and symptoms. In addition, there are differences in allergic mediators when guinea pigs and humans are compared. Leukotrienes are the principal mediators of allergic reactions in the guinea pig. In humans, histamine, heparin, and trypase are the mediators.

From a practical perspective, the inhalation response is dependent on the quality of the protein conjugate (Botham *et al.,* 1988). The hapten must be covalently linked to protein with a high molar substitution ratio (haptedprotein) without altering the allergenic characteristics of the hapten. Generally, the binding of 8–10 moles of hapten per mole of protein creates an immunogenic molecule. In addition, the newly synthesized conjugate must be soluble under physiological conditions and easily aerosolized by conventional means. Excepting highly reactive haptens, it is often difficult to synthesize conjugates meeting these criteria.

The dose of putative allergen used in the challenge phase is critical. High concentrations of respiratory tract irritants can cause bronchoconstriction. Nonspecific bronchoconstriction can be avoided by using subirritant concentrations of protein conjugates in the study. When free chemicals are used in the challenge phase, it is difficult to determine the subirritating concentration. Minimally irritating doses may be necessary to elicit a pulmonary reaction when haptens are used in exposure challenges.

Mouse IgE Model

In the BALB/c mouse, topical administration of human respiratory sensitizers (e.g., phthalic anhydride, trimellitic anhydride) significantly increases total and hapten-specific IgE levels (Dearman and Kimber, 1992). Presumably, this response is mediated by CD4 Th2 cells. Contact sensitizers such as dinitro-

chlorobenzene do not increase IgE levels (Briatico-Vangosa *et al.,* 1994). Therefore, the data suggest that changes in serum IgE levels may be used to identify respiratory sensitizers.

Although the mouse IgE test requires few animals and is cost effective, the assay does have several disadvantages. Only a limited number of chemicals have been tested in the model system, and the model needs to be formally and systematically validated. To adequately evaluate the model, a wide range of positive and negative chemicals need to be tested in multiple laboratories (Briatico-Vangosa *et al.,* 1994).

Inherent in the mouse model are the assumptions that IgE is the sole mediator of murine allergic reactions and that elevations of IgE are always associated with exposure to respiratory allergens. These assumptions may not be correct. Murine hypersensitivity may involve mechanisms other than IgE. For example, exposure to picryl chloride, which classically evokes a Th1 response, causes respiratory responses in mice (Garssen *et al.,* 1991). In addition, anaphylaxis can be induced in mice deficient in IgE (Oettigen *et al.,* 1994). Some studies have also demonstrated a decrease in IgE following respiratory exposure to allergens. Decreased IgE levels in mice are found following exposure to toluene diisocyanate (TDI), a known respiratory sensitizer (Satoh *et al.,* 1995).

Rat Model for Hypersensitivity

Inbred Sprague–Dawley rats exhibit classical allergic lung responses with an underlying airway hyperactivity. However, mediators of the response are different in rats and humans. Human mediators include histamine, heparin, and trypase. In contrast, responses in the rat are mediated by serotonin. This model needs additional validation before it can be used as a standard toxicology test.

Rabbit Model

The rabbit has been used to study hypersensitivity reactions to plicatic acid, the agent responsible for red cedar asthma in exposed workers (Chan *et al.,* 1987). Specific IgG and IgE were demonstrable in immunized animals. When challenged with the acid, the rabbits responded with increases in respiratory frequency and pulmonary resistance.

DELAYED HYPERSENSITIVITY (CONTACT DERMATITIS)

The most commonly used animal tests for contact sensitivity induction are the guinea pig maximization test (GPMT) and the Buehler Test (BT). From an

immunological perspective, these tests measure a Th1-mediated delayed hypersensitivity reaction.

Guinea Pig Maximization Test

In the GPMT, three groups of animals are used with 20–25 animals in each group. One group receives intradermal injections of Freund's complete adjuvant (FCA). The second group receives an intradermal bolus of the test material. The induction group is immunized with both FCA and the test material at a dose that causes mild to moderate skin irritation. After 7 days, the animals receive an epicutaneous application of test material in petrolatum. This challenge dose is usually the highest nonirritating concentration. To promote contact with the skin, the material is covered with a surgical dressing for 48 hr. Twenty-one days after the initial exposure, the animals are challenged for 24 hr with a topical application of the test material and the intensity and duration of the skin reactions are determined relative to controls (Burrell *et al.,* 1992).

The GPMT method has several disadvantages (Robinson *et al.,* 1989) and does not provide an absolute standard. The method bypasses the skin barrier that normally prevents chemical penetration. By bypassing the skin barrier at induction, the GPMT can overstate the sensitization risk of epicutaneous exposure to weak sensitizers (Robinson *et al.,* 1989) and can understate the risk to very strong sensitizers possibly through tolerance induction (Buehler and Ritz, 1985). Also, the method gives false-negative responses with weaker contact allergens (Goodwin *et al.,* 1981).

Because the FCA is a potent immune system stimulator, caution should be used in the interpretation of data from sensitization methods using FCA (Buehler, 1996). FCA increases responses to the vehicle. It is also possible that FCA increases hyperreactivity to several alternate vehicles. Nonspecific hyperreactivity in the vehicle control group may contribute to the poor reproducibility in repetitive testing studies (Marzulli and McGuire, 1982).

Buehler Test

The BT was originally designed to detect strong and weak sensitizers before human studies are conducted. It mimics the closed patch occlusion used in the human repeat insult patch test (Buehler, 1965). Validation data suggest that the test is sufficiently sensitive to detect weak sensitizers and flexible enough to be useful in risk assessment paradigms (Buehler and Ritz, 1985).

The standard BT uses 20 animals in three groups: the sensitized or induced group, a vehicle control group, and an unsensitized control group. Often a positive control group of animals sensitized with dinitrofluorobenzene is included in the assay. The induction and challenge doses are similar to those described for

the GPMT. Once a week for 3 weeks, a minimally irritating dose of test material is placed on the same skin site and covered with a surgical dressing. After a 2-week rest period, the induced and control animals undergo a 6- to 24-hr topical patch challenge with the test material placed on a naive skin site (Burrell *et al.,* 1992).

The incidence of positive skin reactions and the relative sensitization rate are determined following the challenge. Reactivity is determined by the incidence of positive skin tests in the induction group and the reaction intensity. The incidence is the number of animals with skin test scores of 1 or greater. The scoring is based on a scale of 1 (mild erythema or redness) to 3 (severe erythema). Relative sensitization incidence is determined by dividing the number of sensitized animals by the number in the test group. The relative sensitization rate is the most useful and definitive measure of sensitization. Severity is determined by summing the test grades and dividing by the number of test subjects.

The BT is an effective screen for predicting sensitization induced by most technical-grade chemicals or formulations used for human topical exposure. Threshold sensitization or potency and antigenic cross-reactivity issues can be addressed and resolved using the BT (Robinson *et al.,* 1990a). The accuracy rate of the BT may be as high as 97% (Robinson *et al.,* 1989). However, the test may underestimate the risk of sensitization.

Limitation of the GPMT and BT

The GPMT and BT have some limitations. Severe local lesions often occur because of adjuvant, test material, or the occlusive patch at the application site. In addition, dyes or colored test materials may impair the visualization of the erythematous lesion. These factors, along with some coexisting irritant reactions, may yield false-negative reactions in the assay. Finally, the data may be skewed because the original classification system has no scale 0 and negative, nonreactive compounds cannot be classified as nonsensitizers.

Extrapolating Guinea Pig Data to Humans

Data from animal sensitization studies may not be extrapolated to humans. In fact, there is a 51% *(n* = 86) false-positive reaction rate when the guinea pig tests are followed by testing in human repeat insult patch test (HRIPT) protocols. The data discordance may be related to several factors. Chemicals are tested in guinea pig assays at concentrations that are several hundred times higher than the actual anticipated human exposure.

Test article concentration used in the HRIPT is usually tenfold lower than concentrations eliciting reactions in guinea pigs. Alternatively, the test article

concentration may be much lower but similar to anticipated human exposure (Robinson *et al.,* 1989).

Some data suggest that neither guinea pig tests nor the HRIPT predict human sensitization. Pesticides such as malathion, captan, benomyl, maneb, and naled are sensitizers in the GPMT but give a negative response in the human predictive test. Barban, a carbamate insecticide, is a potent sensitizer in both the GPMT and the repeat human insult test, yet there are few reports of sensitization pesticides or insecticides in farm workers.

Other factors may also contribute to the discordance between human and animal sensitization data. The human exposure paradigm may be different. Exposure is a function of the concentration of the substance in the final product, duration of exposure, and the size of the exposed population. Some evidence suggests that strong sensitizers can be used when there is minimal contact at low concentrations.

HUMAN PATCH TESTING

Patch Testing

The most common method used for diagnosis of DTH reactions is the patch test. Usually, nonirritating concentrations of test compound are placed on the skin for 24–48 hr. The presence of antigen-specific T cells in the skin or peripheral circulation induces an inflammatory response at the site of topical application. Within 48 hr, the skin becomes indurated and erythematous.

In the design of skin test studies, it is critical that nonirritating test chemical concentrations are determined before skin testing in humans. Any chemical, under the proper conditions and concentrations, can induce an irritant reaction in naive subjects. In the skin, these reactions resemble the pathophysiology of a DTH reaction. Over 80% of the human skin reactions to xenobiotics are the result of irritant, toxic, or nonimmunological reactions (Mathias, 1983).

Human Repeat Insult Patch Test

An HRIPT is usually performed to confirm product safety using conditions relative to consumer use levels. As described previously, the test article concentration is at least tenfold lower than the dose sensitizing guinea pigs. The concentration may be further lowered if the test article causes irritation in the human skin (Robinson *et al.,* 1989).

During the induction phase, 100 or more normal subjects are topically exposed to the chemical. Subjects are patch tested three times a week for 3 weeks. All test sites are visually scored before and 24 hr following application. After 14–17 days,

subjects arechallenged with alower dose by patch testing at the induction site on the alternate arm. The reaction is scored at 24, 48, and 72 hr. To confirm sensitization, the positive subjects can be rechallenged later. Reactions are scored from 0 (no visible reaction) to 5 (erythema, induration, and blisters).

Extrapolation of HRIPT data from a large population to the public is possible. When negative skin reactions to a single chemical are reported in a population of 100, statistical analysis shows that there may be as many as 30 positive reactions per 1000 persons (95% confidence level). If the test population is increased to 200 subjects and the data show no skin reactions, one might expect 15 sensitizations/1000 persons (95% confidence interval) or 22/1000 persons (99% confidence level) (Henderson and Riley, 1945).

In many countries, there is a reluctance to use the HRIPT. This may reflect ethical considerations centered on the use of humans in biomedical experiments or the fear of sensitizing test subjects during the experiment. Some sensitization may occur because of testing, but the reactions are usually confined to the test site and do not evoke systemic sensitization (Robinson *et al.,* 1989). Moreover, subjects sensitized during the HRIPT can use the sensitizing chemical under normal conditions without recurrence of skin reactions (Weaver and Herrman, 1981).

ALTERNATE TESTS FOR DELAYED HYPERSENSITIVITY

Mouse Ear Swelling Test (MEST)

In the assay, the highest nonirritating dose of test material is used (Gad et al., 1986). At day zero, FCA is injected into the skin of the abdomen. Following tape stripping, the test agent is topically applied on days 1, 3, and 5. After a 5-day rest period, the animals are challenged by topical application to the ear. Ear thickness is measured relative to the control ear. A 20% increase in ear swelling relative to the control ear is considered a positive response.

The original MEST design had some limitations that restricted common usage. MEST did not identify weak and moderate sensitizers (Dunn *etal.,* 1990). Moreover, using the highest possible test material did not universally increase the sensitization potential of moderate and strong sensitizers. Repeated, low-dose exposure to sensitizers does, however, increase the sensitization rate (Thorne *et al.,* 1986).

In addition to using multiple exposures, other improvements have been made in the original MEST protocol. The most significant improvement was the addition of vitamin A acetate (250 IU/g feed) to feed before the initiation of testing. Vitamin A acetate has been reported to increase the numbers of Langerhans cells (APCs) in the skin, which could in turn enhance the cellular immune response (Sailstad *et al.,* 1995). The enhanced response allows the detection of weak and moderate sensitizers (Gad, 1995).

Local Lymph Node Assay (LLNA)

Strong allergens induce measurable changes in the draining lymph node weights when contact sensitizers are applied to the mouse ear. Rapid proliferation and pyroninophilia increase the node weight (Kimber and Weisenberger, 1989). To increase the sensitivity and reproducibility of the LLNA, a modified assay was developed using [^{3}H]thymidine incorporation to measure proliferation.

In the LLNA, test material is administered to the ear over the course of several days. After a rest period of 5 days, mice receive a challenge dose on the ear and treated thymidine via an intravenous injection. A threefold increase in thymidine incorporation is considered a positive response.

This assay has been validated in several national and international interlaboratory validation studies (Kimber *et al.,* 1995; Chamberlain and Basketter, 1996; Scholes *et al.,* 1992). With respect to moderate or severe sensitizers, data from the LLNA correlate well with the standard guinea pig assays. Often the LLNA assay fails to predict weak or moderate sensitizers that are positive in the GPMT. However, the GPMT is an artificial system bypassing the skin barrier and may overestimate the risk of sensitization. Occasionally, strong irritants will give a strong reaction in the LLNA. Thus, substances with exclusively irritating properties could falsely be classified as sensitizers by the method. Alternatively, chemicals with both sensitizing and irritating properties could be overestimated (Montelius *et al.,* 1994).

Differences between irritant reactions and contact sensitivity can be ascertained by phenotyping cells from the lymph node (Sikorski *et al.,* 1996). Sensitizers increase the percentage of CD4+ and CD8+ cells expressing $CD62^{lo}$ and $CD44^{hi}$ (Gerberick *et al.,* 1992). Irritant reactions do not increase the number of cells in the preparation or the specific lymphocyte subsets.

The nature of the vehicle or the test material may influence the performance of the assay. For most test compounds, the LLNA uses a 4:1 acetone/olive oil vehicle (Basketter and Kimber, 1996). Other vehicles such as water or aqueous vehicles are ineffectual and often give false–negative results (Ikarashi *et al.,* 1992). When metal salts are used in the assay, special vehicles must be used. Sensitization is only observed when the compounds are applied in dimethyl sulfoxide or aqueous ethanol solution.

In Vitro Sensitization

A practical *in vitro* model has not been developed (Botham *et al.,* 1991) because sensitization depends on several defined and interconnected steps involving blood, skin cells, and lymphatics.

Limited success has been achieved with strong sensitizers. There was a correlation between *in vivo* and *in vitro* sensitization (Hauser and Katz, 1988) using *in vitro* sensitization of antigen-specific helper T cells. In the assay system,

helper T cells are reacted with hapten-modified, syngeneic Langerhans cells. Cells recovered from the assays can be rested, rechallenged with the sensitizing material, and proliferation measured by [^{3}H]thymidine incorporation. Like any assay, *in vitro* sensitization has advantages and disadvantages. One advantage is that many chemicals can be tested with a relatively short turnaround time and the endpoint can be quantified. The major disadvantage of the system is that the roles of skin metabolism and skin penetration are bypassed. Therefore, there would be false-negative reactions for sensitizers requiring metabolism and exaggerated responses to weak sensitizers.

Cutaneous Basophil Hyperreactivity (CBH)

Irritant and contact sensitization reactions are similar in chronology and appearance in the guinea pig. The presence of basophils in lesions may be the differentiating factor between sensitization and irritant reactions in guinea pigs (Robinson, 1995). Basophils are normal constituents of inflammatory reactions and are driven by immunological processes. However, basophils are not normally found in irritant reactions (Medenica and Rostenberg, 1971). Enumeration of basophils in lesions can detect strong and relatively weak sensitizers. Moreover, irritation and sensitization could be differentiated even near threshold doses (Robinson, 1995).

The CBH assay is often used as an additional endpoint in the standard Buehler Guinea Pig Sensitization Test. After the challenge phase of the BT, skin reactions are quantified and graded on a five-point scale. At the same time, punch biopsies are removed from the center of each test site, fixed, and stained with Giemsa. The number of basophils per 400 leukocytes is determined.

IMMUNE SUPPRESSION

There are two approaches to assessing the biological significance of standard study endpoints. The NTP established statistical correlations between *in vivo* and *ex vivo* immune function tests and changes in host resistance. An alternative approach uses animal infectivity and tumor models to define biologically relevant effects. Mortality is the usual endpoint measured.

Statistical Approach to Modeling Immunosuppression

Data from the NTP study showed that compounds negative for immunotoxicity in immune function assays are not likely to affect host resistance. Conversely, compounds immunotoxic in *ex vivo* assays have a 50% chance of altering host resistance (Table 2). When one host resistance assay was used for each chemical tested, the concordance between immune function and host resistance decre-

Table 2. Sensitivity, Specificity, andConcordance of Host Defense and Tumor Models in Detecting Relevant Immunosuppression

		Specificity	Sensitivity	
Challenge Agent	Tests No.	–/–	+/+	Total
L. monocytogenes	34	100	52	65
PYB6 tumor	24	100	39	54
S. pneumoniae	19	100	38	58
B16F10 tumor	19	100	40	68
P. yoelii	11	100	38	55
Influenza	9	100	17	44
Any test	46	100	68	78

Modified from *Fundamental and Applied Toxicology,* Luster *et al.,* 1992b, 21:71-82, with permission of Academic Ress.

ments increased to 78% (Luster *et al.,* 1993). Therefore, alteration in ex vivo immune function tests predicts biologically relevant immunosuppression approximately 80% of the time.

Animal Model Approach to Testing

Immunosuppression. To draw meaningful conclusions from host resistance studies, the animal model must fulfill certain criteria. The criteria are that (1) the model must simulate a relevant disease or infection with known adverse health effects, (2) the immune effector mechanism active in the disease or infection must be the same in humans and the test animal, (3) microorganisms must be administered by the natural route, (4) the concentration of the challenge agent should not overwhelm the host defense system, and (5) relative resistance or susceptibility to microorganisms and viruses is genetically determined (Bradley, 1985). Based on these criteria, the major considerations in the design of host defense or tumor models are the genetic background, the dose, and the number of animals used in the study.

The genetic background has long been known to control resistance or susceptibility to disease. In mice, influenza-induced macrophage antiviral activity is controlled by the presence of the *Mx* gene. Viruses can replicate in macrophages lacking the *Mx* gene even when high concentrations of interferon are present. In contrast, the virus does not replicate in cells carrying the *Mx* gene (Haller *et al.,* 1980).

Resistance to murine cytomegalovirus (MCMV) is carried in the H-2^k MHC

locus. Mice expressing this marker have augmented NK cell activity and are ten times more resistant to the virus than are mouse strains lacking the *H-2*^{k}. Therefore, MCMV host resistance studies use C3H/HeJ or B6C3F1 mouse strains.

The response in host defense assays depends, in part, on the dose of the organism used in the study. Clearly, a high dose of a virulent organism will overwhelm the host and kill most of the animals. In contrast, low concentrations will fail to detect changes in immunocompetence.

Two different dose regimens can be used in host resistance studies. The LD_{20}-LD_{30} is the most common dose used in host defense studies. This dose will kill 20–30% of the animals in the control group. In the second regimen, a dose slightly below the dose inducing effects in the control group (ED_0) is administered. The determination of the LD_{20} or the ED_0 is difficult and depends on the test species. In practice, extreme accuracy in administration of the dose is required (Anonymous, 1997).

Statistically significant changes in host defense are a reflection of the number of animals in the test groups treated with xenobiotics (Table 3). For example, when 15 animals in each test group are immunized with an LD_{20}-LD_{30} inoculum, 13 of 15 animals must be affected before changes are statistically significant. If the number of animals per group is increased to 30, only 18/30 must be

Table 3. Effect of Dose of Bacteria (ED) on the Number of Animals Required for a Host Defense Study

	ED_{30}		ED_{30}		ED_0	
	No. affected (n = 15)	p value*	No. affected (n = 30)	p value*	No. affected (n = 15)	p value*
Control	3/15		6/30		0115	
Test						
1	4/15	0.532	8/30	0.428	1/15	0.5 16
2	5/15	0.41 1	10/30	0.273	2/15	0.274
3	6/15	0.312	12/30	0.165	3/15	0.150
4	7/15	0.233	14/30	0.096	4/15	0.084
5	8/15	0.173	16/30	0.055	5/15	0.048**
6	9/15	0.128	18/30	0.031**		
7	10/15	0.094				
8	11/15	0.069				
9	12/15	0.051				
10	13/15	0.038**				

Modified from *International Program on Chemical Safety, Principles and methods for assessing direct immunotoxicity associated with exposure to chemicals,* Anonymous, 1995a. Geneva.
*Chi-square one tailed test.
**Statistically significant $p < 0.05$.

Table 4. Biologically Relevant Immunotoxicity Induced by Chemicals in Host Defense Models

Model	Chemical	Reference
Listeria	Δ^9-Tetrahydrocannabinol	Morahan *et al.* (1979)
Streptococcus	TCDD	White *et al.* (1986)
Cytomegalovirus	Organotin	Garssen *et al.* (1993)
Influenza virus	Benzo[a]pyrene	Munson and White (1990)
	Benzo [e] pyrene	
	Methyl isocyanate	Luster *et al.* (1986)
	TCCD	House *et al.* (1990)
	Dimethylnitrosamine	Thomas *et al.* (1985)
Trichinella	Organotin	Vos *et al.* (1990)
	Acyclovir	Stahlmann *et al.* (1992)
Plasmodium	4,4'-Thiobis (6-r-butyl-m-cresol)	Holsapple *et al.* (1988)
	Fish oil supplement	Blok *et al.* (1992)
	Styrene	Dogra *et al.* (1992)

affected to achieve statistical significance. Only a limited number of chemicals have been tested in host defense (Table 4) or tumor models (Table 5).

The dose of the infectious agents also influences the number of animals needed in host defense assays. When the subclinical dose (ED_0) is used in the assay, only 5 of 15 animals must be affected for statistical significance (Anonymous, 1997).

Data from the host resistance assays can be analyzed by Fisher's exact test. The use of this test requires several basic assumptions. First, it is assumed that death is the result of direct or indirect action of the test chemical. Second, it is assumed that death is the result of viral, bacterial, or fungal infections. Third, it is assumed that death from infection is the event of interest.

Table 5. Biologically Relevant Immunotoxicity Induced by Chemicals in Tumor Models

Model	Chemical	Reference
B16F10 melanoma	Phorbol myristate acetate	Murray *et al.* (1985)
	Gallium arsenide	Sikorski *et al.* (1989)
	4,4'-Thiobis(6-r-butyl-m-cresol)	Holsapple *et al.* (1988)
PYB6 fibrosarcoma	Aroclor	Luster *et al.* (1986)
	Dimethylbenzanthracene	Dean *et al.* (1986)
	Benzene	Rosenthal and Snyder (1987)
MAD106	Nickel chloride	Smialowicz *et al.* (1987)

Often overlooked in the statistical analyses of host defense data is the power of the results. This measurement determines the frequency that the expected result would occur. For example, if the results were expected 9 of 10 times, the power to detect would be 90%. Assuming approximately 20% mortality in the control group and three dose levels, a mortality rate of 63% in the highest dose group would yield a power of 90%.

Listeria monocytogenes Model. The immune response to intracellular listeria involves several different immune effector mechanisms. An initial response is mediated by macrophages activated by T-cell-derived IFN-γ. The activated macrophages attempt to kill the extracellular organisms by phagocytosis (Van Loveren *et al.,* 1987). After 2 or 3 days, CD8 cytotoxic cells lyse infected phagocytic cells releasing bacterial protein antigens. CD4 Th1 cells respond to the proteins when presented in context with MHC II. A cell-mediated response is elicited resulting in granuloma formation in the liver and spleen. In mice, additional IFN-γ production causes the B cells to synthesize IgG2a that opsonizes the bacteria and activates complement.

In the typical listeria challenge study, three groups of mice (12 mice per group) are injected intravenously with bacterial numbers that produce 20,50, and 80% mortality. The intracellular bacteria begin to grow in the liver and spleen within 24 to 72 hr. Mortality is observed at 5 to 14 days, depending on the dose. Several endpoints can be measured: (1) the number of microorganisms retrieved from organs relative to the controls (Reynolds and Thomson, 1973), (2) the percent mortality relative to controls, (3) histopathology caused by listeria. Lesions are characteristic lymphocyte foci with histiocytic cells. Both the severity and the duration of the lesion can be altered by immunotoxicants.

Streptococcus pneumoniae. In the early phase of infection, C3 is deposited on the bacterial surface and the alternate complement pathway is activated. Opsonization bacteria are ingested by monocytes and other phagocytic cells. During the late phase of the infection, streptococcal polysaccharide (a T-cell-independent antigen) elicits production of antibodies. IgM antibody provides protection against circulating bacteria and prevents dissemination. Tissue invasion is prevented by the production of IgG antibodies. Activation of the classical complement pathway, opsonization, and phagocytosis terminate the infection (Winkelstein, 1981).

In summary, host defense against *S. pneumoniae* requires several effector mechanisms including antibody response to a T-cell-independent antigen (Moxon, 1981), phagocytosis by monocytes and PMNs, and activation of complement (Schiffman, 1983).

Depending on whether the early or late phase of the infection is being

probed, two strains of streptococcus are used in the defense model. Smooth, encapsulated *S. pneumoniae,* which causes rapid death, is used to probe the complement activation observed early in the disease. Another strain of streptococcus is used to probe the late–phase immune response. *S. zooepidemicus* tests for antibody-mediated resistance during the late phase of the infection. *S. zooepidemicus* animals have a much longer time to death (Fugmann *et al.,* 1983).

The effect of immunotoxicants on the complement system can be defined in acute-phase response by using different strains of *S. pneumoniae.* For example, type 14 *S. pneumoniae* ATCC 6314 activates both pathways whereas *S. pneumoniae* type 25 ATCC 6325 activates only the alternative pathway.

In the assay, bacteria are usually administered intravenously in large doses at 20, 50, and 80% of the lethal dose. Deaths occurring within the first 48 hr are related to a complement defect. During the 2- to 4-day period, deaths can be attributed to decreases in phagocytosis. Defects in antibody production result in deaths at 5–8 days.

Cytomegalovirus (CMV) Model. Infection with CMV elicits a number of different immune responses. Interferon is synthesized during the first 48 hr. This is followed by a vigorous NK cell response that peaks at day 6 (Butowski *et al.,* 1986). Virus-specific cytotoxic T cells appear in large numbers by day 10. Neutralizing antibodies are also produced late in the response. A role for macrophages in CMV infection is doubtful. However, the CMV may infect macrophages that serve as reservoirs for future infections (Boos, 1980). Termination of the infection is primarily brought about by NK cells, cytotoxic T cells, and ADCC (Selgrade et al., 1982a).

Two routes of administration have been used in the model. Mice are generally immunized by the intraperitoneal or intratracheal route. Intraperitoneal administration initiates a systemic infection. The highest virus concentration is found in the salivary glands followed by the liver, spleen, and lung. When the virus is administered by the intratracheal route, the virus usually localizes in the salivary glands and death is a rare event. Generally, the viral replication is slow with peak viral loads at 15–20days. Mortality or the concentration of virus in tissue are common endpoints (Bruggeman *et al.,* 1983, 1985).

With intratracheal administration in the rat, there are species-specific differences in virus localization (Bruggeman *et al.,* 1983). In PVG rats, the highest viral load is in the salivary glands. In other rat strains such as Lewis or LN, the viral load is higher in the kidney than in the salivary gland (Bruning, 1985).

In the assay system, pathogen-free, 3-week-old CD-1 mice are the strain of choice. This strain is susceptible to the virus. Other strains are resistant. Resistance or susceptibility to CMV is genetically determined. Mouse strains carrying the $H\text{-}2^k$ haplotype are 10–20 times more resistant to the virus than are strains

carrying the *H-2*^{b} or *H-2*^{d} haplotype (Chalmers *et al.,* 1977). Resistance may be related to the fact that strains with the *H-2*^{k} haplotype respond more vigorously with an NK cell response (Bancroft *et al.,* 1981).

The CMV model is used as a surrogate for human opportunistic infections in immunosuppressed subjects and chemicals that suppress NK cell function. There is a significant correlation between immunosuppression and the appearance of CMV infections (Rubin *et al.,* 1981). CMV infections are commonly observed in patients purposefully (e.g., transplantation), accidentally (AIDS), or naturally (e.g., the elderly) immunosuppressed (Rubin, 1990). In addition, there is a correlation between chemically induced suppression of NK cells and infections with CMV.

Influenza Virus Model (A/Port Chalmers/1/72/H3N2). Host response to influenza infection is a cascade of effector mechanisms that include (1) interferons, (2) interleukins, (3) alveolar macrophages, and (4) NK cells, cytotoxic T cells, and antibody production (Burleson *et al.,* 1987). However, resistance to the virus is associated with antibody production (Vireligier, 1975) and interferon (Hoshino *et al.,* 1983). Mortality is commonly measured at 14 days. However, virus concentration in the lung may also be determined by the virus plaque assay.

In theory, mice are inoculated with the LD_{20} virus concentration. In practice, the LD_{20} is difficult to define accurately. Continued viral passage in mice increases the viral virulence. Increased virulence reduces the number of viruses necessary to achieve an LD_{20} and increases the variability around the LD_{20}. To abrogate the variability, three different viral concentrations near the 0.2 $\log_{10}$ challenge dose are usually administered (Burleson, 1995).

The model has limitations. It is a nasal instillation model with effects in the lung. Frequently, the lung acts as an autonomous immunological organ that requires no interdiction from the peripheral blood immune system. Therefore, it is conceivable that chemicals lowering systemic antibody levels will have no effect on the host defense against in the inhalation model.

Trichinella spiralis Model. *T spiralis* has a complicated life cycle. Following ingestion of the encysted helminth larvae, the larvae excyst in the acid environment of the stomach. They enter the small intestine where they mature within 3–4 days. After copulation, the gravid females penetrate the intestinal mucosa and produce offspring larvae. This event results in an inflammatory response consisting of mast cells and eosinophils. Over a 3-week period, viviparous larvae enter the lymphatic system and migrate to striated muscle. In the muscle, the larvae become encapsulated and remain viable for months or, perhaps, years. The encysted larvae evoke a vigorous T-cell-dependent inflammatory response.

The adult worms resident in the gut are expelled approximately 6 days after infection. Expulsion is mediated by a specific inflammatory response consisting of gut mast cell activation, goblet cell hyperplasia, and accumulations of eosinophils, PMNs, lymphocytes, and plasma cells. Evidence suggests that the lymphocytic response is T cell dependent (Vos *et al.,* 1983). Antibodies (IgM, IgG, and IgA) are produced and directed toward surface antigens. The IgG-coated parasites are lysed via ADCC mediated by neutrophils and eosinophils (Ruitenberg *et al.,* 1983). Complement and enzymes released from granulocytes may also play a role in destruction of the parasites in the early stages.

This model is unique in that different effector mechanisms are involved in host resistance to life cycle changes of the parasite. T cells are involved in the expulsion of parasites during the first infection. T, B cells, and eosinophils are involved in preventing parasite migration and limiting parasite reproduction (Van Loveren *et al.,* 1995).

A number of different endpoints can be measured in the trichinella model. An increased worm load in the gut and larvae in the muscle are hallmarks of immunosuppression. Histopathology can be used to compare the inflammatory responses to encysted larvae in treated and control animals. Serum antibody titers are also reduced in compromised animals (Ruitenberg *et al.,* 1983).

Malaria Host Defense Model. Resistance to this organism involves antibody production, macrophage activation, and T-cell functions (Luster *et al.,* 1988). Multiple effector cells are involved because malaria has a complicated life cycle using several different host organs and tissues. Sporozoites released from the mosquito salivary gland enter the bloodstream and ultimately infect hepatocytes. After sexual reproduction in the liver, merozoites are released and infect circulating red blood cells. The merozoites mature in the red cell becoming tropozoites. When the red blood cell ruptures, the merozoites infect other red cells. Some merozoites mature in gametocytes transmitted to a feeding mosquito. Sexual reproduction of the gametocytes occurs in the mosquito.

Several different strains of plasmodium have been used in model systems. Lethal (Py17L) and nonlethal (Py17NL) strains of *Plasmodiumyoelii* are commonly used in model mouse systems. Susceptibility to these organisms is influenced by genes within and outside of the MHC complex. C57BL/6 mice are susceptible to infection and DBA/2 mice are resistant (Sayles and Wassom, 1988).

Less frequently used in assays are *Plasmodium chabaudi* and *P. adami* that produce nonlethal infections in some strains of mice. R chabaudi is lethal for BALB/c and C3H/HeJ mice but C57BL/6, C57L, DBA/2, and B 10.A are resistant to infection (Stevenson et al., 1982).

Plasmodium berghei initiates a self-limiting disease in most mouse species. However, peak parasitemia, anemia, and other hallmarks of infection differ with

the mouse strain (Eling *et al.,* 1977). Host resistance to this strain of plasmodium is a result of T and B cells and macrophage activation (Bradley and Morahan, 1982).

Usually, 10^6 parasitized red cells are inoculated in naive hosts via intravenous or intraperitoneal routes. Blood samples are taken at days 10, 12, and 14. In most mouse strains, peak parasitemia occurs at day 12. Endpoints include the number of parasites in the blood, the number of parasitized red cells, and the number of red cells in the circulation (Dockrell and Playfair, 1983). These measurements are made manually or by flow cytometry (Whaun *et al.,* 1983).

B16F10 Melanoma. Host defense to melanoma is mediated by NK cells, macrophages, and some T cells (Parhar and Lala, 1987). Using an artificial tumor metastasis model in syngeneic C57BL/6 mice, mice are challenged with different number of tumor cells (Fidler *et al.,* 1978). Because cells are injected into the tail vein, the cells lodge in the lung, which is the first capillary bed encountered. Two endpoints are usually measured. The proliferation rate of cells in the lung can be measured by injecting fluorodeoxyuridine, which blocks *de novo* nucleotide synthesis, followed by [^{125}I]iododeoxyuridine. The latter nucleotide is incorporated into the DNA of newly divided cells (White, 1992). As a second endpoint, the number of tumor nodules per lung can be determined. Tumor cells form characteristic black nodules on a background of white or yellow lung tissue. Up to 200–250 nodules can be enumerated in each lung section.

Immune responses to the melanoma tumor are time dependent. IL-2 production and an increased NK cell cytolysis occur early in the tumor response (Makovic and Murasko, 1991). The intermediate response consists of macrophages and T cells. Macrophages release TNF that kills tumor cells and inhibits tumor growth (Loveless and Munson, 1981). T cells release lymphokines that initiate proliferation of cytotoxic T cells. Cytotoxic T cells participate in the late responses to the tumor.

PYB6 Fibrosarcoma. This model was originally developed as a screening tool for immunosuppression. The tumor produces a vigorous NK and cytotoxic T cell response (Urban *et al.,* 1982). In the assay, tumor cells are introduced subcutaneously in the thigh and observed for 42 days. Endpoints include the incidence of tumors, time to tumor development, and tumor size.

MAD106. This mammary adenocarcinoma, which is syngeneic in the Fischer 344 rat, is the only rat tumor model. The host response consists of NK and LAK cells (Barlozzari *et al.,* 1985). Following the intravenous administration of tumor cells, the time to death is usually 3 weeks.

SCID MOUSE AS AN EXPERIMENTAL MODEL

The SCID mouse is being validated to extrapolate murine data to humans. SCID mice have a genetic defect in chromosome 16 reflected in a defective DNA recombinase enzyme. The faulty recombinase prevents the expression of a T-cell receptor and an antibody repertoire.

Two different models have been developed. In one model, human peripheral blood lymphocytes are injected into C.B.17 scid/scid (SCID) mice. However, there is considerable variability in the reconstitution rate and in antibody production. In the second model, fetal human thymus and liver cells are transplanted into the kidney capsule of SCID mice (McCune *et al.,* 1988). Usually, tissue from a 15- to 20-week fetus is used for reconstitution. Liver cells are a necessary component in transplantation as the liver contains precursor thymocytes and thymic epithelial cells.

Successful engraftment is reflected by the presence of thymic architecture that is similar to that of an age-matched human thymus. There are high numbers of double-negative CD4/CD8 thymocytes under the kidney capsule. In addition, functional T cells of donor origin are found in the blood (Vandekerckhove *et al.,* 1991).

Data from the SCID mouse model must be interpreted with caution. Results should be confirmed in mice with thymuses in the anatomically correct position. It is conceivable that thymic tissue transplanted to the kidney may behave differently because of its unique location. Moreover, the SCID model only detects xenobiotic-induced damage when the thymus is the target organ.

REGULATORY POSITION AND BIOLOGICAL SIGNIFICANCE

In standard toxicology studies, there is no validated method to determine the autoimmune potential of a chemical. Regulatory agencies agree that the PLNA is useful for screening commercial candidates for T-cell activation. There is controversy, however, between the European and the US. agencies concerning the need for additional validation studies to assess PLNA's utility in predicting autoimmunity.

Chemicals are not routinely tested for their ability to induce immediate hypersensitivity reactions in animals. Agencies will request testing when (1) the test compound has structure–activity relationships to known allergens (e.g., phthalates, isocyanates) or (2) the exposed population is very large. Most companies voluntarily perform tests to determine whether a new compound is more allergic than the one that it is replacing.

There is a critical need to develop and validate rapid, cost-effective *in vivo*

or *in vitro* models that predict reactions in the lung or skin. Animal models for asthma should mimic the pathophysiology and immunopathology of the human disease in that (1) airway hyperactivity should be demonstrable either before or following antigenic challenge, (2) pulmonary function changes elicited by challenge with allergen should be consistent with reversible airway obstructive disease, and (3) the presence of allergen-specific IgE should be documented by serological assays or appropriate *in vivo* methods.

Tests for delayed hypersensitivity skin reactions are mandated by the EPA and OECD. However, Americans and Europeans differ on specific tests required for registration of chemicals. The EPA accepts data from either the BT or GPMT assay. Because methods using FCA are considered more sensitive than non-FCA sensitization protocols, the Europeans prefer that data from GPMT be submitted for registration of new compounds.

In Europe, arbitrary thresholds of ≥30% for the GPMT and >15% for the BT are used as a basis for sensitizer classification. The classification considers only the threshold and does not consider the intensity of the reaction. Moreover, the test results are not evaluated in the context of positive control data in the same assay (Basketter *et al.,* 1993). Therefore, the data are treated the same regardless of whether the testing laboratory obtains a 30% or a 100% response with the positive control. If a chemical is classified a sensitizer, it must be labeled with the phrase "may cause sensitization by skin contact" (Anonymous, 1994).

In the United States, GPMT data are expressed as the sensitization rate (number of animals with a positive reaction/number of animals tested X 100). When 9–28% of the animals show a moderate and diffuse skin reaction (Grade II), the chemical is considered a mild sensitizer. A strong sensitizer has a 65–80% sensitization rate with intense reddening and swelling. Reactions in the BT are graded differently. The sensitization incidence (number of animals showing a positive reaction/number of animals tested) and the reaction severity (sum of the test grades/number of animals tested) is determined. Slight, confluent erythema or moderate, patchy erythema (Grade I) reactions are considered sensitization unless the same response is seen in the controls.

Regulatory agencies recognize and accept data from alternate sensitization assays. The EPA accepts the MEST or the LLNA as screening assays to detect strong or moderate sensitizers. If a positive result is seen in either assay, the test substance would be designated a potent sensitizer and guinea pig tests would be unnecessary. If the LLNA or MEST does not indicate sensitization, accepted guinea pig studies must be used to confirm the results. Either the GPMT or the BT is acceptable to the agency as a confirmatory test.

Host defense and tumor models have been validated by the NTP. A major concern is that the models were validated in the mouse and most standard toxicology studies are performed in the rat. Efforts are currently under way to validate the host defense and tumor models in the rat.

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Risk Characterization

INTRODUCTION

Risk assessment is a generic term used by industrial and governmental toxicologists to describe the process that establishes qualitative and quantitative estimates of adverse effects (Scala, 1991). Included in the process are (1) hazard identification that identifies adverse effects and the assessment of the biological relevance of the effect, (2) a dose–response assessment that defines the relationship between the effect of exposure and the severity of effects, (3) exposure assessment that defines the actual dose or exposure to which humans are exposed, and (4) risk characterization that estimates the frequency and severity of adverse effects in a population because of exposure (Anonymous, 1983). Risk characterization can be formalized to yield a quantitation of the incidence and severity of adverse effects in a population. Risk reduction is the process used to reduce the likelihood of adverse effects.

THRESHOLD EFFECTS

Immunological risk assessment assumes that there is a final concentration (threshold) below which there is no effect on the immune system. Thresholds are the consequence of dynamic and complex interactions in which modest changes give rise to compensating factors that reverse the effect and/or restore biological homeostasis.

It is necessary to differentiate between the individual and population thresh-

old. Because most toxicology studies use colony derived rats or inbred rats, animal studies only define the individual threshold. The population threshold is the dose that will not affect a single individual in a mixed population with differing sensitivities. Sensitivities, in turn, depend on differences in the absorption rate, metabolic activation, rates of detoxification, age, diet, and functional reserve of the immune system.

DOSE–RESPONSE RELATIONSHIPS

No Observable Adverse Effect Level (NOAEL)

Because there is a threshold, it is possible to determine the NOAEL from standard immunotoxicology studies. To determine the NOAEL, animals are dosed over a range that includes the maximum tolerated dose (MTD), one-half the MTD, and lower doses. The primary basis for risk evaluation is the determination of the lowest observable adverse effect level (LOAEL) that causes a "critical effect." This is the lowest dose at which there is an adverse effect or an increase in the frequency or severity of the effect. In practice, the LOAEL is rarely achieved because the dose selection is faulty.

Rather than repeat studies with lower doses to determine the LOAEL, an alternate approach is used. The NOAEL is determined from the study, and can be defined in two ways. The NOAEL may be the highest exposure level without an adverse effect. Also, it may be the exposure level at which there are no biological differences in the frequency or severity of adverse effects when the treated and control populations are compared.

The NOAEL approach has been criticized for many reasons that include:

1. The dependence of the NOAEL on the background incidence of the adverse effect in the control group.
2. The size of the test group at each interval and the interval between doses are critical factors.
3. The NOAEL must be a dose level in the study.
4. It is dependent on the experimental design, particularly dose selection, and interval spacing between doses, concentration range, and alpha levels.
5. There is no control of variability in the data and a study with fewer animals often has a higher NOAEL than one with more animals.
6. Choosing an adverse or critical effect for the NOAEL is subjective and not based on scientific judgment.

The major drawback of the NOAEL approach is that it does not use all information available from the dose–response curve. It considers only one point

on the dose–response curve, neglecting the remaining points on the curve. Moreover, the variability around the NOAEL and the slope of the curve is not taken into consideration. As a consequence, the NOAEL approach cannot detect small changes over background. Poorly designed studies yield high NOAEL levels.

Benchmark Dose (BMD)

Because of the criticisms directed toward the NOAEL, the BMD was developed as an alternate approach (Crump, 1984). The BMD is a mathematically derived, dose–responsemodel that yields a statistical lower confidence level on the dose producing a predetermined level of change in a "critical" adverse response (Kimmel and Gaylor, 1988). For example, the BMD could denote a 99% statistical lower confidence limit on a dose corresponding to a 1% increase in adverse effects (Crump *et al.,* 1995).

The BMD approach overcomes all of the criticisms of the NOAEL. It uses response within the experimental range of the study and extrapolates the NOAEL to the lowest doses. The full dose response and the slope of the curve are considered in the calculations. As examples, a steep slope in the BMD denotes a threshold just below the experimental range; a shallow slope signals effects well below the range. By using confidence intervals, it considers sample size, experimental and interspecies variability and acknowledges better designed experiments. Finally, the BMD is less dependent on spacing between doses and the number of doses (Crump *et al.,* 1995).

The BMD also has a major advantage over the NOAEL approach. A single BMD for all studies can be derived regardless of route of administration or duration. Because the BMD does not involve extrapolation far beyond the experimental data, it is not dependent on the dose response model used for analyses (Crump *et al.,* 1995).

The BMD measured by mathematical models cannot be used for "low dose extrapolation" at doses that cannot be measured experimentally. Predication of low dose extrapolation will be seriously in error without incorporating mechanistic information on the adverse effect.

The first step in the determination of the BMD is the selection of the "critical effects" to be modeled. From a review of the literature, effects may be evident in high-quality studies using the same route and duration. In studies using chemicals with unknown toxicity, the toxicologist may show that some endpoints are unsuitable for modeling because of the route of exposure, inappropriate range of health effects, or the lack of a dose–response effect. After the data are culled, one or more relevant points may remain, each with a different dose–response pattern.

There are several options to the modeling of different endpoints. One option is to model all relevant responses. However, data may be difficult to interpret

from multiple dose response analyses. Alternatively, modeling only "critical effects" restricts the number of parameters to be modeled.

Effects observed at the LOAEL can be modeled. Because this effect is independent of the slope of the dose response, the calculated BMD may have a higher LOAEL but a lower BMD than an effect with a steeper dose response and a lower BMD (Crump *et al.,* 1995).

In statistical models for determining the BMD, continuous data are usually transformed into quantal formats. Then, only the presence or absence of the response is reported. There are advantages and disadvantages to the conversion of data sets. A disadvantage of data transformation is that information on the magnitude of the response is lost. An advantage is that comparisons among various responses are relatively easy if they are all quantal in nature. Also, recoding of data into quantal form may relate more directly to an adverse effect. For example, body weight changes of 10% are considered an adverse effect. When the 10% change is used to define the adverse response, the data are expressed quantally.

Although the BMD calculation is model independent, certain statistical considerations must be satisfied. There must be evidence of a dose–effect relationship in treated animals. When data are expressed quantally, it is assumed that each animal responds independently and all animals have an equal chance of responding. A different assumption is used when the data are continuous. The assumption is that each animal responds independently and the response has a normal probability distribution.

Continuous data can be converted to quantal data and expressed in several ways. Data may be expressed as differences in the means normalized by background means or absolute differences in means normalized by background standard error. It is also possible to express the data as some percentile of the population being abnormal.

There are various models for continuous or quantal data (Tables 1 and 2). In each model, the experimental data are fitted through maximum likelihood meth-

Table 1. Dose–Response Models Proposed for Estimating Benchmark Doses of Quantal Data

Model	Formula
Quantal linear regression (QLR)	$P(d) = c + (1 - c)\{1 - \exp[-q_1(d - d_0)]\}$
Quantal quadratic regression (QQR)	$P(d) = c + (1 - c)\{1 - \exp[-q_1(d - d_0)^2]\}$
Quantal polynomial regression (QPR)	$P(d) = c + (1 - c)\{1 - \exp[-q_1d \cdots q_kd^k]\}$
Quantal Weibull (QW)	$P(d) = c + (1 - c)\{1 - \exp[q_1Id^k]\}$
Log normal (LN)	$P(d) = c + (1 - c)N(a + b \log d)$

P(d) is the probability of a response at the dose, *d.* Reprinted from the *Risk Assessment Forum,* Crump *et al.,* 1995, US. Environmental Protection Agency, p. 17.

Table 2. Dose Response Models Proposed for Estimating Benchmark Doses of Continuous Data

Model	Formula
Continuous linear regression (CLR)	$m(d) = c + q_1(d - d_0)$
Continuous quadratic regression (CQR)	$m(d) = c + q_1(d - d_0)^2$
Continuous linear-quadratic regression (CLQR)	$m(d) = c + q_1 d + q_2 d^2$
Continuous polynomial regression (CPR)	$m(d) = c + q1_d + \cdots + q_k d^k$
Continuous power	$m(d) = c + q_1(d)^k$

m(d) is the mean response at the dose, *d.* Reprinted from the *Risk Assessment forum,* Crump *et al.,* 1995, US. Environmental Protection Agency, p. 17.

ods (quantal data) that estimate the probability of response or the mean response (continuous data). In the selection of a model, biological considerations may be used. For example, threshold or nonthreshold models could be selected. Quantal models QLR and QQR define a threshold dose, d_0. Doses below the threshold will not alter the response probability. In contrast, models QPR, QW, and LN do not consider a threshold dose. Hence, any dose will increase the probability of a response. The BMD is not dependent on the model and it makes no difference whether a threshold or nonthreshold model is used.

Certain data sets cannot be fitted by any of the proposed models. This is because there are altered responses in the high doses that are inconsistent with the dose–responseeffects at lower doses. There are several reasons for the high-dose effects. Most likely reasons are competing mechanisms of toxicity, saturation of metabolic and of delivery systems, or chemical interferences. Because the high-dose response is of least interest, a simple solution to the problem is to drop the high-dose data from consideration and model only the lower doses (Crump *et al.,* 1995).

For quantal data, "additional risk" and "extra risk" are additional measurements of increased response. When *P(d)* is the probability of a response at dose d and *P(0)* is the probability of the response in the absence of exposure $(d = 0)$, additional risk means the additional proportion of total animals that respond in the presence of the dose. Additional risk can be derived as

$$\text{AR } (d) = P(d) - P(0)$$

Extra risk is determined by dividing the additional risk by the proportion of animals that will not respond in the absence of exposure. In essence, extra risk is the fraction of animals responding when exposed to a dose *d* among animals that would otherwise not respond. Risk can be determined as

$$\text{ER } (d) = [P(d) - P(0)]/1 - P(0)$$

Both responses will coincide for responses that do not occur spontaneously.

When continuous data are analyzed, measurements analogous to the additional and extra risk are determined. These measurements include the difference between the mean response expected after exposure to dose *d* of a chemical and the mean response without the exposure. Mathematically, the response can be expressed as

$$|m(d) - m(0)|$$

where m(d) is the mean value of the continuous response for dose *d.* Vertical lines in the equation indicate absolute values. Another measure is the normalized difference in the mean responses divided by the background mean response:

$$|m(d) - m(0)|/|m(0)|$$

In defining the BMD, a number of decisions are made at the initiation of the analyses. Initially, the percent change is determined between 1 and 100%. Because a l–5% change is outside the range that could be determined in typical experiments, 10% is usually chosen. Ten percent represents a level that can be reliably detected in most studies.

A second decision involves selecting and calculating the lower confidence limit. The lower confidence limit is used for several reasons. First, it is not influenced by the sample size. Second, it is more stable to minor changes in the data set. Third, the lower limit can usually be defined when maximum likelihood estimates from the asymptotic distribution of the likelihood ratio statistic cannot be derived. The lower confidence limit can range from 90 to 99%. However, most governmental regulatory agencies use a one-sided 95% confidence level.

The analyses may yield BMDs derived from different studies or different endpoints with a single or multiple studies. Additional studies are needed to determine endpoint-specific reference doses (RfDs) based on multiple endpoints. However, the BMD from multiple endpoints is determined by simply taking the lowest BMD. Determining the geometric mean of multiple BMDs from a single study is also an acceptable means of determining the BMD (Crump *et al.,* 1995).

Uncertainty Factors

In the extrapolation of risk, the EPA applies uncertainty factors (UFs) and modifying factors (MFs) to the NOAEL for the most sensitive species. It is anticipated that the same factors will be applied to the BMD. The LOAEL is multiplied by factors of 10 to account for (1) variation in sensitivities within the human population, (2) extrapolation of data from animals to humans, (3) differences in acute or subchronic to chronic exposure, (4) inadequacy of the data base, and (5) a conversion from LOAEL to NOAEL.

MFs are also used in risk assessment. These factors are usually greater than zero and less than or equal to 10. The magnitude of the MF is dependent on a professional assessment of scientific uncertainties that are not considered in the UF approach (e.g., number of animals or route of administration). The usual default value is 1.

After applying the UFs and MFs to the LOAEL, it is possible to calculate the reference dose (RfD). As defined by the EPA, the RfD is "an estimate (with uncertainties over several orders of magnitude) of a daily exposure of a human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Anonymous, 1995a). The RfD can be calculated by dividing the adjusted NOAEL or LOAEL by the product of UFs X MFs.

The RfD, which is expressed in milligrams per kilogram body weight per day, takes into account the overall quality of the studies. However, different RfDs are obtained from different routes of exposure (e.g., oral versus inhalation), specific endpoints, and exposure duration.

RISK ASSESSMENT AND IMMUNOLOGICAL DATA

Most risk assessment paradigms were developed to assess health risks for cancer. Less attention has been given to modeling non-cancer-related effects. The lack of attention reflects a lack of consensus regarding the shape of the dose–response curve below the NOAEL and the diverse nature of noncancer effects (Crump *et al.,* 1995).

For the immune system, analyses are further complicated by the fact that the immune system consists of effector cells with overlapping and/or redundant function and the presence of a functional reserve. The function reserve must be exceeded before the effect is biologically relevant.

Assessment of noncancer effects by traditional risk assessment paradigms creates problems. Traditionally, risk has been determined by the NOAEL approach or the newer BMD approach. However, there is little correlation between NOAELs and BMDs (Faustman *et al.,* 1994). The lack of correlation may be related to the sensitivity of the endpoints and the type of data (e.g., quantal, continuous or categorical).

Immunological data derived from testing create problems in risk assessment using either the NOAEL or BMD approach. Most immunological data are continuous in nature. With the conventional NOAEL approach, modeling of continuous data may result in a 5% false-positive rate. The rate may be reduced to 3–4% by using the Fisher exact test in statistical analyses. Conversion of continuous data to a quantal or categorical format may increase the false-positive rate to 10% and

reduce the sensitivity of the test. This reflects the fact that each subject responds independently and all animals have an equal chance of responding.

Another problem resides in the mathematical conversion of continuous to quantal data sets. Demarcation between normal variation and adverse effects is often difficult. However, some studies have successfully used absolute changes in the mean or an abnormal percentile of the population in the conversions (Crump *et al.,* 1995).

In the BMD approach, there is the loss of sensitivity when continuous data are converted to the quantal form. For example, changes in *ex vivo* immune function assays, expressed as continuous data, may be statistically significant when there is a 15–20% difference between treated and control animals. Conversely, host defense models are expressed as categorical data (survival or tumor frequency) and require an 80% change to achieve statistical significance. This assumes a group size of 15 animals and infective doses of ED,, (Anonymous, 1995c).

Only one study has compared the NOAEL and a modified BMD for immunosuppression using the same chemical (McGrath *et al.,* 1996). In the study, the effects of TCCD on plaque-forming cells/10^6 spleen cells were modeled. Both approaches relied on a single value in studies that varied widely with the number and range of doses in many different test species. In addition, test chemical was administered by oral and parenteral routes in both males and females.

The RfD derived from the classical NOAEL approach differed by three orders of magnitude. In contrast, the modified BMD approach provided RfD estimates in the same order of magnitude (McGrath *et al.,* 1996).

RISK CHARACTERIZATION

The RfD is generally compared to the estimated exposure dose (EED) for a population. If the EED is less than the RfD, it is assumed that there are no safety issues. The margin of exposure (MOE) is occasionally used by the EPA to characterize the risk. This defines the magnitude by which the NOAEL of the critical effect exceeds the estimated exposure concentration.

The Europeans have formulated a histopathology-based (Fig. 1), risk characterization process (Anonymous, 1994). However, it is generally agreed that there is no evidence to refute or support the use of histopathology and lymphoid organs as accurate predictors of immunotoxicity or immunomodulation. Additional studies must be undertaken to define the utility of histopathology in immunotoxicity (Anonymous, 1995b).

Although conventional histopathology is an insensitive method to detect immunotoxic effects, the sensitivity of histopathology can be enhanced by detailed examination of the spleen, thymus, local and distant lymph nodes, and the lymphoid organ weights.

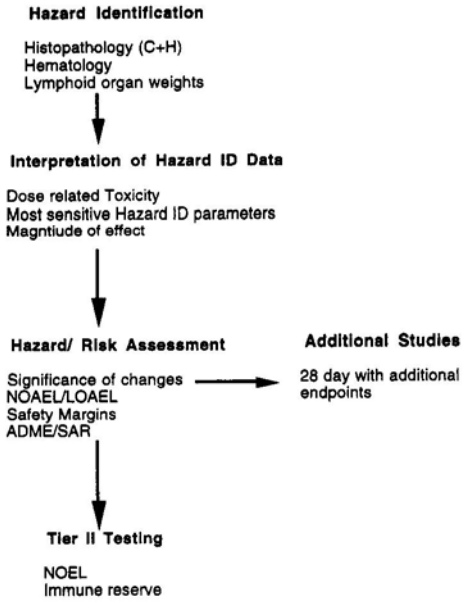

Figure 1. The European histopathology-based risk assessment paradigm. Reprinted from *Immunotoxicity: Hazard Identification and Risk Characterization,* Anonymous, 1994, ECETOC, Brussels, 21:1-35.

RISK MANAGEMENT

Risk management decisions are usually made on a "case by case" basis. The different risk factors, regulatory options, and governmental mandates are evaluated to arrive at an "adequate safety margin." This margin is formalized into the regulatory dose (RgD) (Anonymous, 1995a).

ASSESSMENT OF HUMAN HEALTH RISK

Nonoccupational Approaches

There are a number of different approaches that can be used to assess the human health risk (Table 3). A classical approach is the EPA NOAEL/safety factor risk assessment paradigm described above. The Renwick and the Lewis/ Lynch/Nikiforov approaches are also commonly used in human risk assessment.

Renwick Approach. The two elements in the extrapolation of data from animal to human can be subdivided in toxicokinetics (e.g., delivery to the site of

Table 3. Comparison of the Default Factors Used in Different Risk Assessment Paradigms

	LLN		Renwick		EPA	
	Range	Default	Range	Default	Range	Default
Interspecies						
Kinetic	(S) > 0	1	>0–4	4		10
Dynamic	(R) 0.3–3	1	>0–2.5	2.5		
Intraspecies						
Kinetic	(H) 1–5	3	>0–4	3.2		10
Dynamic			>0–2.5	3.2		
Relevance	(Q1) 0.1–1	1				
Duration	(Q1) 1–5	3				10
LOAEL/NOAEL	(43) 1–5	3				10
Database adequacy			1–10		>1–10	10
Uncertainty	(U) 1–10	1			1–10	

Modified from *Assessment Factors in Human Health Risk Assessment,* Anonymous, 1995 by Technical Report 68, ECETOC, Brussels.

toxicity) and toxicodynamics (e.g., potency at the site of toxicity). Renwick retains the tenfold factors for interspecies and interindividual variation used in other paridigms, but allows modification of these values (correction factor) when appropriate data are available (Renwick, 1993). A major advantage of the method is that data can quantitatively contribute to the tolerable intake (TI) calculation (Renwick, 1991).

Critical decisions in the process are the nature of data and the weight given to each correction factor. Useful data for defining the toxicokinetic correction factor are bioavailability, total clearance from the body, and bioaccumulation. Data from the toxicodynamic component could include *in vitro* sensitivity information and dose–effect relationships(Anonymous, 1995a). Because of an inability to distinguish between the importance of toxicokinetics and toxicodynamics, the default values are usually set at 3.2 and 3.2, respectively (Anonymous, 1995a).

The Lewis/Lynch/Nikoforov Approach. This method was originally reported in 1990 (Lewis *et al.,* 1990) as a flexible, science-based risk assessment paradigm. It separates scientific conclusions or inferences from nonscientific influences. Moreover, the evaluator estimates the most likely values for each parameter and the degree of uncertainty for each factor. This allows the method to be used for deriving ambient environmental or occupational exposure (Anonymous, 1995a). The human No Observable Adverse Effect Level is estimated from animal and laboratory data (Fig. 2).

$$NAEL_{human} = \frac{NOAEL_{animal} \times S}{R \times H \times Q1 \times Q2 \times U}$$

S = Scaling factor

R = Interspecies adjustment factor

H = Heterogeneity factor

Q1= Crtical Human Health Effect

Q2= Study duration factor

Q3= LOAEL TO NOAEL factor

U = Uncertainity factor

Figure 2. Mathematical derivation of the human No Adverse Effect Level. Reprinted from *Assessment Factors in Human Health Risk Assessment,* Anonymous, 1995b, Technical Report 68, ECETOC, Brussels.

REGULATORY POSITION ON NOAEL VERSUS BMD

A classical NOAEL safety factor approach to risk assessment has been used by the EPA for many years. The agency has embraced the RfD concept because it distinguishes between risk assessment and risk characterization while promoting consistency in the decision making process. For regulatory purposes, all doses over the RfD are considered to produce adverse effects and are unacceptable. Similarly, not all doses below the RfD will be acceptable or will be risk free (Anonymous, 1995a).

In 1991, the U.S. EPA began to move toward the BMD as a replacement for the classical NOAEL approach. Publication of draft guidelines for the Risk Assessment of Reproductive Toxicity signaled the agency's intention for a wider use of the technique. Both industry and the regulatory agencies agree that the benchmark dose has significant scientific and practical advantages over the conventional NOAEL especially in risk assessments derived from developmental toxicity studies (Foster and Auton, 1995). Additional agency initiatives have extended the BMD to neurotoxicity and immunotoxicity.

There are concerns about the more conservative BMD as a replacement for the NOAEL approach. Industry scientists believe that the NOAEL is adequate to protect human health. Moreover, there is no evidence to suggest that the NOAEL approach has failed over the last 30 years. If increased conservatism of the NOAEL is necessary or desirable, increasing the minimum acceptable sample size would be appropriate. More importantly, there is a concern

the regulators will extrapolate and model below the mathematically derived BMD.

The NOAEL/BMD approaches have not been embraced by other countries. When modeling is acceptable, the European method of choice is the Renwick modification of the NOAEL approach. As a matter of policy, other countries are reluctant to model biological data.

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References

Abbas, A. K., Lichtman, A. H., and Pober, J. S. 1994. *Cellular and Molecular Immunology,* Saunders, Philadelphia, pp. 325–228.

Abrahmsen, L. 1995. Superantigen engineering. *Current Opinion in Structural Biology* 5:464–70.

Acha-Orbea, H. 1993. Bacterial and viral superantigens: roles in autoimmunity? *Annals of the Rheumatic Diseases* 52:S6–16.

Ackermann, B., Staege, M. S., Reske-Kunz, A. B., Dienes, H. P., Meyer zum Buschenfelde, K. H., and Marker-Hermann, E. 1997. Enterobacteria-infected T cells as antigen-presenting cells for cytotoxic CD8 T cells: a contribution to the self-limitation of cellular immune reactions in reactive arthritis? *Journal of Infectious Diseases* 175:1121-7.

Adair, J. R., Athwal, D. S., Bodmer, M. W., Bright, S. M., Collins, A. M., Pulito, V. L., Rao, P. E., Reedman, R., Rothermel, A. L., and Xu, D. 1994. Humanization of the murine anti-human CD3 monoclonal antibody OKT3. *Human Antibodies and Hybridomas* 1994:41-7.

Adams, L., Franblau, S., Taintor, R., Hibbs, J., and Krahenbuhl, J. 1990. L-Arginine (ARG) dependent pathway (DP) of activated macrophages (act Mo) affects metabolic pathway of Mycobacterium leprae. *FASEB* 4:A2032.

Adelstein, S., Pritchard-Briscoe, H., Loblay, R. H., and Basten, A. 1990. Suppressor T-cell memory. *Current Topics in Microbiology & Immunology* 159:123–38.

Adkinson, N. F. 1974. Measurement of total serum IgE and allergen specific immunoglobulin E antibody, American Society for Microbiology, Washington, DC, pp. 591–602.

Adlard, B. F. F., and Smart, J. L. 1972. Adrenocortical function in rats subjected to nutritional deprivation in early life. *Journal of Endocrinology* 54:99.

Aeschlimann, A., Graf, U., and Bourgeois, P. 1991. Environmental chemicals: their clini-

cal significance in rheumatology. *Schweizerische Rundschau fur Medizin Praxis* 80:1190-6.

Afeltra, A., Russo, S., Pen, C., and Paggi, A. 1995. CD5 positive B lymphocytes in autoimmunity: possible role in Graves disease. *Rivista Europea Per Le Scienzw Mediche e Fannacologiche* 17:167-74.

Agrawal, S. 1992. Antisense oligonucleotides as antiviral agents. *Trends in Biotechnology* 10:152–8.

Ahmed, R., and Gray, D. 1996. Immunological memory and protective immunity: understanding their relation. *Science* 272:54-60.

Ahuja, S. K., Gao, J.-L., and Murphy, P. M. 1994. Chemokine receptors and molecular mimicry. *Immunology Today* 15:281–87.

Airas, L., and Jalkanen, S. 1996. CD73 mediates adhesion of B cells to follicular dendritic cells. *Blood* 88: 1755–64.

Akerblom, H. K. 1992. Which factors cause type-1 diabetes? *Nordisk Medicin* 107:204–6, 230.

Akira, S., and Kishimoto, T. 1992. IL-6 and NF-IL6 in acute-phase response and viral infection. *Immunological Reviews* 127:25–50.

Akira, S., Yoshida, K., Tanaka, T., Taga, T., and Kishimoto, T. 1995. Targeted disruption of the IL-6 related genes: gp130 and NF-IL-6. *Immunological Reviews* 148:221–53.

Akiyama, Y., Suzuki, T., Imai, F., Ishibashi, T., and Fujimaki, T. 1995. Low reactivity peripheral blood dendritic cells respond to IL-1 and GM-CSF in SLE patients. *Advances in Experimental Medicine & Biology* 378:385–7.

Alam, R. 1997. Chemokines in allergic inflammation. *Journal of Allergy & Clinical Immunology* 99:273–7.

Alam, R., and Grant, J. A. 1995. The chemokines and the histamine-releasing factors: modulation of function of basophils, mast cells and eosinophils. *Chemical Immunology* 61:148–60.

Albani, S., and Roudier, J. 1992. Molecular basis for the association between HLA DR4 and rheumatoid arthritis. From the shared epitope hypothesis to a peptidic model of rheumatoid arthritis. *Clinical Biochemistry* 25:209–12.

Albani, S., Tuckwell, J. E., Esparza, L., Carson, D. A., and Roudier, J. 1992. The susceptibility sequence to rheumatoid arthritis is a cross-reactive B cell epitope shared by the Escherichia coli heat shock protein dnaJ and the histocompatibility leukocyte antigen DRB 10401 molecule. *Journal of Clinical Investigation* 89:327–31.

Albani, S., Ravelli, A., Massa, M., De Benedetti, F., Andree, G., Roudier, J., Martini, A., and Carson, D. A. 1994. Immune responses to the Escherichia coli dnaJ heat shock protein in juvenile rheumatoid arthritis and their correlation with disease activity. *Journal of Pediatrics* 124:56 1–5.

Albright, J. W., Jiang, D., and Albright, J. F. 1997. Innate control of the early course of infection in mice inoculated with Trypanosoma masculi. *Cellular Immunology* 176:146–52.

Alcalay, J., Bucana, C., and Kripke, M. L. 1989. Effect of psoralens and ultraviolet radiation on murine dendritic epidermal cells. *Journal of Investigative Dermatology* 92:657–62.

Alcami, A., and Smith, G. L. 1995. Cytokine receptors encoded by poxviruses: a lession in cytokine biology. *Immunology Today* 16:474–8.

Alexander, J., Snoke, K., Ruppert, J., Sidney, J., Wall, M., Southwood, S., Oseroff, C., Arrhenius, T., Gaeta, F. C., Colon, S. M., et al. 1993. Functional consequences of engagement of the T cell receptor by low affinity ligands. *Journal of Immunology* 150:1-7.

Alexander, J., Ruppert, J., Page, D. M., Hedrick, S. M., Franco, A., Ishioka, G. Y., Grey, H. M., and Sette, A. 1995. Antigen analogs as therapeutic agents. *Advances in Experimental Medicine & Biology* 386:109-18.

Altmann, A., Mustelin, T., and Coggeshall, K. M. 1990. T lymphocyte activation: a biological model of signal transduction. *Critical Reviews in Immunology* 10:348–91.

Ambrose, J. D., Rajamahendran, R., Yoshiki, T., and Lee, C. Y. 1996. Anti-bull sperm monoclonal antibodies: I. Identification of major antigenic domains of bull sperm and manifestation of interspecies cross-reactivity. *Journal of Andrology* 17:567-78.

An, L. L., and Whitton, J. L. 1997. A multivalent minigene vaccine, containing B-cell, cytotoxic T-lymphocyte, and Th epitopes from several microbes induces appropriate responses in vivo and confers protection against more than one pathogen. *Journal of Virology* 71:2292-302.

Andersen, K. E., Volund, A., and Frankild, S. 1995. The guinea pip maximization test—with a multiple dose design [published erratum appears in *Acta Dermato-Venereologica* 1996 76(4):335]. *Acta Dermato-Venereologica* 75:463–9.

Anderson, C., Hehr, A., Robbins, R., Hasan, R., Athar, M., Mikthat, H., and Elmets, C. A. 1995. Metabolic requirements for induction of contact sensitivity to immunotoxic polyaromatic hydrocarbons. *Journal of Immunology* 155:3530–7.

Anderson, G. P., and Coyle, A. J. 1994. TH2 and 'TH2-like' cells in allergy and asthma: pharmacological perspectives. *Trends in Pharmacological Sciences* 15:324–32.

Anderson, J. A. 1996. Allergic reactions to food. *Critical Reviews in Food Science and Nutrition* 36:S19-S38.

Anderson, P., Caliqiuri, M., O'Brien, C., Manley, T., Ritz, J., and Schlossman, S. F. 1990. Fc receptor type III (CD16) is included in the zeta NK cell receptor complex expressed by human natural killer cells. *Proceedings of the National Academy of Sciences, USA* 87:2274–8.

Anderson, S. J., and Perlmutter, R. M. 1995. A signaling pathway governing early thymocyte maturation. *Immunology Today* 16:99–105.

Andre, A. 1967. Current concepts on serum complement. *Transfusion* 10:231-45.

Angulo, I., Rodriguez, R., Garcia, B., Medina, M., Navarro, J., and Subiza, J. L. 1995. Involvement of nitric oxide in bone marrow-derived natural suppressor activity. Its dependence on IFN-gamma. *Journal of Immunology* 155:15-26.

Anonymous. 1983. *Risk Assessment in the Federal Government.* Managing the Process. National Academy Press, Washington, DC.

Anonymous. 1989. Lung defensive system: damages and treatment. XIth World Congress on Sarcoidosis. Milan, September 6–11, 1987. Proceedings. *Respiration* 55:41–96.

Anonymous. 1994. *Immunotoxicity and Risk Characterization, European Centre for Ecotoxicology and Toxicology of Chemicals,* Brussels, pp. 1–35.

Anonymous, 1995a. *Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals,* WHO, Geneva.

Anonymous. 1995b. *Assessment Factors in Human Health Risk Assessment,* ECETOC, Brussels.

Anonymous. 1995~. Immunotoxicity testing and risk assessment: Summary of a 1994 workshop. *Food and Chemical Toxicology* 33:887–94.

Anonymous. 1996. *International Collaborative Immunotoxicity Study. Vol. 2: General Conclusions from the Collaborative Studies of Azathioprine and Cyclosporin A,* Inter-Organization Programme for the Sound Management of Chemicals (IOMC), Geneva, pp. 1–14.

Anonymous. 1997. *Scientific Principles and Methods for Assessing Allergic Hypersensitization Associated with Exposure to Chemicals,* WHO, Geneva.

Antia, R., Levin, B., and Williamson, P. 1991. A quantitative model suggests immune memory involves the colocalization of B and Th cells. *Journal of Theoretical Biology* 153:371–84.

Anton, I. M., Sune, C., Meloen, R. H., Borras-Cuesta, F., and Enjuanes, L. 1995. A transmissible gastroenteritis coronavirus nucleoprotein epitope elicits T helper cells that collaborate in the in vitro antibody synthesis to the three major structural viral proteins. *Virology* 212:746–51.

Appella, E., Padlan, E. A., and Hunt, D. F. 1995. Analysis of the structure of naturally processed peptides bound by class I and class II major histocompatibility complex molecules. *EXS* 73:105-19.

Aragane, Y., Schwarz, A., Luger, T. A., Ariizumi, K., Takashima, A., and Schwarz, T. 1997. Ultraviolet light suppresses IFN-gamma-induced IL-7 gene expression in murine keratinocytes by interfering with IFN regulatory factors. *Journal of Immunology* 158:5393–9.

Armandola, E. A., Nijenhuis, M., Momburg, F., and Hammerling, G. J. 1995. TAP polymorphism does not influence transport of peptide variants in mice and humans. European *Journal of Immunology* 25:2170–6.

Armant, M., Delespesse, G., and Sarfati, M. 1995. IL-2 and IL-7 but not IL-12 protect natural killer cells from death by apoptosis and up-regulate bc1-2 expression. *Immunology* 85:331–7.

Ameson, L. S., and Miller, J. 1995. Efficient endosomal localization of major histocompatibility complex class 11-invariant chain complexes requires multimerization of the invariant chain targeting sequence. *Journal of Cell Biology* 129:1217-28.

Ashby, J., Basketter, D. A., Paton, D., and Kimber, I. 1995. Structure activity relationships in skin sensitization using the murine local lymph node assay. *Toxicology* 103: 177–94.

Ashworth, J., Kahan, M. C., and Breathnach, S. M. 1989. Flow cytometric analysis and sorting of HLA-DR+CDl + Langerhans cells. *British Journal of Dermatology* 121:11-8.

Askenase, P. 1973. Cutaneous basophil hypersensitivity in contact sensitized guinea pigs. *Journal of Experimental Medicine* 138:1144–55.

Askenase, P. W. 1977. Role of basophils, mast cells, and vasoamines in hypersensitivity reactions with a delayed time course. *Progress in Allergy* 23:199–320.

Atassi, M. Z. *Advances in Experimental Medicine and Biology* 98:9.

Attur, M. G., Patel, R. N., Abramson, S. B., and Amin, A. R. 1997. Interleukin-17 up-regulation of nitric oxide production in human osteoarthritis cartilage. *Arthriris & Rheumatism* 40: 1050–3.

Auger, I., Toussirot, E., and Roudier, J. 1997. Molecular mechanisms involved in the association of HLA-DR4 and rheumatoid arthritis. *Immunologic Research* 16:121-6.

Austen, K. F. 1971. Inborn and acquired abnormalities of the complement system of man. *Johns Hopkins Medical Journal* 12857–74.

Badolato, R., and Oppenheim, J. J. 1996. Role of cytokines, acute-phase proteins, and chemokines in the progression of rheumatoid arthritis. *Seminars in Arthritis & Rheumatism* 26:526–38.

Baggiolini, M., and Wymann, M. P. 1990. Turning on the respiratory burst. *Trends in Biochemical Sciences* 15:69–72.

Baggiolini, M., Loetscher, P., and Moser, B. 1995. Interleukin-8 and the chemokine family. *International Journal of Immunopharmacology* 17: 103–8.

Baggiolini, M., Dewald, B., and Moser, B. 1997. Human chemokines: an update. *Annual Review of Immunology* 15:675–705.

Baker, C. C., Yamada, A. H., Faist, E., and Kupper, T. S. 1987. Interleukin-1 and T cell function following injury. *Journal of Burn Care & Rehabilitation* 8:503-8.

Bakker, A. B., van der Burg, S. H., Huijbens, R. J., Drijfhout, J. W., Melief, C. J., Adema, G. J., and Figdor, C. G. 1997. Analogues of CTL epitopes with improved MHC class-I binding capacity elicit anti-melanoma CTL recognizing the wild-type epitope. *International Journal of Cancer* 70:302–9.

Balasa, B., and Sarvetnick, N. 1996. The paradoxical effects of interleukin 10 in the immunoregulation of autoimmune diabetes. *Journal of Autoimmunity* 9:283–6.

Baldari, C. T., Heguy, A., Di Somma, M. M., and Telford, J. L. 1994. Inhibition of T-cell antigen receptor signaling by overexpression of p120GAP. *Cell Growth & Differentiation* 5:95–8.

Baldo, B. A. 1984. Milk allergies. *Australian Journal of Dairy Technology* 39:120.

Balk, S. P., Polischuk, J. E., Probert, C., Stevens, C., Ebert, E., She, J., Terhorst, C., and Blumberg, R. S. 1995. Composition of TCR-CD3 complex in human intestinal intraepithelial lymphocytes: lack of Fc epsilon RI gamma chain. *International Immunology* 7:1237–41.

Ballow, M., and Weiping, W. 1995. Retinoic-acid-induced enhancing effects of immunoglobulin synthesis by human B cells. *International Archives of Allergy and Immunology* 107:370–1.

Ballow, M., Wang, W., and Xiang, S. 1996. Modulation of B-cell immunoglobulin synthesis by retinoic acid. *Clinical Immunology and Immunopathology* 80:S73–81.

Banas, B., Wenzel, U., Stahl, R. A., and Schlondorff, D. 1996. Role of chemokines in glomerular diseases. *Kidney & Blood Pressure Research* 19:270–80.

Bancroft, G. J. 1993. The role of natural killer cells in innate resistance to infection. *Current Opinion in Immunology* 5:503-10.

Bancroft, G. J., Shellman, G. R., and Chalmer, J. 1981. Genetic influences on the augmentation of natural killer (NK) cells during murine cytomegalovirus infection. *Journal of Immunology* 126:988–94.

Bancroft, G. J., Kelly, J. P., Kaye, P. M., McDonald, V., and Cross, C. E. 1994. Pathways of macrophage activation and innate immunity. *Immunology Letters* 43:67–70.

Bang, H., Mollenhauer, J., Schulmeister, A., Nager, C., van Eden, W., Wand-Wurttenberger, A., Kaufmann, S. H., and Brune, K. 1994. Isolation and characterization of a

cartilage-specific membrane antigen (CH65): comparison with cytokeratins and heat-shock proteins. *Immunology* 81:322–9.

Banos, D. M., Lopez, S., Arias, C. F., and Esquivel, F. R. 1997. Identification of a T-helper cell epitope on the rotavirus VP6 protein. *Journal of Virology* 71: 419–26.

Barbara, J. A., Van ostade, X., and Lopez, A. 1996. Tumour necrosis factor-alpha (TNF-alpha): the good, the bad and potentially very effective. *Immunology & Cell Biology* 74:434–43.

Barclay, A. N., Brady, R. L., Davis, S. J., and Lange, G. 1993. CD4 and the immunoglobulin superfamily. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* 342:7–12.

Barinaga, M. 1992. Getting some "backbone": how MHC binds peptides. *Science* 257:880–1.

Barlozzari, T., Leonhardt, J., Wiltrout, R. H., Herberman, R. B., and Reynolds, C. W. 1985. Direct evidence for the LGL in the inhibition of experimental tumor metastases. *Journal of Immunology* 134:2783–9.

Bamaba, V. 1996. Viruses, hidden self-epitopes and autoimmunity. *Immunological Reviews* 152:47–66.

Barnes, P. J., and Liew, F. Y. 1995. Nitric oxide and the inflammatory response. *Immunology Today* 16:128–30.

Bamett, D., and Howden, W. E. H. 1986. Partial characterization of an allergenic glycoprotein from peanut (Arachis hypogaea). *Biochimica et Biophysica Acta* 882:97–104.

Barratt, M. D., and Langowski, J. J. 1997. Validation and subsequent development of the DEREK skin sensitization rule base by analysis of the BgVV list of contact allergens. *Toxicology in Vitro* 8:837-9.

Barratt, M. D., Basketter, D. A., Chamberlain, M., Payne, M. P., Adams, G. D., and Langowski, J. J. 1994. Development of an expert system rule base for identifying contact allergens. *Toxicology in Vitro* 8:837-9.

Barry, T. S., and Haynes, B. F. 1992. In vivo models of human lymphopoiesis and autoimmunity in severe combined immune deficient mice. *Journal of Clinical Immunology* 12:3 11-24.

Bartholomew, W. R., and Shanahan, T. C. 1990. Complement components and receptors: deficiencies and disease associations. *Immunology Series* 52:33–51.

Bartsch, H., and Montesano, R. 1984. Relevance of nitrosamines to human cancer. *Carcinogenesis* 5:1381–93.

Bas, S., Cunningham, T., Kvien, T. K., Glennas, A., Melby, K., and Vischer, T. L. 1996. Synovial fluid and serum antibodies against Chlamydia in different forms of arthritis: intra-articular IgA production in Chlamydia sexually acquired reactive arthritis. *British Journal of Rheumatology* 35:548-52.

Basketter, D. A., and Gerberick, G. F. 1996. An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitizers. *Contact Dermatitis* 35:146-51.

Basketter, D. A., and Kimber, I. 1996. Olive oil: suitability for use as a vehicle in the local lymph node assay. *Contact Dermatitis* 35: 190–1.

Basketter, D. A., and Scholes, E. W. 1992. Comparison of the local lymph node assay with the guinea-pig maximization test for the detection of a range of contact allergens. *Food and Chemical Toxicology* 30:65–9.

Basketter, D. A., Scholes, E. W., Cumberbatch, M., Evans, C. D., and Kimber, I. 1992a. Sulphanilic acid: divergent results in the guinea pig maximization test and the local lymph node assay. *Contact Dermatitis* 27:209–13.

Basketter, D. A., Roberts, D. W., Cronin, M., and Scholes, E. W. 1992b. The value of the local lymph node assay in quantitative structure–activity investigations. *Contact Dermatitis* 27: 137–42.

Basketter, D. A., Selbie, E., Scholes, E. W., Lees, D., Kimber, I., and Botham, P. A. 1993. Results with OECD recommended positive control sensitizers in the maximization, Buehler and local lymph node assays. *Food and Chemical Toxicology* 31:63–7.

Basketter, D. A., Scholes, E. W., and Kimber, I. 1994. The performance of the local lymph node assay with chemicals identified as contact allergens in the human maximization test. *Food and Chemical Toxicology* 32:543-7.

Basketter, D. A., Scholes, E. W., Chamberlain, M., and Barratt, M. D. 1995. An alternative strategy to the use of guinea pigs for the identification of skin sensitization hazard. *Food and Chemical Toxicology* 33:1051-6.

Basketter, D. A., Scholes, E. W., Fielding, I., Dearman, R. J., Hilton, J., and Kimber, I. 1996a. Dichloronitrobenzene: a reappraisal of its skin sensitization potential. *Contact Dermatitis* 34:55-8.

Basketter, D. A., Gerberick, G. F., Kimber, I., and Loveless, S. E. 1996b. The local lymph node assay: a viable alternative to currently accepted skin sensitization tests. *Food and Chemical Toxicology* 34:985–97.

Bauer, K. 1986. Antigenic structure of human Cl esterase inhibitor, factor H and factor B. *Experimental & Clinical Immunogenetics* 3:224–9.

Beaven, M. A., and Baumgartner, R. A. 1996. Downstream signals initiated in mast cells by Fc epsilon RI and other receptors. *Current Opinion in Immunology* 8:766–72.

Beaven, M. A., and Metzger, H. 1993. Signal transduction by Fc receptors: the Fc epsilon RI case. *Immunology Today* 14:222–6.

Beck, S., Belich, M., Gruneberg, U., Jackson, A., Kelly, A., Sanseau, P., Sanderson, F., Trowsdale, J., and Van Ham, M. 1996. Organization of class II genes and molecules. *DNA Sequence* 7:21–3.

Becker, F., and Federlin, K. 1991. Autoantigens in type-I diabetes. *Immunitat und Infektion* 19:167–9.

Beckman, E. M., and Brenner, M. B. 1995. MHC class I-like, class 11-like and CD1 molecules: distinct roles in immunity. *Immunology Today* 16:349–52.

Beckman, E. M., Melian, A., Behar, S. M., Sieling, P. A., Chatterjee, D., Furlong, S. T., Matsumoto, R., Rosat, J. P., Modlin, R. L., and Porcelli, S. A. 1996. CD1c restricts responses of mycobacteria-specific T cells. Evidence for antigen presentation by a second member of the human CD1 family. *Journal of Immunology* 157:2795–803.

Bedford, P. A., and Knight, S. C. 1989. The effect of retinoids on dendritic cell function. *Clinical & Experimental Immunology* 75:48 1–6.

Bedin, C., Brazillet, M. P., Texier, B., and Charreire, J. 1992. T cell mapping of one

epitope from thyroglobulin inducing experimental autoimmune thyroiditis (EAT). *International Reviews of Immunology* 9:125-33.

Bell, S., Brand, K., and Meurer, M. 1992. Toxic oil syndrome — an example of an exogenously-induced autoimmune disease. *Hautarzt* 43:339–43.

Bell, S. C., Carter, S. D., May, C., and Bennett, D. 1995. Antibodies to heat shock proteins in dogs with rheumatoid arthritis and systemic lupus erythematosus. *British Veterinary Journal* 151:271–9.

Bendelac, A. 1995. CD1: presenting unusual antigens to unusual T lymphocytes. *Science* 269: 185–6.

Beninga, J., Kalbacher, H., and Mach, M. 1996. Analysis of T helper cell response to glycoprotein H (gpUL75) of human cytomegalovirus: evidence for strain-specific T cell determinants. *Journal of Infectious Diseases* 173:1051–61.

Benjamin, D., Berzofsky, J., and East, I. 1984. The antigenic structure of proteins: a reappraisal. *Annual Review of Immunology* 2:67.

Benne, C. A., Benaissa-Trouw, B., van Strijp, J. A., Kraaijeveld, C. A., and van Iwaarden, J. F. 1997. Surfactant protein A, but not surfactant protein D, is an opsonin for influenza A virus phagocytosis by rat alveolar macrophages. *European Journal of Immunology* 27:886-90.

Benson, R. W., and Roberts, D. W. 1982. Evaluation of the immune response to type III pneumococcal polysaccharide as a means to evaluate T-independent immune function in the rat. *Journal of Toxicology Environmental Health* 10:859–65.

Berek, C. 1992. The development of B cells and the B cell repertoire in the microenvironment of the germinal center. *Immunology Reviews* 126:5-19.

Berek, C., and Milstein, C. 1987. Mutational drift and repertoire shift in the maturation of the immune response. *Immunology Reviews* 96:23–41.

Berek, C., and Ziegner, M. 1993. The maturation of the immune response. *Immunology Today* 14:400–4.

Berg, T. L. O., and Johansson, S. G. O. 1974. Allergy diagnosis with the radioallergosorbent test. A comparison with the results of skin and provocation sites in an unselected group of children with asthma and hay fever. *Journal* of *Allergy and Clinical Immunology* 54:207–21.

Berger, M., Gaither, I. A., and Frank, M. M. 1981. Complement receptors. *Clinical Immunology Reviews* 1:471-545.

Bergmann, C. C., Yao, Q., Ho, C. K., and Buckwold, S. 1996. Flanking residues alter antigenicity and immunogenicity of multi-unit CTL epitopes *Journal of Immunology* 151:3242–3249.

Bemhisel-Broadbent, J. 1995. Allergenic cross-reactivity of foods and characterization of food allergens and extracts. *Annals of Allergy, Asthma, & Immunology* 75:295–303.

Bemstein, D. I., Patterson, R., and Zeiss, C. R. 1982. Clinical and immunological evaluation of trimellitic anhydride and phthalic anhydride-exposed workers using a questionnaire and comparative analysis of enzyme linked immunosorbent and radioimmunoassays. *Journal of Allergy and Clinical Immunology* 69:311-8.

Bernstein, M., Day, J. H., and Welsh, A. 1982. Double blind food challenge in the diagnosis of food sensitivity in the adult. *Journal of Allergy and Clinical Immunology* 70:205–11.

Betts, R. F., and Hanshaw, J. B. 1977. Cytomegalovirus in the compromised host. *Annual Review of Medicine* 28:103–10.

Beug, H., Van Kirchbach, A., Doderlein, G., Conscience, J.-F., and Graf, T. 1979. Chicken hematopoietic cells transformed by seven strains of defective avian leukemia viruses display three distinct phenotypes of differentiation. *Cell* 18:375–90.

Beutler, B., and Grau, G. E. 1993. Tumor necrosis factor in the pathogenesis of infectious diseases. *Critical Care Medicine* 21:S423-35.

Beverley, P. C. 1990a. Human T-cell memory. *Current Topics in Microbiology & Immunology* 159:111-22.

Beverley, P. C. 1990b. Is T-cell memory maintained by crossreactive stimulation? *Immunology Today* 11:203–5.

Beverley, P. C. 1996. Generation of T-cell memory. *Current Opinion in Immunology* 8:327–30.

Bhardwaj, N., Friedman, S. M., Cole, B. C., and Nisanian, A. J. 1992. Dendritic cells are potent antigen-presenting cells for microbial superantigens. *Journal of Experimental Medicine* 175:267-73.

Bhardwaj, N., Seder, R. A., Reddy, A., and Feldman, M. V. 1996. IL-12 in conjunction with dendritic cells enhances antiviral CD8+ CTL responses in vitro. *Journal of Clinical Investigation* 98:715-22.

Bieber, T. 1986. The Langerhans cell: outpost of the immune system in the epidermis. *Hautarzt* 37:424–31.

Bindslev-Jenson, C., Hansen, T. K., Norgaard, A., Vestergaard, H., and Lars, K. 1994. *New Controversial Techniques in the Diagnosis of Food Hypersensitivity,* Huber, Toronto, pp. 468–74.

Binstat, B. A., Brumbaugh, K. M., and Leibson, P. J. 1997. Signal transduction by human NK MHC-recognizing receptors. *Immunological Reviews* 155:197-203.

Birk, O. S., and Cohen, I. R. 1993. T-cell autoimmunity in type 1 diabetes mellitus. *Current Opinion in Immunology* 5:903–9.

Birkelund, S., Larsen, B., Holm, A., Lundemose, A. G., and Christiansen, G. 1994. Characterization of a linear epitope on Chlamydia trachomatis serovar L2 DnaK-like protein. *Infection and Immunity* 62:2051-7.

Biron, C. A., and Gazzinelli, R. T. 1995. Effects of IL-12 on immune responses to microbial infections: a key mediator in regulating disease outcome. *Current Opinion in Immunology* 7:485–96.

Biron, C. A., and Orange, J. S. 1995. IL-12 in acute viral infectious disease. *Research in Immunology* 146:590–600.

Biron, C. A., Bryon, K. S., and Sullivan, J. L. 1989. Severe herpes virus infection in an adolescent without NK cells. *New England Journal of Medicine* 320: 1731-3.

Bjorkman, P. J., and Burmeister, W. P. 1994. Structures of two classes of MHC molecules elucidated: crucial differences and similarities. *Current Opinion in Structural Biology* 4:852-6.

Black, L. E., Farrelly, J. G., Cavagnaro, J. A., Ahn, C.-H., DeGeorge, J. J., Taylor, A. S., DeFelice, A. F., and Jordan, A. 1994. Regulatory considerations of oligonucleotide drugs: updated recommendations for pharmacology and toxicology testing. *Antisense Research and Development* 4:299–301.

Blackman, M. A., and Woodland, D. L. 1996. Role of the T cell receptor alpha-chain in superantigen recognition. *Immunologic Research* 15:98–113.

Blaser, K. 1996. Allergen dose dependent cytokine production regulates specific IgE and IgG antibody production. *Advances in Experimental Medicine and Biology* 409:295–303.

Blauvelt, A., Katz, S. I., and Udey, M. C. 1995. Human Langerhans cells express E-cadherin. *Journal of Investigative Toxicology* 194:293-6.

Blazek, I., and Mathe, G. 1983. Interleukins and immunosuppressive factors: a regulatory system? *Biomedicine and Pharmacotherapy* 37:258–65.

Bleecker, E. R. 1985. Airways reactivity and asthma: significance and treatment. *Journal of Allergy* 75:21–4.

Bliss, J., Van Cleave, V., Murray, K., Wiencis, A., Ketchum, M., Maylor, R., Haire, T., Resmini, C., Abbas, A. K., and Wolf, S. F. 1996. IL-12, as an adjuvant, promotes a T helper 1 cell, but does not suppress a T helper 2 cell recall response. *Journal of Immunology* 156:887-94.

Bloemena, E., Van Oers, M. H., Weinreich, S., and Schellekens, P. T. 1988. Cyclosporin A and prednisolone do not inhibit the expression of high-affinity receptors for interleukin 2. *Clinical and Experimental Immunology* 71:308-13.

Blok, W. L., Vogels, M. T., Curfs, J. H., Eling, W. M., and Buurman, W. A. a. V. D. M., J. W. 1992. Dietary fish-oil supplementation in experimental gram negative infection and in cerebral malaria in mice. *Journal of Infectious Diseases* 165:898–903.

Blumberg, R. S., Gerdes, D., Chott, A., Porcelli, S. A., and Balk, S. P. 1995. Structure and function of the CD1 family of MHC-like cell surface proteins. *Immunological Reviews* 147:5-29.

Blythe, S. A., and Lemanske, R. F., Jr. 1988. Pulmonary late-phase allergic reactions. *Pediatric Pulmonology* 4:173-80.

Bock, S. A., and Sampson, H. A. 1994. Food allergy in infancy. *Pediatric Clinics ofNorth America* 41:1047–67.

Boehm, U., Klamp, T., Groot, M., and Howard, J. C. 1997. Cellular responses to interferon-gamma. *Annual Review of Immunology* 15:749–95.

Boehme, S. A., and Lenardo, M. J. 1993. Ligand-induced apoptosis of mature T lymphocytes (propriocidal regulation) occurs at distinct stages of the cell cycle. *Leukemia* 7:S45-9.

Bohm, S. K., Grady, E. F., and Bunnett, N. W. 1997. Regulatory mechanisms that modulate signaling by G-protein-coupled receptors. *Biochemical Journal* 322:1-18.

Boman, H. G. 1996. Peptide antibiotics: Holy grail or heretic grails of innate immunity? *Scandinavian Journal of Immunology* 43:475–82.

Boniface, J. J., Lyons, D. S., Wettstein, D. A., Allbritton, N. L., and Davis, M. M. 1996. Evidence for a conformational change in a class II major histocompatibility complex molecule occurring in the same pH range where antigen binding is enhanced. *Journal of Experimental Medicine* 183:119-26.

Bonnefoy, J. Y., Lecoanet-Henchoz, S., Aubry, J. P., Gauchat, J. F., and Graber, P. 1995a. CD23 and B-cell activation. *Current Opinion in Immunology* 7:355–9.

Bonnefoy, J. Y., Gauchat, J. F., Life, P., Graber, P., Aubry, J. P., and Lecoanet-Henchoz, S. 1995b. Regulation of IgE synthesis by CD23/CD21 interaction. *International Archives of Allergy and Immunology* 107:40–2.

Bonnefoy, J. Y., Gauchat, J. F., Life, P., Graber, P., Mazzei, G., and Aubry, J. P. 1996. Pairs of surface molecules involved in human IgE regulation: CD23–CD21 and CD40–CD40L. *European Respiratory Journal* Supplement 22:63s-66s.

Bonnetblanc, J. M., and Roux, J. 1979. Betablockers and skin. *Annales de Dermatologie et de Venereologie* 106:927–30.

Booss, J. 1980. Establishment of cytomegalovirus infection in mice: role of a macrophage-enriched subpopulation. *Journal* of *Infectious Disease* 141:466–72.

Borg, H., Fernlund, P., and Sundkvist, G. 1997. Measurement of antibodies against glutamic acid decarboxylase 65 (GADA): two new 125I assays compared with [^{35}S]GAD 65-ligand binding assay. *Clinical Chemistry* 43:779–85.

Bos, G. M., Majoor, G. D., Willighagen, R. G., and van Breda Vriesman, P. J. 1989. Chronic cyclosporine induced autoimmune disease in the rat: a new experimental model for scleroderma. *Journal* of *Investigative Dermatology* 93:610–5.

Botham, P. A. 1992. Classification of chemicals as sensitizers based on new test methods. *Toxicology Letters* 64–65:165–71.

Botham, P. A., Hext, P. M., Rattray, N. J., Walsh, S. T., and Woodcock, D. R. 1988. Sensitization of guinea pigs: inhalation exposure to low molecular weight compounds. *Toxicology Letters* 41:41.

Botham, P. A., Basketter, D. A., Maurer, T., Mueller, D., Potokar, M., and Bontinck, W. J. 1991. Skin sensitization — a critical review of predictive test methods in animals and man. *Food and Chemical Toxicology* 29:275–86.

Bottazzo, G. F., Bosi, E., Bonifacio, E., Mirakian, R., Todd, I., and Pujol-Borrell, R. 1989. Pathogenesis of type I (insulin-dependent) diabetes: possible mechanisms of autoimmune damage. *British Medical Bulletin* 45:37–57.

Bottazzo, G. F., Genovese, S., Bosi, E., Dean, B. M., Christie, M. R., and Bonifacio, E. 1991. Novel considerations on the antibody/autoantigen system in type I (insulin-dependent) diabetes mellitus. *Annals of Medicine* 23:453–61.

Boussiquet-Leroux, C., Durand-Cavagna, G., Herlin, K., and Holder, D. 1995. Evaluation of lymphocyte proliferation by immunohistochemistry in the local lymph node assay. *Journal* of *Applied Toxicology* 15:465–75.

Boyd, R. L., and Hugo, P. 1991. Toward an integrated view of thymopoiesis. *Immunology Today* 12:71–9.

Bradley, S. G. 1985. *Immunologic Mechanisms of Host Resistance to Bacteria and Parasites,* Raven Press, New York, pp. 45–53.

Bradley, S. G., and Morahan, P. S. 1982. Approaches to assessing host resistance. *Environmental Health* 43:61–9.

Brady, R. L., and Barclay, A. N. 1996. The structure of CD4. *Current Topics in Microbiology & Immunology* 205:1-18.

Brady, R. L., Lange, G., and Barclay, A. N. 1993. Structural studies of CD4: crystal structure of domains 3 and 4 and their implication for the overall receptor structure. *Biochemical Society Transactions* 21:958-63.

Brai, M., Misiano, G., Maringhini, S., Cutaja, I., and Hauptmann, G. 1988. Combined homozygous factor H and heterozygous C2 deficiency in an Italian family. *Journal of Clinical Immunology* 8:50-6.

Braley-Mullen, H. 1975. Secondary IgG responses to type III pneumococcal polysaccharide. I. Kinetics and antigen requirements. *Journal of Immunology* 115:1194.

Branellec, D., and Chouaib, S. 1991. TNF: antitumoral agent at the border lines of immunity and inflammation. *Pathologie Biologie* 39:230–9.

Braun, J., Grolms, M., Distler, A., and Sieper, J. 1994. The specific antibacterial proliferation of reactive arthritis synovial T cells is not due to their higher proportion of CD45RO+ cells compared to peripheral blood. *Journal of Rheumatology* 21:1702-7.

Bremell, T., Abdelnour, A., and Tarkowski, A. 1992. Histopathological and serological progression of experimental Staphylococcus aureus arthritis. *Infection & Immunity* 60:2976–85.

Brennan, F. M., and Feldmann, M. 1996. Cytokines in autoimmunity. *Current Opinion in Immunology* 8:872–7.

Brennan, M., Davison, P. E., and Paulus, H. 1985. Preparation of bispecific antibodies by chemical recombination of monoclonal G1 fragments. *Science* 289:81.

Brenner, B. G., Gryllis, C., and Wainberg, M. A. 1991. Role of antibody dependent cellular cytotoxicity and lymphokine activated killer cells in AIDS and related diseases. *Journal of Leukocyte Biology* 50:628–40.

Breslow, E., Daniel, R., Ohba, R., and Tate, S. 1986. Inhibition of ubiquitin-dependent proteolysis by non-ubiquitinatable proteins. *Journal of Biological Chemistry* 261 :6530–5.

Briatico-Vangosa, G., Braun, C. L. J., Cookman, G., Hoffman, T., Kimber, I., Loveless, S. E., Morrow, T., Pauluhn, J., Sorensen, T., and Neissen, H. J. 1994. Respiratory allergy: hazard identification and risk assessment. *Fundamental and Applied Toxicology* 23:145–58.

Broliden, K., Von Gegerfelt, A., Persson, C., Horal, P., Svennerholm, B., Wahren, B., Bjorling, E., and Vahlne, A. 1996. Identification of cross-reactive antigenic target regions for HIV type 1-specific antibody-dependent cellular cytotoxicity. *AIDS Research & Human Retroviruses* 12:1699-702.

Bromberg, J. S. 1995. IL-10 immunosuppression in transplantation. *Current Opinion in Immunology* 7:639–43.

Bronaugh, R. L., Roberts, C. D., and McCoy, J. L. 1994. Dose-response relationship in skin sensitization. *Food and Chemical Toxicology* 32:113-7.

Brouckaert, P., Libert, C., Everaerdt, B., Takahashi, N., Cauwels, A., and Fiers, W. 1993. Tumor necrosis factor, its receptors and the connection with interleukin 1 and interleukin 6. *Immunobiology* 187:317–29.

Brouhard, B. H. 1992. Cytokines and the pathogenesis of insulin-dependent diabetes mellitus. *Cleveland Clinic Journal* of *Medicine* 59:629–33.

Brown, D. L. 1975. Complement and coagulation. *British Journal of Haematology* 30:377-82.

Brown, M. A., and Hural, J. 1997. Functions of IL-4 and control of its expression. *Critical Reviews in Immunology* 17:1-32.

Brown, M. G., Scalzo, A. A., Matsumato, K., and Yokoyama, W. M. 1997. The natural killer cell gene complex: a genetic basis for understanding natural killer cell function and innate immunity. *Immunological Reviews* 155:53-65.

Brown, Z., Robson, R. L., and Westwick, J. 1996. Regulation and expression of chemokines: potential role in glomerulonephritis. *Journal of Leukocyte Biology* 59:75–80.

Brudzynski, K. 1993. Insulitis-caused redistribution of heat-shock protein HSP60 inside beta-cells correlates with induction of HSP60 autoantibodies. *Diabetes* 42:908–13.

Bruggeman, C. A., Debie, W. M. H., Grauls, G., Majoor, G., and Boven, C. P. A. 1983. Infection of laboratory rats with a new cytomegalovirus-like virus. *Archives of Virology* 76: 189–99.

Bruggeman, C. A., Meijer, H., Bosman, F., and Boven, C. P. A. 1985. Biology of rat cytomegalovirus infection. *Intervirology* 24:1-9.

Brummer, E., and Stevens, D. A. 1994. Macrophage stimulating factor induction of enhanced macrophage anticryptococcal activity: synergy with fluconazole for killing. *Journal of Infectious Diseases* 1870: 173–9.

Bruning, J. H. 1985. Cytomegalovirus infections in renal transplant patients. Study in a rat model. Thesis, Utrecht University, Utrecht.

Buchanan, J. R., Burka, L. T., and Relnick, R. L. 1997. Purpose and guidelines for toxokinetic studies within the National Toxicology Program. *Environmental Health Perspectives* 105:468–71.

Buehler, E. V. 1965. Delayed contact hypersensitivity in the guinea pig. *Archives of Dermatology* 91:171-77.

Buehler, E. V. 1996. Nonspecific hypersensitivity: false-positive responses with the use of Freund's complete adjuvant. *Contact Dermatitis* 34:111-4.

Buehler, E. V., and Ritz, H. L. 1985. *Methods and Approaches for Assessment of Contact Hypersensitivity,* Raven Press, New York, pp. 123–31.

Buelens, C., Willems, F., Pierard, G., Delvaux, A., Velu, T., and Goldman, M. 1995. IL-10 inhibits the primary allogeneic T cell response to human peripheral blood dendritic cells. *Advances in Experimental Medicine & Biology* 378:363–5.

Burkholder, P. M., and Littleton, C. E. 1971. Immunobiology of complement. *Pathobiology Annual* 1:215–39.

Burleson, G. R. 1995. Influenza virus host resistance model for assessment of immunotoxicity, immunostimulation and antiviral compounds. In *Methods in Immunotoxicology,* Volume 2 (G. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 181–202.

Burleson, G. R., and Keyes, L. L. 1989. Natural killer cell activity in Fischer 344 rat lungs as a method to assess pulmonary immunocompetence: immunosuppression by phosgene inhalation. *Immunopharmacology and Immunotoxicology* 11:421-43.

Burleson, G. R., Fuller, L. B., Manache, M. G., and Graham, J. A. 1987. Poly(I):poly(C)-enhanced alveolar and peritoneal macrophage phagocytosis: quantification of a new method utilizing fluorescent beads. *Proceedings of the Society for Experimental Biology and Medicine* 184:468–74.

Burmester, G. R., Daser, A., Kamradt, T., Krause, A., Mitchison, N. A., Sieper, J., and Wolf, N. 1995. Immunology of reactive arthritides. *Annual Review of Immunology* 13:229-50.

Burrell, R., Flaherty, D., and Sauers, L. J. 1992. *Toxicology of the Immune System: A Human Approach,* Van Nostrand–Reinhold, New York.

Busch, R., Vturina, I. Y., Drexler, J., Momburg, F., and Hammerling, G. J. 1995. Poor loading of major histocompatibility complex class II molecules with endogenously synthesized short peptides in the absence of invariant chain. *European Journal of Immunology* 25:48–53.

Busse, W. W., Nagata, M., and Sedgwick, J. B. 1996. Characteristics of airway eosinophils. *European Respiratory Journal* Supplement 22: 132s–135s.

Butcher, B., Jones, R. N., and O'Neil, C. E. 1977. Longitudinal studies of workers employed in the manufacture of toluene diisocyanate. *American Review of Respiratory Disease* 116:411–21.

Butcher, B., O'Neil, C. E., Reed, N. A., and Salvaggio, J. E. 1980. Radioallergosorbent testing of toluene diisocyanate-reactive individuals using p-tolyl diisocyanate antigen. *Journal of Allergy and Clinical Immunology* 66:213–6.

Butowski, J. F., Woda, B. A., and Welsh, R. M. 1986. Pathogenesis of murine cytomegalovirus infection in natural killer cell depleted mice. *Journal of Virology* 52:119-28.

Cahill, R. N. P., Frost, H., and Trnka, Z. 1976. Antipolysaccharide antibodies of restricted heterogeneity secreted by a single lymph node. *Journal of Experimental Medicine* 143:870–88.

Calvanico, N. J. 1993. The humoral immune response in autoimmunity. *Dermatologic Clinics* 11:379–89.

Campbell, I. L., and Hamson, L. C. 1990a. A new view of the beta cell as an antigen-presenting cell and immunogenic target. *Journal of Autoimmunity* 3:53-62.

Campbell, I. L., and Hamson, L. C. 1990b. Molecular pathology of type 1 diabetes. *Molecular Biology & Medicine* 7:299-309.

Capron, A., Dessaint, J. P., Capron, M., Joseph, M., Ameisen, J. C., and Tonnel, A. B. 1986. From parasites to allergy: a second receptor for IgE. *Immunology Today* 7:15-8.

Careless, D. J., and Inman, R. D. 1995. Etiopathogenesis of reactive arthritis and ankylosing spondylitis. *Current Opinion in Rheumatology* 7:290–8.

Carini, C., and Essex, M. 1994. Interleukin 2-independent interleukin 7 activity enhances cytotoxic immune response of HIV- 1-infected individuals. *AIDS Research & Human Retroviruses* 10:121-30.

Carmenes, R. S., Freije, J. P., Molina, M. M., and Martin, J. M. 1989. Predict7, a program for protein structure prediction. *Biochemical and Biophysical Research Communications* 159:687–74.

Carotenuto, P., Pontesilli, O., Cambier, J. C., and Hayward, A. R. 1986. Desferoxamine blocks IL-2 receptor expression on human T lymphocytes. *Journal of Immunology* 136:2342–7.

Carulli, G. 1996. Applications of flow cytometry in the study of human neutrophil biology and pathology. *Hematopathology and Molecular Hematology* 10:39–61.

Casali, P., Borzini, P., Pioltelli, P., Invernizzi, F., and Zanussi, C. 1978. Acquired C1-inhibitor deficiency in essential cryoglobulinemia and macrocryoglobulinemia. *Acta Haematologica* 59:277–84.

Castano, L., and Eisenbarth, G. S. 1990. Type-I diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annual Review of Immunology* 8:647–79.

Castro, R. F., and Perlman, S. 1996. Differential antigen recognition by T cells from the spleen and central nervous system of coronavirus-infected mice. *Virology* 222:247–51.

Cello, J., Strannegard, O., and Svennerholm, B. 1996. A study of the cellular immune response to enteroviruses in humans: identification of cross-reactive T cell epitopes on the structural proteins of enteroviruses. *Journal of General Virology* 77:2097–108.

Cerottini, J. C., and MacDonald, H. R. 1989. The cellular basis of T-cell memory. *Annual Review of Immunology* 7:77–89.

Chalmers, J. E., MacKenzie, J. S., and Stanley, N. F. 1977. Resistance to murine cytomegalovirus linked to the major histocompatibility complex of the mouse. *Journal of General Virology* 37:107-14.

Chamberlain, M., and Basketter, D. A. 1996. The local lymph node assay: status of validation. *Food and Chemical Toxicology* 34:999–1002.

Chan, E., Fossati, G., Giuliani, P., Lucietto, P., Zaliani, A., Coates, A. R., and Mascagni, P. 1995. Sequence and structural homologies between M. tuberculosis chaperonin 10 and the MHC class I/II peptide binding cleft. *Biochemical & Biophysical Research Communications* 211:14-20.

Chan, H., Tse, J., and van Oostdam, J. 1987. A rabbit model of hypersensitivity to plicatic acid, the agent for red cedar asthma. *J. Allergy and Clinical Immunology* 79:762-767.

Chang, C. H., Furue, M., and Tamaki, K. 1995. B7–1 expression of Langerhans cells is up-regulated by proinflammatory cytokines, and is down-regulated by interferon-gamma or by interleukin-10. *European Journal of Immunology* 25:394–8.

Chatenoud, L., and Bach, J. F. 1990. Monoclonal antibodies to CD3 as immunosuppressants. *Seminars in Immunology* 2:437–47.

Chehimi, J., and Trinchieri, G. 1994. Interleukin-12: a bridge between innate resistance and adaptive immunity with a role in infection and acquired immunodeficiency. *Journal* of *Clinical Immunology* 14:149-61.

Chen, W., Qin, H., Chesebro, B., and Cheever, M. A. 1996. Identification of a gag-encoded cytotoxic T-lymphocyte epitope from FBL-3 leukemia shared by Friend, Moloney, and Rauscher murine leukemia virus-induced tumors. *Journal of Virology* 70:7773–82.

Chevailler, A., Arlaud, G., Ponard, D., Pernollet, M., Carrere, F., Renier, G., Drouet, M., Hurez, D., and Gardais, J. 1996. C-1-inhibitor binding monoclonal immunoglobulins in three patients with acquired angioneurotic edema. *Journal of Allergy & Clinical Immunology* 97:998-1008.

Chevrier, D., Giral, M., Braud, V., Bourbigot, B., Muller, J. Y., Bignon, J. D., and Solollou, J. P. 1995. Effects of MHC encoded TAP1 and TAP2 gene polymorphism and matching kidney graft rejection. *Transplantation* 60:292–6.

Chicz, R. M., Urban, R. G., Lane, W. S., Gorga, J. C., Stem, L. J., Vignali, D. A., and Strominger, J. L. 1992. Predominant naturally processed peptides bound to HLA-DR1 are derived from MHC-related molecules and are heterogeneous in size. *Nature* 358:764–8.

Chihara, J. 1996. The roles of adhesion molecules, cytokines and chemokines in relation to eosinophil activation in allergic inflammation. *Rinsho Byori—Japanese Journal of Clinical Pathology* 44:1147-51.

Christen, S., Hagen, T. M., Shigenaga, M. K., and Ames, B. N. 1996. *Chronic Infection and Inflammation Lead to Mutation and Cancer,* Oxford University Press, London, pp. 1–81.

Christensen, N. D., Dillner, J., Eklund, C., Carter, J. J., Wipf, G. C., Reed, C. A., Cladel, N. M., and Galloway, D. A. 1996. Surface conformational and linear epitopes on HPV-16 and HPV-18 L1 virus-like particles as defined by monoclonal antibodies. *Virology* 223:174-84.

Ciccone, E., Grossi, C. E., and Velardi, A. 1996. Opposing functions of activatory T-cell

receptors and inhibitory NK-cell receptors on cytotoxic T cells. *Immunology Today* 17:450-3.

Ciechanover, A. 1994. The ubiquitin–proteasomeproteolytic pathway. *Cell* 79:13–21.

Ciechanover, A., Gonen, H., Elias, S., and Mayer, A. 1990. Degradation of proteins by the ubiquitin-mediated proteolytic pathway. *New Biologist* 2:227–34.

Citro, M., Gadaleta, G., Gamba Ansaldi, S., Molinatti, M., Salvetti, E., and Bruni, B. 1983. The HLA system and insulin-dependent diabetes mellitus. A review and personal studies. *Annali dell Ospedale Maria Vittoria di Torino* 26:108-42.

Clark, I. A., and Cowden, W. B. 1992. Roles of TNF in malaria and other parasitic infections. *Immunology Series* 56:365–407.

Clarke, A. G., and Kendall, M. D. 1989. Histological changes in the thymus during mouse pregnancy. *Thymus* 14:65–74.

Clarke, D. J., Jacq, A., and Holland, I. B. 1996. A novel DnaJ-like protein in Escherichia coli inserts into the cytoplasmic membrane with a type III topology. *Molecular Microbiology* 20:1273-86.

Clore, G. M., and Gronenborn, A. M. 1995. Three-dimensional structures of alpha and beta chemokines. *FASEB Journal* 9:57-62.

Clottens, F. L., Breyssens, A., De Raeve, H., Demedts, M., and Nemery, B. 1996. Assessment of the ear swelling test and the local lymph node assay in hamsters. *Journal of Pharmacological & Toxicological Methods* 35:167-72.

Coffman, R. L., and Cohn, M. 1977. The class of surface immunoglobulin on virgin and memory B lymphocytes. *Journal of Immunology* 118:1806–15.

Coffman, R. L., O'Hara, J., Bond, M. W., Carty, J., Zlotnik, A., and Paul, W. E. 1986. B-cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide activated B cells. *Journal of Immunology* 136:4538–41.

Cohen, I. R., and Mor, F. 1993. On the regulation of EAE. *International Reviews of Immunology* 9:243–9.

Cohen, J. 1997. Exploiting the HIV–chemokine nexus. *Science* 275:1261–3.

Cohen, M. C., and Cohen, S. 1996. Cytokine function: a study in biologic diversity. *American Journal of Clinical Pathology* 105:589–98.

Collado, A., Gratacos, J., Ebringer, A., Rashid, T., Marti, A., Sanmarti, R., and Munoz-Gomez, J. 1994. Serum IgA anti-Klebsiella antibodies in ankylosing spondylitis patients from Catalonia. *Scandinavian Journal of Rheumatology* 23:119-23.

Collee, J.-H., Le Moael, M. A., and Truffa-Bachi, P. 1990. Immunological memory. *Critical Reviews in Immunology* 10:259–84.

Colomb, M. G., Villiers, C. L., Villiers, M. B., Gabert, F. M., Santoro, L., and Rey-Millet, C. A. 1996. The role of antigen-bound C3b in antigen processing. *Research in Immunology* 147:75–82.

Conrad, D. H., Campbell, K. A., Bartlett, W. C., Squire, C. M., and Dierks, S. E. 1994. Structure and function of the low affinity IgE receptor. *Advances in Experimental Medicine & Biology* 347:17–30.

Conti, P. 1991. Interleukin-1 (IL-1) and interleukin-1 receptor antagonist (IL-1ra). *Annales de Medecine Interne* 142:521-5.

Cook, D. N. 1996. The role of MIP-1 alpha in inflammation and hematopoiesis. *Journal of Leukocyte Biology* 59:61–6.

Coombs, R. R., and McLaughlan, P. 1984. Allergenicity of food proteins and its possible modification. *Annals* of *Allergy* 53:592-6.

Coonrod, J. D., and Yoneda, K. 1983. Detection and partial characterization of antibacterial factor(s) in alveolar lining material of rats. *Journal of Clinical Investigation* 71:129-41.

Cooper, D. S. 1984. Antithyroid drugs. *New England Journal of Medicine* 311:1353–62.

Cooper, M. D., and Lawton, A. R. 1972. *Contemporary Topics in Immunology* (M. G. Hanna, ed.), Plenum Press, New York, pp. 49–68.

Cooper, N. R. 1973. Activation of the complement system. *Contemporary Topics in Molecular Immunology* 2:155-83.

Cooper, N. R. 1985. The classical complement pathway: activation and regulation of the first complement component. *Advances in Immunology* 37:151–216.

Cooper, N. R., Moore, M. D., and Nemerow, G. R. 1988. Immunobiology of CR2, the B lymphocyte receptor for Epstein–Barr virus and the C3d complement fragment. *Annual Review* of *Immunology* 6:85-113.

Corbett, J. A., and McDaniel, M. L. 1992. Does nitric oxide mediate autoimmune destruction of beta-cells? Possible therapeutic interventions in IDDM. *Diabetes* 41:897-903.

Costello, R., Brailly, H., Mallet, F., Mawas, C., and Olive, D. 1993. Interleukin-7 is a potent co-stimulus of the adhesion pathway involving CD2 and CD28 molecules. *Immunology* 80:451-7.

Craddock, C. G. 1978. Corticosteroid-induced lymphopenia, immunosuppression, and body defense. *Annals of Internal Medicine* 88:564-6.

Craig, S. W., and Cebra, J. J. 1971. Peyer's patches: an enriched source of precursors for IgA producing immunocytes in the rat. *Journal of Experimental Medicine* 134:188–200.

Creamer, P., Edmonds, J., Sullivan, J., and Matthews, S. 1992. Measurement of HLA class I expression in ankylosing spondylitis. *Annals of the Rheumatic Diseases* 51:1138-42.

Crespo, P., Schuebel, K. E., Ostrom, A. A., Gutkind, J. S., and Bustello, X. R. 1997. Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by Vav proto-oncogene product. *Nature* 385:169–72.

Cresswell, P. 1996. Invariant chain structure and MHC class II function. *Cell* 84:505-7.

Croft, M. 1994. Activation of naive, memory and effector T cells. *Current Opinion in Immunology* 6:43 1–7.

Cronin, M. T., and Basketter, D. A. 1994. Multivariate QSAR analysis of a skin sensitization database. *SAR and QSAR in Environmental Research* 2:159-79.

Crump, K. 1984. A new method for determining allowable daily intakes. *Fundamental and Applied Toxicology* 4:854-7 1.

Crump, K., B., A., and Faustman, E. 1995. *The Use of the Benchmark Dose Approach in Health Risk Assessment,* U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC.

Crutcher, J. M., Stevenson, M. M., Sedegah, M., and Hoffman, S. L. 1995. Interleukin-12 and malaria. *Research in Immunology* 146:552-9.

Cumberbatch, M., Hope, J. C., Dearman, R. J., Hopkins, S. J., and Kimber, I. 1995.

Migration of interleukin-6 producing Langerhans cells to draining lymph nodes following skin sensitization. *Advances in Experimental Medicine & Biology* 378:531-3.

Cunningham, A. J., and Szenberg, A., 1968. Further Improvements in the plaque technique for detecting single antibody forming cells. *Immunology* 14:599-600.

Cunningham-Rundles, C. 1994. Clinical and immunological studies of common variable immunodeficiency. *Current Opinion in Pediatrics* 6:676–81.

Cuvelier, C. A., De Wever, N., Mielants, H., De Vos, M., Veys, E. M., and Roels, H. 1992. Expression of T cell receptors alpha beta and gamma delta in the ileal mucosa of patients with Crohn's disease and with spondylarthropathy. *Clinical & Experimental Immunology* 90:275–9.

Daeron, M. 1995. Intracytoplasmic sequences involved in the biological properties of low-affinity receptors for IgG expressed by murine macrophages. *Brazilian Journal of Medical & Biological Research* 28:263–74.

Daher, K. A., Selsted, M. E., and Lehrer, R. I. 1986. Direct inactivation of viruses by human granulocyte defensins. *Journal of Virology* 60: 1068–74.

Dai, W. J., Kohler, G., and Brombacher, F. 1997. Both innate and acquired immunity to Listeria monocytogenes infection are increased in IL-10 deficient mice. *Journal of Immunology* 158:2259-67.

D'Alessio, D. J. 1992. A case–controlstudy of group B coxsackievirus immunoglobulin M antibody prevalence and HLA-DR antigens in newly diagnosed cases of insulin-dependent diabetes mellitus. *American Journal of Epidemiology* 135:1331-8.

Dalgleish, A. G. 1995. The immune response to HIV: potential for immunotherapy. *Immunology Today* 16:356–7.

Damle, N. K., and Bradley, E. C. 1988. Interleukin 2-activated killer lymphocytes in man. *Year in Immunology* 31:138-43.

Daneshmend, T. K. 1984. The neurotoxicity of dapsone. *Adverse Drug Reactions & Acute Poisoning Reviews* 3:43–58.

Davies, J. M. 1997. Molecular mimicry: can epitope mimicry induce autoimmune disease? *Immunology and Cell Biology* 75:113–26.

Davies, R. J., Butcher, B., O'Neil, C. E., and Salvaggio, J. E. 1977. The in vivo effect of toluene diisocyanate on lymphocyte cyclic adenosine monophosphate production by isoproterenol, prostaglandin and histamine. *Journal of Allergy and Clinical Immunology* 60:223-9.

Davis, D. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., and Wood, W. B. 1973. *Microbiology,* 2nd ed., Harper and Row, New York.

Dawkins, R. L., Garlepp, M. J., McDonald, B. L., Williamson, J., Zilko, P. J., and Carrano, J. 1981. Myasthenia gravis and D-penicillamine, *Journal of Rheumatology Supplement* 7:169-74.

Day, N. K. 1986. Deficiencies in regulator proteins. 2. Factor I and H. *Progress in Allergy* 39:335-8.

Dayan, C. M., Elsegood, C. M., and Maile, R. 1997. FasL expression on epithelial cells: the Botazzo–Feldman hypothesis revisited. *Immunology Today* 18:203.

Dean, J. H., Ward, E. C., Murray, M. J., Lauer, L. D., and House, R. V. 1986. Immunosuppression following 7,12-dimethylbenz-a-anthracene exposure in B6C3F1 mice: II Altered cell-mediated immunity and tumor resistance. *International Journal of Immunopharmacology* 8:189-98.

Dearman, J., and Kimber, I. 1992. Divergent immune responses to respiratory and contact chemical allergens: antibodies elicited by phthalic anhydride and oxazolone. *Clinical and Experimental Allergy* 22.

Dearman, R. J., Basketter, D. A., and Kimber, I. 1995. Differential cytokine production following chronic exposure of mice to chemical and respiratory allergens. *Immunology* 86:545–50.

de Boer, B. A., Kruize, Y. C., Rotmans, P. J., and Yazdanbakhsh, M. 1997. Interleukin-12 suppresses immunoglobulin E production but enhances immunoglobulin G4 production by human peripheral blood mononuclear cells. *Infection and Immunity* 65:1122–5.

De Carli, M., D'Elios, M. M., Zancuoghi, G., Romagnani, S., and Del Prete, G. 1994. Human Th1 and Th2 cells: functional properties, regulation of development and role in autoimmunity. *Autoimmunity* 18:301–8.

de Crouy-Chanel, A., Kohiyama, M., and Richarme, G. 1995. A novel function of Escherichia coli chaperone DnaJ. Protein-disulfide isomerase. *Journal of Biological Chemistry* 270:22669–72.

de Graeff-Meeder, E. R., Rijkers, G. T., Voorhorst-Ogink, M. M., Kuis, W., van der Zee, R., van Eden, W., and Zegers, B. J. 1993. Antibodies to human HSP60 in patients with juvenile chronic arthritis, diabetes mellitus, and cystic fibrosis. *Pediatric Research* 34:424–8.

De Lau, W. B., Van Loon, A. E., Heije, K., Valerio, D., and Bast, B. J. 1989. Production of hybridomas based on HAT(s)-neomycin(r) double mutants. *Journal of Immunological Methods* 117:1-8.

De Lau, W. B., Heije, K., Neefjes, J. J., Oosterwegel, M., Rosemuller, E., and Bast, B. J. 1991. Absence of preferential homologous H/L chain association in hybridomas. *Journal of Immunology* 146:906–13.

Delepresse, G., Sarfati, M., Wu, C. Y., Fournier, S., and Letellier, M. 1992. The low affinity receptor for IgE. *Immunological Reviews* 125:77–97.

DeLibero, G. 1997. Sentinel function of broadly reactive human γδ T cells. *Immunology Today* 22:22–5.

del Pozo, M. A., Sanchez-Mateos, P., and Sanchez-Madrid, F. 1996. Cellular polarization induced by chemokines: a mechanism for leukocyte recruitment? *Immunology Today* 17:127–31.

de Martino, M., Peruzzi, M., de Luca, M., Amato, A. G., Galli, L., Lega, L., Azzari, C., and Vierucci, A. 1993. Fish allergy in children. *Annals of Allergy* 71:159–65.

Denis, M. 1991. Involvement of cytokines in determining resistance and acquired immunity in murine tuberculosis. *Journal of Leukocyte Biology* 50:495–501.

Deo, Y. M., Graziano, R. F., Repp, R., and van de Winkel, J. G. J. 1997. Clinical significance of IgG Fc receptors and FcγR-directed immunotherapies. *Immunology Today* 18:127–33.

Descotes, J. 1990. *Drug Induced Immune Diseases,* Elsevier Science, Amsterdam, pp. 28–46.

Descotes, J., and Verdier, F. 1995. Popliteal lymph node assay. In *Methods in Immunotoxicology,* Volume 1 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 189–96.

de Waal, E. J., Rademakers, L. H., Schuurman, H. J., and van Loveren, H. 1993. Ultra

structure of interdigitating cells in the rat thymus during cyclosporin A treatment. *Advances in Experimental Medicine & Biology* 329:153-7.

de Weck, A. L. 1977. HLA and allergy. *Monographs in Allergy* 11:3–18.

de Weck, A. L. 1984. Pathophysiologic mechanisms of allergic and pseudo-allergic reactions to foods, food additives and drugs. *Annals of Allergy* 53:583-6.

Dezutter-Dambuyant, C. 1994. Current data on epidermal Langerhans cells. *Pathologie Biologie* 42:767–74.

Dietert, R. R., Hotchkiss, J. H., Austic, R. E., and Sung, Y.-J. 1997. Production of reactive nitrogen intermediates by macrophages. In *Methods in Immunotoxicology,* Volume 2 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, *pp. 99–117.*

Dillman, J. B. 1988. Toxicity of monoclonal antibodies in the treatment of cancer. *Seminars in Oncology Nursing* 4:107–11.

Dinarello, C. A. 1992. The role of interleukin-1 in host responses to infectious diseases. *Infectious Agents & Disease* 1:227-36.

Dinauer, M. C., and Orkin, S. H. 1992. Chronic granulomatous disease. *Annual Review of Medicine* 43:117–24.

Ding, A. H., Nathan, C. F., and Stuehr, D. J. 1988. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. *Journal of Immunology* 141:2407-12.

Dixon, F. J. 1982. Murine SLE models of autoimmune disease. *Hospital Practice* 17:63–73.

Djeu, J. Y. 1990. Role of tumor necrosis factor and colony-stimulating factors in phagocyte function against Candida albicans. *Diagnostic Microbiology and Infectious Disease* 13:383–6.

Dobersen, M. J. 1982. Humoral autoimmunity in insulin-dependent (type I) diabetes mellitus. A current assessment. *Survey of Immunologic Research* 1:329–38.

Dobersen, M. J. 1985. Humoral autoimmune aspects of insulin-dependent (type I) diabetes mellitus. *Concepts in Immunopathology* 2:47–64.

Dockrell, H. M., and Playfair, J. H. L. 1983. Killing of bloodstage murine malaria parasites by hydrogen peroxide. *Infection and Immunity* 39:456–9.

Dogra, R. K., Chandra, K., Chandra, S., Gupta, S., Khanna, S., Snvastava, S. N., Shukla, L. J., Katiyar, J. C., and Shanker, R. 1992. Host resistance assays as predictive models in styrene immunomodulation. *International Journal of Immunopharmacology* 14:1003–9.

Doherty, P. 1995. Immunity to viruses. *The Immunologist* 3:231–4.

Doherty, P. C. 1996. Cytotoxic T cell effector and memory function in viral immunity. *Current Topics in Microbiology and Immunology* 206:1-14.

Donahoe, R. M., Bueso-Ramos, C., Donahoe, F., Madden, J. J., Falek, A., Nicholson, J. K., and Bokos, P. 1987. Mechanistic implications of the findings that opiates and other drugs of abuse moderate T-cell surface receptors and antigenic markers. *Annals of the New York Academy of Sciences* 496:711-21.

Drapier, J. C. 1995. NO, ambivalent effector of non-specific immunity and of inflammation. *Comptes Rendus des Seances de la Societe de Biologie et des See Filiales* 189:1081-96.

Drapier, J.-C., and Hibbs, J. B., Jr. 1988. Differentiation of murine macrophages to express nonspecific cytotoxicity for tumor cells results in L-arginine dependent inhibition of mitochondrial iron-sulfur enzymes in the macrophage effector cell. *Journal of Immunology* 140:2826–38.

Drees, C. 1991. Aseptic meningitis and Muromonab-CD3 therapy. DCIP 25: 138.

Dreyfus, D. H., Kelleher, C. A., Jones, J. F., and Gelfand, E. W. 1996. Epstein–Barrvirus infection of T cells: implications for altered T-lymphocyte activation, repertoire development and autoimmunity. *Immunological Reviews* 152:89–110.

D'Souza, M. P., and Harden, V. A. 1996. Chemokines and HIV-1 second receptors. Confluence of two fields generates optimism in AIDS research. *Nature Medicine* 2:1293-300.

Dubois, E. L. 1975. Serologic abnormalities in spontaneous and drug-induced systemic lupus erythematosus. *Journal of Rheumatology* 2:204–14.

Duchrnann, R., May, E., Ackermann, B., Goergen, B., Meyer zum Buschenfelde, K. H., and Marker-Hermann, E. 1996. HLA-B27-restricted cytotoxic T lymphocyte responses to arthritogenic enterobacteria or self-antigens are dominated by closely related TCRBV gene segments. A study in patients with reactive arthritis. *Scandinavian Journal of Immunology* 43:101-8.

Dudzinski, W. 1977. Autoimmunological phenomena in diabetes, particularly of the juvenile type. *Polski Tygodnik Lekarski* 32:1525-7.

Dugas, B., Mossalayi, M. D., Damais, C., and Kolb, J. P. 1995. Nitric oxide production by human monocytes: evidence for a role of CD23. *Immunology Today* 16:574-80.

Dunn, B. J., Rusch, G. M., Siglin, J. C., and Blaszeak, D. L. 1990. Variability of a mouse ear swelling test (MEST) in predicting weak and moderate contact sensitizers. *Fundamental and Applied Toxicology* 15:242-8.

Dunon, D., Piali, L., and Imhof, B. A. 1996. To stick or not to stick: the new leukocyte homing paradigm. *Current Opinion in Cell Biology* 8:714-23.

Durham, S. R. 1990. Late asthmatic responses. *Respiratory Medicine* 84:263–8.

Durie, F. H., Foy, T. M., Masters, S. R., Laman, J. D., and Noelle, R. J. 1994. The role of CD40 in the regulation of humoral and cell mediated immunity. *Immunology Today* 15:406-10.

Duschl, A., Muller, T., and Sebald, W. 1995. Antagonistic mutant proteins of interleukin-4. *Behring Institute Mitteilungen* 1995:87–94.

Ebner, C., Hirschwehr, R., Bauer, L., Breiteneder, H., Valenta, R., Hoffmann, K., Krebitz, M., Kraft, D., and Scheiner, 0. 1996. Identification of allergens in apple, pear, celery, carrot and potato: cross-reactivity with pollen allergens. *Monographs in Allergy* 32:73–7.

Edelbaum, D., Mohamadzadeh, M., Bergstresser, P. R., Sugamura, K., and Takashima, A. 1995. Interleukin (IL)-15 promotes the growth of murine epidermal gamma delta T cells by a mechanism involving the beta- and gamma c-chains of the IL-2 receptor. *Journal of Investigative Dermatology* 105:837–43.

Edwards, S. W. 1995. Cell signaling by integrins and immunoglobulin receptors in primed neutrophils. *Trends in Biochemical Sciences* 20:362–7.

Eisenbarth, G. S. 1986. Type I diabetes mellitus. A chronic autoimmune disease. *New England Journal of Medicine* 314:1360-8.

Elies, R., Ferrari, I., Wallukat, G., Lesbesgue, D., Chiale, P., Elizari, M., Rosenbaum, M.,

Hoebeke, J., and Levin, M. J. 1996. Structural and functional analysis of the B cell epitopes recognized by anti receptor autoantibodies in patients with Chagas' disease. *Journal of Immunology* 157:4203–311.

Eling, W., vob Zon, A., and Jerusalem, C. 1977. The course of Plasmodium berghei infection in six different mouse strains. *Zeitschrift fuer Parasitologie* 54:29–45.

Elliott, M. J., Feldmann, M., and Maini, R. N. 1995. TNF alpha blockade in rheumatoid arthritis: rationale, clinical outcomes and mechanisms of action. *International Journal of Immunopharmacology* 17:141–5.

Ellis, J., Chain, B. M., Davies, D. H., Ibrahim, M. A., Katz, D. R., Kaye, P. M., and Lightstone, E. 1991. Antigen presentation by dendritic cells provides optimal stimulation for the production of interleukin (IL)-2, IL 4 and interferon-gamma by allogeneic T cells. *European Journal of Immunology* 21:2803–9.

Elson, C. J., Barker, R. N., Thompson, S. J., and Williams, N. A. 1995. Immunologically ignorant autoreactive T cells, epitope spreading and repertoire limitation. *Immunology Today* 16:71–6.

Emekdas, G., Rota, S., Kustimur, S., and Kocabeyoglu, 0. 1992. Antibody levels against Coxsackie B viruses in patients with type 1 diabetes mellitus. *Mikrobiyoloji Bulteni* 26:116-20.

Emonard, H., and Grimaud, J. A. 1990. Matrix metalloproteinases. A review. *Cellular and Molecular Biology* 36:131-53.

Enberg, R. N. 1991. Food induced oropharyngeal symptoms. *Immunology Allergy Clinics of North America* 11:767.

Engel, I., Letourneur, F., Houston, J. T., Ottenhoff, T. H., and Klausner, R. D. 1992. T cell receptor structure and function: analysis by expression of portions of isolated subunits. *Advances in Experimental Medicine and Biology* 323:1-7.

Enk, A. H., and Katz, S. I. 1995. Contact sensitivity as a model for T cell activation in the skin. *Journal of Investigative Dermatology* 105:80S–83S.

Epplen, C., Mitreiter, R., Hatz, H., Helmke, K., Albert, E., and Epplen, J. T. 1991. Induction of autoreactive T-cells by bacterial stress proteins in HLA-DR4 donors. *Immunitat und Infektion* 19:63–4.

Erb, K. J., and Le Gros, G. 1996. The role of Th2 type CD4+ T cells and Th2 type CD8+ T cells in asthma. *Immunology and Cell Biology* 74:206–8.

Erlich, H. A. 1990. HLA class II polymorphism and genetic susceptibility to insulin-dependent diabetes mellitus. *Current Topics in Microbiology & Immunology* 164:41–55.

Erlich, J. P., and Burleson, G. R. 1991. Enhanced and prolonged pulmonary influenza virus infection following phosgene inhalation. *Journal of Toxicology and Environmental Health* 34:259–73.

Etienne, I., Joannides, R., Dhib, M., and Fillastre, J. P. 1992. Drug-induced nephropathies. *Revue du Praticien* 42:2210–6.

Etlinger, J. D., Li, S. X., Guo, G. G., and Li, N. 1993. Phosphorylation and ubiquitination of the 26S proteasome complex. *Enzyme and Protein* 47:325–9.

Fabian, I., Kietler, Y., Mor, S., Geller-Berstein, C., Ben-Yaakov, M., Volovitz, B., and Golde, D. W. 1992. Activation of human eosinophil and neutrophil function by hematopoietic growth factors: comparison of IL-1, IL-3, IL-5 and GM-CSF. *British Journal of Hematology* 80:137-43.

Fairchild, P. J., and Wraith, D. C. 1992. Peptide–MHC interaction in autoimmunity. *Current Opinion in Immunology* 4:748–53.

Fakhfakh, F., Ayadi, H., Bahloul, Z., Jarraya, A., Sioud, M., and Zouali, M. 1996. Antibody epitopes probed by immunoselected phage-display library peptides in members of a family with various rheumatic manifestations. *Clinical and Experimental Rheumatology* 14:607–11.

Falkoff, R. J. M., Zhu, L., and Fauci, A. S. 1982. Separate signals for human B cell proliferation in response to Staphylococcus aureus: evidence for a two signal model of B cell activation. *Journal of Immunology* 129:97–102.

Fanger, M. W., Morganelli, P. M., and Guyre, P. M. 1992. Bispecific antibodies. *Critical Reviews in Immunology* 12:101-24.

Farber, J. M. 1997. Mip and IP-10: CXC chemokines that target lymphocytes. *Journal of Leukocyte Biology* 61:246–57.

Faustman, E., Allen, B. C., Kavlock, R. J., and Kimmel, C. A. 1994. Dose–response assessment of developmental toxicity. *Fundamental and Applied Toxicology* 23:478–86.

Fearon, D. T., Austen, K. F., and Ruddy, S. 1975. The properdin pathway: mechanisms of complement activation and analogies to the classical pathway. *Rheumatology* 6:2–16.

Ferrari, I., Levin, M. J., Wallukat, G., Elies, R., Lebesgue, D., Chiale, P., Elizari, M., Rosenbaum, M., and Hoebeke, J. 1995. Molecular mimicry between the immunodominant ribosomal protein PO of Trypanosoma cruzi and a functional epitope on the human beta 1 adrenergic receptor. *Journal ofExperimental Medicine* 182:59–65.

Fidler, U. 1973. Selection of successive tumor lines for metastasis. *Nature New Biology* 242:148-9.

Fidler, U., Gersten, D. M., and Hart, I. R. 1978. The biology of cancer invasion and metastases. *Advances in Cancer Research* 28:149-250.

Fiebiger, E., Stingl, G., and Maurer, D. 1996. Anti-IgE and anti-Fc epsilon RI autoantibodies in clinical allergy. *Current Opinion in Immunology* 8:784–9.

Field, L. L., McArthur, R. G., Shin, S. Y., and Yoon, J. W. 1987. The relationship between Coxsackie-B-virus-specific IgG responses and genetic factors (HLA-DR, GM, KM) in insulin-dependent diabetes mellitus. *Diabetes Research* 6:169-73.

Fielder, M., Pirt, S. J., Tarpey, I., Wilson, C., Cunningham, P., Ettelaie, C., Binder, A., Bansal, S., and Ebringer, A. 1995. Molecular mimicry and ankylosing spondylitis: possible role of a novel sequence in pullulanase of Klebsiella pneumoniae. *FEBS Letters* 369:243–8.

Finklestein, D. M., and Miller, R. G. 1990. Cell surface recognition determinants involved in triggering the lymphokine activated killer cell phenomenon: enhanced killing of modified "anti-self'' targets by varying LAK culture conditions. *Journal of Otolaryngology* 19:294–8.

Finley, D., and Chau, V. 1991. Ubiquitination. Annual Review of Cell Biology 7:25–69.

Finnegan, A., Needleman, B. W., and Hodes, R. J. 1990. Function of autoreactive T cells in immune responses. *Immunological Reviews* 116:15-31.

Firestein, G. S. 1992. Mechanisms of tissue destruction and cellular activation in rheumatoid arthritis. *Current Opinion in Rheumatology* 4:348–54.

Fischer, A. 1990. Primary immunodeficiencies. *Current Opinion in Immunology* 2:439–44.

Fischer, A., and Amaiz-Villena, A. 1995. Immunodeficiencies of genetic origin. *Immunology Today* 16:510-4.

Fischer, A., and Wood, W. J. 1995. Inherited immunodeficiencies. *The Immunologist* 3:237–240.

Fishman, P., and Shoenfeld, Y. 1993. Cytokines, interleukin-3 and autoimmunity. *Israel Journal of Medical Sciences* 29:159-64.

Fitzmaurice, C. J., Brown, L. E., McInerney, T. L., and Jackson, D. C. 1996. The assembly and immunological properties of non-linear synthetic immunogens containing T-cell and B-cell determinants. *Vaccine* 14:553-60.

Flajnik, M. F., Canel, C., Kramer, J., and Kasahara, M. 1991. Which came first, MHC class I or class II? *Immunogenetics* 33:295–300.

Fleischer, B. 1991a. Stimulation of human T cells by microbial "superantigens." Immunologic Research 10:349–55.

Fleischer, B. 1991b. The human T cell response to mitogenic microbial exotoxins. *Current Topics in Microbiology & Immunology* 174:53-62.

Fleischer, B. 1991c. T lymphocyte-stimulating microbial toxins as "superantigens." *Medical Microbiology & Immunology* 180:53-8.

Fleischer, B., and Hartwig, U. 1992. T-lymphocyte stimulation by microbial superantigens. *Chemical Immunology* 55:36–64.

Fletcher, F. A., and Williams, D. E. 1992. Recent progress in the discovery and invention of novel hematopoietic cytokines. *Critical Reviews in Oncology and Hematology* 13:1–15.

Floret, D., Stamm, D., and Ponard, D. 1991. Increased susceptibility to infection in children with congenital deficiency of factor I. *Pediatric Infectious Disease Journal* 10:615-8.

Flynn, G. L. 1990. *Physiological Determinants in Skin Absorption,* Elsevier, Amsterdam, pp. 93–127.

Fossiez, F., Djossou, O., Chomarat, P., Flores-Romo, L., Ait-Yahia, S., Maat, C., Pin, J. J., Gamone, P., Garcia, E., Saeland, S., Blanchard, D., Gaillard, C., Das Mahapatra, B., Rouvier, E., Golstein, P., Banchereau, J., and Lebecque, S. 1996. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. *Journal of Experimental Medicine* 183:2593–603.

Foster, P. M., and Auton, T. R. 1995. Application of the benchmark dose risk assessment methodology to developmental toxicology: an industrial perspective. *Toxicology Letters* 82-83:555-9.

Foucar, E., Erickson, D. G., and Tung, K. S. 1979. Glomerulonephritis in procainamide induced lupus erythematosus: report of a case and review of the literature. *Journal of Clinical & Laboratory Immunology* 2:79–84.

Fowler, W. J., Garcia-Valcarcel, M., Hill-Perkins, M. S., Murphy, G., Harper, D. R., Jeffnes, D. J., Bums, N. R., Adams, S. E., Kingsman, A. J., and Layton, G. T. 1995. Identification of immunodominant regions and linear B cell epitopes of the gE envelope protein of varicella–zostervirus. *Virology* 214:531-40.

Franco, A., Southwood, S., Arrhenius, T., Kuchroo, V. K., Grey, H. M., Sette, A., and Ishioka, G. Y. 1994. T cell receptor antagonist peptides are highly effective inhibitors of experimental allergic encephalomyelitis. European *Journal of Immunology* 24:940–6.

Franco, A., Ferrari, C., Sette, A., and Chisari, F. V. 1995. Viral mutations, TCR antagonism and escape from the immune response. *Current Opinion in Immunology* 7:524-31.

Frank, M. M. 1986. C1 esterase inhibitor: clinical clues to the pathophysiology of angioedema. *Journal of Allergy & Clinical Immunology* 78:848–50.

Frank, M. M., and Atkinson, J. P. 1975. Complement in clinical medicine. *Disease-A-Month* 1975:1-54.

Frank, R. W., Gennaro, R., Schneider, K., Przybylski, M., and Romeo, D. 1990. Amino acid sequence of two proline rich bactenecins. Anti-microbial peptides of bovine neutrophils. *Journal of Biological Chemistry* 265:18871-4.

Frankild, S., Basketter, D. A., and Andersen, K. E. 1996. The value and limitations of rechallenge in the guinea pig maximization test. *Contact Dermatitis* 35:135-40.

Freeman, J. J., McKee, R. H., and Trimmer, G. W. 1988. Experimental contact sensitization with 3,4,5-trichloropyridazine. *Contact Dermatitis* 18:259–62.

Fremont, D. H., Hendrickson, W. A., Marrack, P., and Kappler, J. 1996. Structures of an MHC class II molecule with covalently bound single peptides. *Science* 272: 1001–4.

Frendl, G. 1992. Interleukin 3: from colony-stimulating factor to pluripotent immunoregulatory cytokine. *International Journal of Immunopharmacology* 14:421–30.

Frenz, A. M., Gibbs, B. F., and Pearce, F. L. 1997. The effect of recombinant stem cell factor on human skin and lung mast cells and basophil leukocytes. *Inflammation Research* 46:35–9.

Fridman, W. H. 1993. Regulation of B-cell activation and antigen presentation by Fc receptors. *Current Opinion in Immunology* 5:355–60.

Friedland, J. S. 1996. Chemokines in viral disease. *Research in Virology* 147:131–8.

Friedman, S. M., Tumang, J. R., and Crow, M. K. 1993. Microbial superantigens as etiopathogenic agents in autoimmunity. *Rheumatic Diseases Clinics of North America* 19:207–22.

Friend, P. J., Hale, G., Waldman, H., Gore, S., Thiru, S., Joysey, V., Evans, D. B., and Calne, R. Y. 1989. CAMPATH-1M prophylactic use after kidney transplantation. *Transplantation* 48:248–53.

Frisk, G., Nilsson, E., Tuvemo, T., Friman, G., and Diderholm, H. 1992. The possible role of Coxsackie A and echo viruses in the pathogenesis of type I diabetes mellitus studied by IgM analysis. *Journal of Infection* 24:13–22.

Fu, T. M., Bonneau, R. H., Epler, M., Tevethia, M. J., Alam, S., Verner, K., and Tevethia, S. S. 1996. Induction and persistence of a cytotoxic T lymphocyte (CTL) response against a herpes simplex virus-specific CTL epitope expressed in a cellular protein. *Virology* 222:269–74.

Fugmann, R. A., Aranyi, C., Barbera, P. W., Bradof, J. N., Gibbons, R. D., and J. D., 1983. The effects of DES as measured by host resistance and tumor susceptibility assays in mice. *Journal of Toxicology and Environmental Health* 11:827-41.

Furst, D. E., Clements, P. J., Graze, P., Gale, R., and Roberts, N. 1979. A syndrome resembling progressive systemic sclerosis after bone marrow transplant. A model for scleroderma? *Arthritis and Rheumatology* 22:904–10.

Furue, M., Nindl, M., Kawabe, K., Nakamura, K., Ishibashi, Y., and Sagawa, K. 1992. Epitopes for CD1a, CD1b, and CD1c antigens are differentially mapped on Lan-

gerhans cells, dermal dendritic cells, keratinocytes, and basement membrane zone in human skin. *Journal of the American Academy of Dermatology* 27:419–26.

Gad, S. C. 1994. The mouse ear swelling test (MEST) in the 1990s. Toxicology 93:33–46.

Gad, S. C. 1995. The mouse ear swelling test. In *Methods in Immunotoxicology* (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 357–72.

Gad, S. C., Dunn, B. J., Dobbs, D. W., Reilly, C., and Walsh, A. E. 1986. Development and validation of an alternate dermal sensitization test. The mouse ear swelling test (MEST). *Toxicology and Applied Pharmacology* 84:93–114.

Gagnadoux, F., Diot, P., Boissinot, E., and Lemarie, E. 1997. Hematopoietic growth factors and anti-infective respiratory defenses. *Revue des Maladies Respiratoires* 14:93–9.

Galbraith, W. M. 1987. *Safety Evaluation of Biotechnology Derived Products,* Wiley–Liss, New York, pp. 3–14.

Galbraith, W. M., Hobson, W. C., Giclas, P. C., Schechter, P. J., and Agrawal, S. 1994. Complement activation and hemodynamic changes following intravenous administration of phosphorothioate oligonucleotides in the monkey. *Antisense Research and Development* 4:201–6.

Gallagher, R. B., Brines, R., and Girdlestone, D. 1993. Why immunopharmacology? *Immunology Today* 14:241.

Gallin, J. I. 1985. Neutrophil specific granule deficiency. *Annual Review of Medicine* 36:263–74.

Ganguly, R., and Waldman, R. H. 1983. T- and B-cell memory on mucosal surfaces. *Annals of the New York Academy of Sciences* 409:603–11.

Ciao, X. M., Wordsworth, P., and McMichael, A. 1994. Collagen-specific cytotoxic T lymphocyte responses in patients with ankylosing spondylitis and reactive arthritis. *European Journal of Immunology* 24:1665-70.

Garratty, G. 1976. Problems in pre-transfusion tests related to drugs and chemicals. *American Journal of Medical Technology* 42:209–19.

Garratty, G., and Petz, L. D. 1975. Drug-induced immune hemolytic anemia. *American Journal of Medicine* 58:398–407.

Garssen, J., Nijkamp, F. P., Van der Wet, H., and Van Lovern, H. 1991. T-cell mediated induction of airway hyperresponsiveness induced by small molecular weight compounds. *American Review of Respiratory Disease* 144:931-8.

Garssen, J., Van der Vliet, H., de Klerk, A., Goettsch, W., Dormans, J. A. M. A., and Bruggeman, C. A. 1993. A rat cytomegalovirus infection model as a tool for immunotoxicity testing. *European Journal of Pharmacology* 292:223–1.

Gascoigne, N. R. 1993. What role does the alpha-chain play in recognition of superantigens? *Research in Immunology* 144: 184–7;discussion 214-22.

Gauggel, D. L., Sarlo, K., and Asquith, T. N. 1993. A proposed screen for evaluating low-molecular weight chemicals as potential respiratory allergens. *Journal of Applied Toxicology* 13:307–13.

Gelfand, J. A., Boss, G. R., Conley, C. L., Reinhart, R., and Frank, M. M. 1979. Acquired C1 esterase inhibitor deficiency and angioedema: a review. *Medicine* 58:321–8.

Geller-Bemstein, C., Keret, N., Zamir, R., Weissglas, L., Lahav, M., Morton, N., and Sela, M. 1984. HLA antigens A, B, C and DR in hay-fever families of similar environmental conditions. *Experimental & Clinical Immunogenetics* 1:66-9.

Genovese, A., Stellato, C., Lamparter-Schummert, B., de Crescenzo, G., Adt, M., and Marone, G. 1996. Contrast media are incomplete secretagogues on human basophils and mast cells isolated from heart and lung but not skin tissue. International Journal of Clinical and Laboratory *Research* 26:192–8.

Genovese, S., Bonfanti, R., Bazzigaluppi, E., Lampasona, V., Benazzi, E., Bosi, E., Chiumello, G., and Bonifacio, E. 1996. Association of IA-2 autoantibodies with HLA DR4 phenotypes in IDDM. *Diabetologia* 39:1223-1226.

Georges, B., Gras-Masse, H., Maes, P., Capron, A., Tartar, A., and Auriault, C. 1996. Analysis of peptides associated with class II MHC molecules, HLA-DR3: implication for the prediction of peptides useful for vaccines. *Comptes Rendus de I'Academie des Sciences, Serie Iii: Sciences de la Vie* 319:1119–25.

Gerberick, G. F., House, R. V., Fletcher, E. R., and Ryan, C. A. 1992. Examination of the local lymph node assay for use in contact sensitization risk assessment. *Fundamental & Applied Toxicology* 19:438–45.

Gerberick, G. F., Cruse, L. W., Miller, C. M., Sikorski, E. E., and Ridder, G. M. 1997. Selective modulation of T cell memory markers CD62L and CD44 on murine draining lymph node cells following allergen and irritant treatment. *Toxicology and Applied Pharmacology* 146:1-10.

Gerdes, J., and Flad, H. D. 1992. Follicular dendritic cells and their role in HIV infection. *Immunology Today* 13:81–3.

Germain, R. N. 1995. MHC-associated antigen processing, presentation and recognition. *The Immunologist* 3:185-92.

Germain, R. N., and Hendrix, L. R. 1991. MHC class II structure, occupancy and surface expression determined by post-endoplasmic reticulum antigen binding. *Nature* 353: 134–9.

German, R. N., Castellino, F., Han, R., Reis e Sousa, C., Romagnoli, P., Sadegh-Nasseri, S., and Zhong, G. M. 1996. Processing and presentation of endocytically acquired protein antigens by MHC class II and class I molecules. *Immunological Reviews* 151:5–30.

Gershwin, M. E., Abplanalp, H. A., Castles, J. J., Ikeds, R. M., Van der Water, J., Eklund, J., and Haynes, D. 1981. Characterization of a spontaneous disease in white leghorn chickens resembling progressive systemic sclerosis (scleroderma). *Journal ofExperimental Medicine* 153:1640-59.

Gery, I., and Streilein, J. W. 1994. Autoimmunity in the eye and its regulation. *Current Opinion in Immunology* 6:938–45.

Gessner, A., Will, A., Vieth, M., Schroppel, K., and Rollinghoff, M. 1995. Stimulation of B-cell lymphopoiesis by interleukin-7 leads to aggravation of murine leishmaniasis. *Immunology* 84:416–22.

Ghosh, P., Amaya, M., Mellins, E., and Wiley, D. C. 1995. The structure of an intermediate in class II MHC maturation: CLIP bound to HLA-DR3. *Nature* 378:457–62.

Giardina, E. G. 1984. Procainamide: clinical pharmacology and efficacy against ventricular arrhythmias, *Annals of the New York Academy of Sciences* 432:177–88.

Gillett, D. G., and Ford, G. T. 1978. Drug-induced lung disease. *Monographs in Pathology* 19:21–42.

Gillis, S. 1983. Interleukin 2: biology and biochemistry. *Journal of Clinical Immunology* 3:1–B.

Giorda, R., and Trucco, M. 1991. NKR-PIA family of genes selectively co-expressed in adherent lymphokine activated killer cells. *Journal of Immunology* 147:1701-8.

Girard, J.-P., and Springer, T. A. 1995a. Cloning from purified high endothelial venule cells of hevin, a close relative of the antiadhesive matrix protein SPARC. *Immunity* 2:113–22.

Girard, J.-P., and Springer, T. A. 1995b. High endothelial venules (HEVs): specialized endothelium for lymphocyte migration. *Immunology Today* 16:449–56.

Gjesing, B., and Lowenstein, H. 1984. Immunochemistry of food antigens. *Annuls of Allergy* 53:602–8.

Glabinski, A. 1993. Cytotoxic cytokines in multiple sclerosis. *Neurologia i Neurochirurgia Polska* 27:869-75.

Gleeson, P. A., and Toh, B. H. 1991. Molecular targets in pernicious anemia. *Immunology Today* 12:233–8.

Gleich, G. J., Loegering, D. A., Frigas, E., and Filley, W. V. 1983. The eosinophil granule major basic protein: biological activities and relationship to bronchial asthma. *Monographs in Allergy* 18:277–83.

Gleichmann, E., and Gleichmann, H. 1976. Graft versus host reactions: a pathogenic principle for the development of drug allergy, autoimmunity and malignant lymphoma in non-chimeric individuals. Hypothesis. *Zeitschriftfuer zeitschriftfur krebforsch* 5:91-109.

Gleichmann, E., Pals, S. T., Rolink, A. G., Radaszkiewicz, T., and Gleichmann, H. 1984. Graft versus host reactions: clues to the etiopathology of a spectrum of immunological diseases. *Immunology Today* 5:324–32.

Glennis, M. J., McBride, H. M., Worth, A. T., and Stevenson, G. T. 1987. Preparation and performance of bispecific F(ab′γ)2 antibody containing thioester-linked F(ab′γ)2 fragments. *Journal of Immunology* 139:2367.

Goddfrey, D. I., and Zlotnik, A. 1993. Control points in early T-cell development. *Immunology Today* 14:547-53.

Goedegebuure, P. S., and Eberlein, T. J. 1995. The role of CD4+ tumor-infiltrating lymphocytes in human solid tumors. *Immunologic Research* 14:119–31.

Goetzl, E. J., Banda, M. J., and Leppert, D. 1996. Matrix metalloproteinases in immunity. *Journal of Immunology* 156:1-4.

Golochevskaia, V. S. 191 1. Side effects of anti-arrhythmic agents. *Klinicheskaya Meditsina* 49:11-5.

Gon, Y., and Yodoi, J. 1996. Low affinity Fc receptor for IgE (Fc epsilon R II/CD23). *Nippon Rinsho, Japanese Journal of Clinical Medicine* 54:456–60.

Gonzalo, J. A., Cuende, E., Ales-Martinez, J. E., Martinez, C., and Kroemer, G. 1992. Interleukin-2: a possible trigger for autoimmunity. *International Archives of Allergy & Immunology* 91:25 1-7.

Good, M. F., and Zevering, Y. 1994. Malaria-specific memory T cells: putative roles of different types of memory responses in immunity and disease. *Research in Immunology* 145:455–60.

Good, R. A., and Lorenz, E. 1992. Nutrition and cellular immunity. *International Journal of Immunopharmacology* 14:361.

Goodacre, J. A., Brownlie, C. E., and Ross, D. A. 1994. Bacterial superantigens in autoimmune arthritis. *British Journal of Rheumatology* 33:413-9.

Gooding, L. R. 1994. Regulation of TNF-mediated cell death and inflammation by human adenoviruses. *Infectious Agents & Disease* 3:106-15.

Goodwin, B. F. J., Crevel, R. W. R., and Johnson, A. W. 1981. A comparison of 3 guinea pig sensitization procedures for the detection of 19 reported human contact sensitizers. *Contact Dermatitis* 7:248–58.

Gordon, J. 1994. B-cell signaling via the C-type lectins CD23 and CD72. *Immunology Today* 15:411-7.

Gorus, F. K., Goubert, P., Semakula, C., Vandewalle, C. L., De Schepper, J., Scheen, A., Christie, M. R., and Pipeleers, D. G. 1997. IA-2-autoantibodies complement GAD65-autoantibodies in new-onset IDDM patients can help predict impending diabetes in their siblings. The Belgian Diabetes Registry. *Diabetologia* 40:95–9.

Goulder, P. J., Bunce, M., Krausa, P., McIntyre, K., Crowley, S., Morgan, B., Edwards, A., Giangrande, P., Phillips, R. E., and McMichael, A. J. 1996. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. *AIDS Research & Human Retroviruses* 12:1691-8.

Gounni, A. S., Lamkhioued, B., Delaporte, E., Dubost, A., Kinet, J. P., Capron, A., and Capron, M. 1994. The high-affinity IgE receptor on eosinophils: from allergy to parasites or from parasites to allergy? *Journal of Allergy & Clinical Immunology* 94:1214-6.

Goust, J. M. 1993. Immediate hypersensitivity. *Immunology* 58:343–359.

Graber, M., June, C. H., Samelson, L. E., and Weiss, A. 1992. The protein tyrosine kinase inhibitor herbimycin A, but not genistein, specifically inhibits signal transduction by the T cell antigen receptor. *International Immunology* 4:1201-10.

Gracie, J. A., and Bradley, J. A. 1996. Interleukin-12 induces interferon-gamma-dependent switching of IgG alloantibody subclass. *European Journal of Immunology* 26:1217-21.

Graham, C., Gealy, R., Macina, 0. T., Karol, M. H., and Rosenkranz, H. S. 1996. QSAR for allergic contact dermatitis. Quantitative Structure Activity Relationships 15: 1–7.

Granfors, K. 1992. Do bacterial antigens cause reactive arthritis? *Rheumatic Diseases Clinics of North America* 18:37–48.

Granger, D. L., and Lehninger, A. L. 1982. Sites of mitochondrial electron transport in macrophage injured neoplastic cells. *Journal of Cell Biology* 95:527–35.

Granucci, F., Girolomoni, G., Lutz, M. B., and Ricciardi-Castagnoli, P. 1995. Recombinant GM-CSF induces cytokine production in mouse dendritic cell clones. *Advances in Experimental Medicine & Biology* 378:3 1–4.

Graves, D. T., and Jiang, Y. 1995. Chemokines, a family of chemotactic cytokines. *Critical Reviews in Oral Biology & Medicine* 6:109-18.

Gray, D. 1996. Viral immunity: interferons jog old T-cell memories. *Current Biology* 6:1254–5.

Gray, D., MacLennan, I. C. M., Bazin, H., and Khan, M. 1986. Migrant m + d+ and static m + d—B lymphocyte subsets. *European Journal of Immunology* 12564–72.

Gray, D., Kosco, M., and Stockinger, B. 1991. Novel pathways of antigen presentation for the maintenance of memory. *International Immunology* 3:141-8.

Greaves, M., Janossy, G., and Doenhoff, H. 1974. Selective triggering of lymphocytes in vitro by polyclonal mitogens. *Journal of Experimental Medicine* 140:1-18.

Green, D. R., Zheng, H., and Shi, Y. 1992. Antisense oligodeoxynucleotides as probes of T-lymphocyte gene function. *Annals of the New York Academy of Sciences* 660:193–203.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. 1982. Analyses of nitrate, nitrite and [15] nitrate in biological fluids. *Analytical Biochemistry* 126:131–8.

Green, S. J., Meltzer, M. S., Hibbs, J. B., Jr., and Nacy, C. A. 1990. Activated macrophages destroy intracellular Leishmania major amastigotes by an arginine dependent killing mechanism. *Journal of Immunology* 144:278–83.

Green, S. J., Scheller, L. F., Marletta, M. A., Seguin, M. C., Klotz, F. W., Slayter, M., Nelson, B. J., and Nacy, C. A. 1994. Nitric oxide: cytokine-regulation of nitric oxide in host resistance to intracellular pathogens. *Immunology Letters* 43:87–94.

Greene, W. C., and Leonard, W. J. 1986. The human interleukin-2 receptor. *Annual Review of Immunology* 4:69–95.

Greenstein, B. D., Fitzpatrick, F. T. A., Kendall, M. D., and Wheeler, M. J. 1987. Regeneration of the thymus in old rats treated with a stable analogue of LHRH. *Endocrinology* 112:345.

Grewal, I. S., and Flavell, R. A. 1996. A central role of CD40L ligand in the regulation of CD4+ T-cell responses. *Immunology Today* 17:410–3.

Grey, H. M., Alexander, J., Snoke, K., Sette, A., and Ruppert, J. 1993. Antigen analogues as antagonists of the T cell receptor. *Clinical & Experimental Rheumatology* 11:S47–50.

Gribben, J. G., Devereux, S., Thomas, N. S., Keim, M., Jones, H. M., Goldstone, A. H., and Linch, D. C. 1990. Development of antibodies to unprotected glycosylation sites on recombinant human GM-CSF. *Lancet* 335:434-7.

Griebel, P. J., and Hein, W. R. 1996. Expanding the role of Peyer's patches in B cell ontogeny. *Immunology Today* 17:30–9.

Griffin, F. M., Jr. 1984. Activation of macrophage complement receptors for phagocytosis. *Contemporary Topics in Immunobiology* 13:57-70.

Griffith, T. S., and Ferguson, T. A. 1997. The role of FasL-induced apoptosis in immune privilege. *Immunology Today* 18:240–4.

Griffiths-Johnson, D. A., and Karol, M. H. 1991. Validation of a non-invasive technique to assess development of airway hyperreactivity in an animal model of immunologic pulmonary hypersensitivity. *Toxicology* 65:283–94.

Groettrup, M., and von Boehmer, H. 1993. A role for a pre-T-cell receptor in T cell development. *Immunology Today* 14:610–4.

Groettrup, M. G., Soza, A., Kuckelkorn, U., and Kloetzel, P.-M. 1996. Peptide antigen production by the proteasome: complexity provides efficiency. *Immunology Today* 17:429-35.

Groisman, E. A. 1994. How bacteria resist killing by host-defense peptides. *Trends in Microbiology* 2:444–9.

Gronski, P., Bodenbender, L., Kanzy, E. J., and Seiler, F. R. 1984. The influence of classical pathway components during alternative pathway-modulated immune complex aggregation: the role of C1 INH. *Behring Institute Mitteilungen* 1984:29–41.

Gruss, H. J., and Dower, S. K. 1995. Tumor necrosis factor ligand superfamily: involvement in the pathology of malignant lymphomas. *Blood* 85:3378–404.

Guery, J. C., and Adorini, L. 1993. Selective immunosuppression of class 11-restricted T cells by MHC class 11-binding peptides. *Critical Reviews in Immunology* 13:195–206.

Guidotti, L. G., and Chisari, F. V. 1996. To kill or cure: options in host defense against viral infections. *Current Opinion in Immunology* 8:478–83.

Gulbenkian, A. R., Egan, R. W., Fernandez, X., Jones, H., Kreutner, W., Payvandi, F., Sullivan, K., Zurcher, J. A., and Watnik, A. S. 1992. Interleukin-5 modulates eosinophil accumulation in allergic guinea pig lung. *American Review of Respiratory Disease* 146:263–6.

Guler, M. L., Gorham, J. D., Hsieh, C. S., Mackey, A. J., Steen, R. G., Dietrich, W. F., and Murphy, K. M. 1996. Genetic susceptibility to Leishmania: IL-12 responsiveness in TH1 cell development. *Science* 271:984-7.

Gupta, S., and Tan, C. 1980. Subpopulations of human T lymphocytes XIV. Abnormality of T-cell locomotion and of distribution of subpopulations of T and B lymphocytes in peripheral blood and spleen from children with untreated Hodgkin's disease. *Clinical Immunology and Immunopathology* 15:133-43.

Haack, B. M., Emmrich, F., and Resch, K. 1993. Cholera toxin inhibits T cell receptor signaling by covalent modification of the CD3-zeta subunit. *Journal of Immunology* 150:2599–606.

Haas, A. L., and Bright, P. M. 1985. The proteolytic inactivation of ubiquitin by rabbit liver. *Progress in Clinical & Biological Research* 180:207–9.

Haddad, Z. H., Kaka, V., and Verma, S. 1979. sIgE antibodies to peptic and peptic–tryptic digests of beta lactoglobin: significance in food hypersensitivity. *Annals of Allergy* 42:368.

Haggerty, H., and Holsapple, M. P. 1990. Role of metabolism in dimethylnitrosamine induced immunosuppression: a review. *Toxicology* 63:1-23.

Hahn, S., Gehri, R., and Erb, P. 1995. Mechanism and biological significance of CD4 mediated cytotoxicity. *Immunological Reviews* 146:57-79.

Haller, O., Arnheiter, H., Lindenmann, J., and Gresser, I. 1980. Host gene influences sensitivity to interferon action selectively for influenza virus. *Nature* (London) 283:660–2.

Halpern, G. M., and Scott, J. R. 1987. Non-IgE antibody mediated mechanisms in food allergy. *Annals of Allergy* 58:14-27.

Haman, A., Andrew, D. P., and Jablonski-Westrich, D. 1994. Role of alpha-4 integrins in lymphocytes homing to mucosal tissues *in vivo Journal of Immunology* 152:3282–93.

Hammarstrom, L., and Smith, C. I. 1986. IgG subclass changes in response to vaccination. *Monographs in Allergy* 19:241–252.

Hanna, N., and Fidler, I. J. 1980. Role of the natural killer cells in the destruction of circulating tumor emboli. *Journal of the National Cancer Institute* 65:801–10.

Hansen, J. A., and Nelson, J. L. 1990. Autoimmune disease and HLA. *Critical Reviews in Immunology* 10:307–28.

Hansen, M. B., Svenson, M., Diamant, M., Abell, K., and Bendtzen, K. 1995. Interleukin-6 autoantibodies: possible biological and clinical significance. *Leukemia* 9:1113–5.

Harlan, D. M., Abe, R., Lee, K. P., and June, C. H. 1995. Potential roles of the B7 and

CD28 receptor families in autoimmunity and immune evasion. *Clinical Immunology & Immunopathology* 75:99–111.

Harris, D. P., Vordermeier, H. M., Arya, A., Bogdan, K., Moreno, C., and Ivanyi, J. 1996. Immunogenicity of peptides for B cells is not impaired by overlapping T-cell epitope topology. *Immunology* 88:348–54.

Harrison, S. C., Wang, J., Yan, Y., Garrett, T., Liu, J., Moebius, U., and Reinherz, E. 1992. Structure and interactions of CD4. *Cold Spring Harbor Symposia on Quantitative Biology* 57:541-8.

Hart, F. D. 1984. Drug-induced arthritis and arthralgia. *Drugs* 28:347–54.

Harton, J. A., and Bishop, G. A. 1993. Length and sequence requirements of the cytoplasmic domain of the αbeta molecule for class 11-mediated B cell signaling. *Journal of Immunology* 151:5282-9.

Haruyama, H., and Hanzawa, H. 1997. Three dimensional structure of CXC chemokines and its implication for the receptor recognition. *Tampakushitsu Kakusan Koso, Protein, Nucleic Acid, Enzyme* 42:982–8.

Hashiguchi, S., Hino, K., Taniguchi, Y., Kurimoto, M., Fukuda, K., Ohyama, M., Fujiyoshi, T., Sonoda, S., Nishimura, Y., Yamada, G., and Sugimura, K. 1996. Immunodominance of seven regions of a major allergen, Cry j 2, of Japanese cedar pollen for T-cell immunity. *Allergy* 51:621–32.

Haurum, J. S., Thiel, S., Jones, I. M., Fischer, P. B., Laursen, S. B., and Jensenius, J. C. 1993. Complement activation upon binding of mannan-binding protein to HIV envelope glycoproteins. *AIDS* 7:1307–13.

Hauser, C., and Katz, S. 1988. Activation and expansion of haptens and protein-specific helper cells from non-sensitized mice. *Proceedings of the National Academy of Sciences (USA)* 85:5625-32.

Havell, E. A. 1992. Role of TNF in resistance to bacteria. Immunology Series 56:341–63.

Hazout, A. 1995. Tumor necrosis factor and underlying infection. *Contraception, Fertilite, Sexualite* 23:631-4.

He, W., and Kabelitz, D. 1993. Differential effects of interleukin-7 and interleukin-2 on T-cell receptor gamma delta-expressing cells within CD4–CD8–postnatal human thymocytes. *International Archives of Allergy & Immunology* 102:321–6.

He, W., and Kabelitz, D. 1995. Differential effects of interleukin-2 and interleukin-7 on the induction of CD4 and CD8 expression by double-negative human thymocytes. Scandinavian *Journal of Immunology* 41:309-12.

Hednck, J. A., and Zlotnik, A. 1996. Chemokines and lymphocyte biology. *Current Opinion in Immunology* 8:343–7.

Hefle, S. L., Nordlee, J. A., and Taylor, S. L. 1996. Allergenic foods. *Critical Reviews in Food Science and Nutrition* 36:S69–89.

Hegde, N. R., and Srikumaran, S. 1996. Prediction of potential cytotoxic T lymphocyte epitopes of bovine herpesvirus 1 based on allele-specific peptide motifs and proteolytic cleavage specificities. *Virus Genes* 13:121-33.

Hein, W. R. 1992. Structure and diversity of ruminant T-cell receptors. *Annales de Recherches Veterinaires* 23:262–5.

Heiner, D. C., and Wilson, J. F. 1986. Delayed immunologic food reactions. *New England & Regional Allergy Proceedings* 7:520-6.

Heinzel, F. P., Ahmed, F., Hujer, A. M., and Rerko, R. M. 1995. Immunoregulation of murine leishmaniasis by interleukin- 12. *Research in Immunology* 146:575-81.

Hiepe, F., Jungnitz, S., and Hiepe, T. 1996. Production of autoantibodies against DNA and collagen after inoculation of rabbits with Trypanosoma equiperdum. *Applied Parasitology* 37:266–74.

Helbling, A. 1997. Important cross-reactive allergens. Schweizerische Medizinische Wochenschrift. *Journal Suisse de Medecine* 127:382–9.

Hellmark, T., Segelmark, M., and Wieslander, J. 1997. Anti-GBM antibodies in Goodpasture syndrome; anatomy of an epitope. *Nephrology, Dialysis, Transplantation* 12:646-8.

Helmbold, P., Rytter, M., Ziegler, V., and Haustein, U. F. 1993. Induction of type IV hypersensitivity to contact allergens in guinea pigs by in vitro haptenized allogeneic peritoneal exudate cells. *Acta Dermato-Venereologica* 73:365–9.

Henderson, C. R., and Riley, E. C. 1945. Certain statistical considerations in patch testing. *Journal of Investigative Dermatology* 6:227-32.

Henry-Toulme, N., Sarthou, P., Seman, M., and Bolard, J. 1989. Membrane effects of the polyene antibiotic amphotericin B and some of its derivatives on lymphocytes. *Molecular and Cellular Biochemistry* 9l:39-44.

Hermann, E. 1995. Enterobacterial antigens with tropism for joint structures and HLA-B27-restricted cytotoxic T-cells in reactive arthritis. *Scandinavian Journal of Rheumatology Supplement* 101:203-6.

Hershko, A. 1991a. The ubiquitin pathway of protein degradation and proteolysis of ubiquitin–protein conjugates. *Biochemical Society Transactions* 19:726–9.

Hershko, A. 1991b. The ubiquitin pathway for protein degradation. *Trends in Biochemical Sciences* 16:265–8.

Hershko, A. 1988. Ubiquitin-mediated protein degradation. *Journal ofBiological Chemistry* 263:15237-40.

Hershko, A., and Ciechanover, A. 1986. The ubiquitin pathway for the degradation of intracellular proteins. *Progress in Nucleic Acid Research & Molecular Biology* 33:19–56, 301.

Hershko, A., and Ciechanover, A. 1992. The ubiquitin system for protein degradation. *Annual Review of Biochemistry* 61:761-807.

Herz, U., Lumpp, U., Da Palma, J. C., Enssle, K., Takatsu, K., Schnoy, N., Daser, A., Kottgen, E., Wahn, U., and Renz, H. 1996. The relevance of murine animal models to study the development of allergic bronchial asthma. *Immunology & Cell Biology* 74:209-17.

Heufelder, A. E. 1995. Involvement of the orbital fibroblast and TSH receptor in the pathogenesis of Graves' ophthalmopathy. *Thyroid* 5:331–40.

Hibbs, J. B., Jr., Vavrin, Z., and Taintor, R. R. 1987. L-Arginine is required for expression of the activated macrophage effector mechanism causing selective metabolic inhibition in target cells. *Journal of Immunology* 138:550-5.

Hibi, M., Nakajima, K., and Hirano, T. 1996. IL-6 cytokine family and signal transduction: a model of the cytokine system. *Journal of Molecular Medicine* 74:1-12.

Higgins, C. F. 1992. ABC transporters from microorganisms to man. *Annual Review of Cell Biology* 8:67–113.

Hilkens, C. M., Snijders, A., Snijdewint, E. G., Wieringa, E. A., and Kapsenberg, M. L. 1996. Modulation of T-cell cytokine secretion by accessory cell-derived products. *European Respiratory Journal* 22:90s-94s.

Hill, H. F. 1979. Penicillamine in rheumatoid arthritis: adverse effects. *Scandinavian Journal of Rheumatology Supplement* 28:94–99.

Hill, S., Stackpoole, A., Kimber, I., and Knight, S. C. 1992. Function of dendritic cells and changes in T cell proliferation in antigen-induced nonresponsiveness. *Cellular Immunology* 139:342–51.

Hiltbold, E. M., Safley, S. A., and Ziegler, H. K. 1996. The presentation of class I and class II epitopes of listeriolysin O is regulated by intracellular localization and by intercellular spread of Listeria monocytogenes. *Journal of Immunology* 157:1163-75.

Hinton, D. 1992. Testing guidelines for evaluation of the immunotoxic potential of direct food additives. *Critical Reviews in Food Science and Nutrition* 32:173-90.

Hiramatsu, K., Tadano, M., Men, R., and Lai, C. J. 1996. Mutational analysis of a neutralization epitope on the dengue type 2 virus (DEN2) envelope protein: monoclonal antibody resistant DEN2/DEN4 chimeras exhibit reduced mouse neurovirulence. *Virology* 224:437-45.

Hiscox, S., and Jiang, W. G. 1997. Interleukin-12, an emerging anti-tumour cytokine. *In Vivo* 11:125–32.

Ho, V. C., Stein, H. B., Ongley, R. A., and McLeod, W. A. 1985. Penicillamine induced pemphigus. *Journal of Rheumatology* 12:583-6.

Hobohm, U., and Sander, C. 1993. Does the HIV Nef protein mimic the MHC? *FEBS Letters* 333:211–3.

Hogquist, K. A., Jameson, S. C., Heath, W. R., Howard, J. L., Bevan, M. J., and Carbone, F. R. 1994. T cell receptor antagonist peptides induce positive selection. *Cell* 76:17–27.

Holgate, S. T., and Kay, A. B. 1985. Mast cells, mediators and asthma. *Clinical Allergy* 15:221-34.

Holland, S. M. 1996. Host defense against non tuberculous mycobacterial infections. *Seminars in Respiratory Infections* 11:217–30.

Holmskov, U., Malhotra, R., Sim, R. B., and Jensenius, J. C. 1994. Collectins: collagenous C-type lectins of the innate immune defense system. *Immunology Today* 15:67–74.

Holowka, D., and Baird, B. 1996. Antigen-mediated IgE receptor aggregation and signaling: a window on cell surface structure and dynamics. *Annual Review of Biophysics & Biomolecular Structure* 25:79–112.

Holsapple, M. P., White, K. L., McCay, J. A., Bradley, S. G., and Munson, A. E. 1988. An immunotoxicological evaluation of 4,4-thiobis(6-t-butyl-m-cresol) in female B6C3F1 mice. *Fundamental and Applied Toxicology* 10:701–16.

Homberg, J. C. 1984. Autoimmunity induced by drugs. Immunological characteristics and etiopathogenic hypotheses. *Presse Medicale* 13:2755-60.

Homey, B., Schuppe, H. C., Assmann, T., Vohr, H. W., Lauerma, A. I., Ruzicka, T., and Lehmann, P. 1997. A local lymph node assay to analyse immunosuppressive effects of topically applied drugs. *European Journal of Pharmacology* 325:199-207.

Hopkin, J. M. 1990. Atopy and genetics. A review. *Journal of the Royal College of Physicians of London* 24:159-60.

Hoppe, H. J., and Reid, K. B. 1994. Collectins–solubleproteins containing collagenous regions and lectin domains and their roles in innate immunity. *Protein Science* 3:1143–58.

Horak, I., Lohler, J., Ma, A., and Smith, K. A. 1995. Interleukin-2 deficient mice: a new model to study autoimmunity and self-tolerance. *Immunological Reviews* 148:35–44.

Horuk, R. 1994. The interleukin-%receptor family from chemokines to malaria. Immunology Today 15:169–74.

Horuk, R., and Peiper, S. C. 1996. Chemokines: molecular double agents. *Current Biology* 6:1581-2.

Horwitz, M. A. 1980. The roles of the Fc and C3 receptors in the phagocytosis and killing of bacteria by human phagocytes. *Journal of the Reticuloendothelial Society* 28:17s-26s.

Hoshino, A., Takenaka, H., Mizukoshi, O., Imanishi, J., Kishida, T., and Tovey, M. G. 1983. Effect of anti-interferon serum on influenza virus infection in mice. *Antiviral Research* 3:59-65.

Hostynek, J. J., Lauerma, A. I., Magee, P. S., Bloom, E., and Maibach, H. I. 1995. A local lymph-node assay validation study of a structure–activity relationship model for contact allergens. *Archives of Dermatological Research* 287:567-7 1.

Hou, J., Said, C., Franchi, D., Dockstader, P., and Chatterjee, N. K. 1994. Antibodies to glutamic acid decarboxylase and P2-C peptides in sera from coxsackie virus B4-infected mice and IDDM patients. *Diabetes* 43:1260-6.

Hounsell, E. F. 1994. Characterization of the glycosylation status of proteins. *Molecular Biotechnology* 2:45–60.

House, R. V., Pallardy, M. J., and Dean, J. H. 1989. Suppression of murine cytotoxic T-lymphocyte induction following exposure to 7,12-dimethylbenz[a]anthracene: dysfunction of antigen recognition. International *Journal of Immunopharmacology* 11:207-15.

House, R. V., Laur, L. D., Murray, M. J., Thomas, P. T., Ehrlich, J. P., Burleson, G. R., and Dean, J. H. 1990. Examination of immune parameters and host resistance mechanisms in B6C3F1 mice following adult exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin. *Journal of Toxicology and Environmental Health* 31:203-15.

Howard, J. C. 1995. Supply and transport of peptides presented by class I MHC molecules. *Current Opinion in Immunology* 7:69–76.

Howard, M., O'Garra, A., Ishida, H., de Waal Malefyt, R., and de Vries, J. 1992. Biological properties of interleukin-10. *Journal of Clinical Immunology* 12:239–47.

Howie, J. B., and Helyer, B. J. 1968. The immunology and pathology of NZB mice. *Advances in Immunology* 9:215–66.

Hruby, Z. 1983. Membrane receptors for the 3d component of complement (C3) on cells of the monocyte–macrophagesystem. *Postepy Higieny i Medycyny Doswiadczalnej* 37:287–308.

Hsiao, L., Takahashi, K., Takeya, M., and Arao, T. 1991. Differentiation and maturation of macrophages into interdigitating cells and their multicellular complex formation in the fetal and postnatal rat thymus. *Thymus* 17:219–35.

Hudson, B. G., Kalluri, R., Gunwar, S., Noelken, M. E., Mariyama, M., and Reeders, S. T. 1993. Molecular characteristics of the Goodpasture autoantigen. *Kidney International* 43:135-9.

Hudson, L. and Hay, F. C. 1989. *Practical Immunology 3rd ed.* Blackwell Scientific, Oxford. pp. 187–190.

Humbria, A., Diaz-Gonzalez, F., Campanero, M. R., Arroyo, A. G., Laffon, A., Gonzalez-Amaro, R., and Sanchez-Madrid, F. 1994. Expression of L-selectin, CD43 and CD44 in synovial neutrophils from patients with inflammatory joint disease: evidence for soluble forms of L-Selectin in the synovial fluid. *Arthritis and Rheumatology* 37:342–8.

Humphrey, J. H., and Frank, M. M. 1967. The localization of nonmicrobial antigens in the draining lymph nodes of tolerant, normal and primed rabbits. *Immunology* 13:87.

Hunter, C. A., and Remington, J. S. 1995. The role of IL-12 in toxoplasmosis. *Research in Immunology* 146:546-52.

Hurtenbach, U., Gleichmann, H., Nagata, N., Radaszkiewicz, T., and Gleichmann, E. 1987. Immunity to D-penicillamine: genetic, cellular and chemical requirements for induction of popliteal lymph node enlargement in the mouse. *Journal ofImmunology* 139:411–6.

Hutchcroft, J. E., and Biere, B. E. 1996. Signaling through CD28/CTLA-4 family receptors. *Journal of Immunology* 43:4071-4.

IARC. 1993. *Beryllium, Cesium, Mercury and Exposures in the Glass Industry,* International Agency for Research on Cancer, Lyon, pp 88–9.

Ikagawa, S., Matsushita, S., Chen, Y. Z., Ishikawa, T., and Nishimura, Y. 1996. Single amino acid substitutions on a Japanese cedar pollen allergen (Cryj 1)-derived peptide induced alterations in human T cell responses and T cell receptor antagonism. *Journal of Allergy & Clinical Immunology* 97:53-64.

Ikarashi, Y., Tsuchiya, T., and Nakamura, A. 1992. Detection of contact sensitivity of metal salts using the murine local lymph node assay. *Toxicology Letters* 62:53-61.

Ikarashi, Y., Tsuchiya, T., and Nakamura, A. 1993. A sensitive mouse lymph node assay with two application phases for detection of contact allergens. *Archives of Toxicology* 67:629–36.

Ilonen, J., Mustonen, A., Akerblom, H. K., and Kouvalainen, K. 1984. Immunological features in the course of type 1 diabetes. *Annals of Clinical Research* 16:64-73.

Indik, Z. K., Park, J. G., Hunter, S., and Schreiber, A. D. 1995. The molecular dissection of Fc gamma receptor mediated phagocytosis. *Blood* 86:4389–99.

Inoue, J. 1997. The TRAF family protein-mediated B cell proliferation signal and the mechanism of LMP1-induced B cell proliferation. *Nippon Rinsho, Japanese Journal of Clinical Medicine* 55:299–304.

Irons, R. D. 1985. *Histopathology of the Immune System: Structure and Function,* Raven Press, New York, pp. 11–22.

Isoe, T., Naito, M., Shirai, A., Hirai, R., and Tsuruo, T. 1992. Inhibition of different steps of the ubiquitin system by cisplatin and aclarubicin. *Biochimica et Biophysica Acta* 1117:131-5.

Itoh, N., Yasunaga, M., Hirashaima, M., Yoshida, O., and Nishikawa, S. I. 1996. Role of IL-7 and KL in activating molecules controlling the G1/S transition of B precursor cells. *International Immunology* 8:317-23.

Izquierdo, M., Balboa, M. A., Lamana, M. L., and Lopez-Botet, M. 1989. High toxic efficiency of ricin immunotoxins specific for the T-cell antigen receptor of a human leukemia T-cell line. *International Journal of Cancer* 43:697–702.

Jacob, C. 0. 1992a. Studies on the role of tumor necrosis factor in murine and human autoimmunity. *Journal of Autoimmunity* 5:133-43.

Jacob, C. 0. 1992b. Tumor necrosis factor alpha in autoimmunity: pretty girl or old witch? *Immunology Today* 13:122–5.

Jacobson, N. G., Szabo, S. J., Guler, M. L., Gorham, J. D., and Murphy, K. M. 1996. Regulation of interleukin-12 signaling during T helper phenotype development. *Advances in Experimental Medicine & Biology* 409:61–73.

Jaffe, I. A. 1986. Adverse effects profile of sulfhydryl compounds in man. *American Journal of Medicine* 80:471–6.

Jalkanen, S. T., Bargatze, R. F., Herron, L. R., and Butcher, E. C. 1986 A lymphoid cell surface glycoprotein involved in endothelial cell recognition and lymphocyte homing in man. *European Journal of Immunology* 16:1195–1202.

James, W., Weiss, R. A., and Simon, J. H. 1996. The receptor for HIV: dissection of CD4 and studies on putative accessory factors. *Current Topics in Microbiology & Immunology* 205:137–58.

Jameson, S. C., and Bevan, M. J. 1995. T cell receptor antagonists and partial agonists. *Immunity* 2:1-11.

Jankovic, B. D., and Radulovic, J. 1992. Enkephalins, brain and immunity: modulation of immune responses by methionine-enkephalin injected into the cerebral cavity. *International Journal of Neuroscience* 67:241-70.

Janossy, G., Veronese, D. F., Klatzman, D., Gluckman, J. C., and Popovic, M. 1987. Monoclonal antibodies to human immunodeficiency virus: their relation to the patterns of lymph node changes in persistent generalized lymphadenopathy and AIDS. *AIDS* 1:95–104.

Janson, R. W., and Arend, W. P. 1992. Receptor-targeted immunotherapy. *Bulletin on the Rheumatic Diseases* 41:6-8.

Janusz, M. 1991. Immunoregulatory function of macrophages. *Postepy Higieny i Medycyny Doswiadczalnej* 45:1-15.

Jelinek, D. F., Spiawski, J. B., and Lipsky, P. 1986. Human peripheral blood B lymphocyte subpopulations: functional and phenotypic analyses of surface IgD positive and negative subsets. *Journal of Immunology* 136:83–92.

Jenkins, M. K., and Miller, R. A. 1992. Memory and anergy: challenges to traditional models of T lymphocyte differentiation. *FASEB Journal* 6:2428–33.

Jentsch, S. 1992. The ubiquitin–conjugation system. Annual Review of Genetics 26:179–207.

Jerner, N. K., and Nordin, A. A., 1963. Plaque formation in agar by single antibody producing cells. *Science* 140:403–408.

Jobanputra, P., Choy, E. H., Kingsley, G. H., Sieper, J., Palacios-Boix, A. A., Heinegard, D., and Panayi, G. S. 1992. Cellular immunity to cartilage proteoglycans: relevance to the pathogenesis of ankylosing spondylitis. *Annals of the Rheumatic Diseases* 51:959-62.

Jones, E. Y. 1997. MHC class I and class II structures. *Current Opinion in Immunology* 9:75–9.

Jorgensen, C., Bologna, C., and Sany, J. 1995. Autoimmunity. Insights provided by the SCID mouse model. *Revue Du Rhumatisme,* English Edition 62:519-24.

Jullien, D., Brossay, L., Sieling, P. A., Modlin, R. L., and Kronenberg, M. 1996. CD1: clues on a new antigen-presenting pathway. *Research in Immunology* 147:321–8.

June, C. H., Bluestone, J. A., Nadler, L. M., and Thompson, C. B. 1994. The B7 and CD28 receptor families. *Immunology Today* 15:321–31.

Jutila, M. A., Rott, L., Berg, E. L., and Butcher, E. C. 1989. Function and regulation of the neutrophil MEL-14 antigen in vivo: comparison with LFA-1 and MAC-1. *Journal of Immunology* 143:3318–24.

Kabelitz, D. 1992. Function and specificity of human $8-positive T cells. *Critical Reviews in Immunology* 11:281–303.

Kaeser, H. E. 1984. Drug-induced myasthenic syndromes. *Acta Neurologica Scandinavica Supplementum* 100:39–47.

Kagan, B. L., Ganz, T., and Lehrer, R. I. 1994. Defensins: a family of antimicrobial and cytotoxic peptides. *Toxicology* 87:131-49.

Kageyama, T., Yonezawa, S., Ichinose, M., Miki, K., and Moriyama, A. 1996. Potential sites for processing of the human invariant chain by cathepsins D and E. *Biochemical and Biophysical Research Communications* 223:549-53.

Kagi, D., and Hengartner, H. 1996. Different roles for cytotoxic T cells in the control of infections with cytopathic versus noncytopathic viruses. *Current Opinion in Immunology* 8:472–7.

Kalden, J. R., Lohmann, E., Peter, H. H., and Hilger, C. 1976. Antibody-dependent cellular cytotoxicity and B- and T-cell activity in the peripheral blood of myasthenia gravis patients. *Annals of the New York Academy of Sciences* 274:421–33.

Kalluri, R., Wilson, C. B., Weber, M., Gunwar, S., Chonko, A. M., Neilson, E. G., and Hudson, B. G. 1995. Identification of the alpha 3 chain of type IV collagen as the common autoantigen in antibasement membrane disease and Goodpasture syndrome. *Journal of the American Society of Nephrology* 6:1178-85.

Kamanaka, M., Yu, P., Yasui, T., Yoshida, K., Kawabe, T., Horii, T., Kishimoto, T., and Kikutani, H. 1996. Protective role of CD40 in Leishmania major infection at two distinct phases of cell-mediated immunity. *Immunity* 4:275–81.

Kamel, 0. W., Van de Rijn, M., and Weiss, L. M. 1993. Reversible lymphoma associated with Epstein Barr virus occurring during methyltrexate therapy for rheumatoid arthritis and dermatomyositis. *New England Journal of Medicine* 328:1317-21.

Kandaswani, C. 1992. Effect of flavonoids on immune and inflammatory cell function. *Biochemical Pharmacology* 17:1167-79.

Kaneshima, H., Chen, B., Namikawa, R., Outzen, H., Rabin, L., Tsukamato, A., and McCune, J. M. 1990. Today's SCID-hu mouse. *Science* 348:561-2.

Kanno, T., Miyata, M., Kazuta, Y., Sato, Y., Nishimaki, T., and Kasukawa, R. 1992. Carbamazepine-induced systemic lupus erythematosus-like disease. *Internal Medicine* 31:1303-5.

Kapasi, K., Chui, B., and Inman, R. D. 1992. HLA-B27/microbial mimicry: an in vivo analysis. *Immunology* 77:456–61.

Kaplan, A. P., Kuna, P., and Reddigari, S. R. 1995. Chemokines and the allergic response. *Experimental Dermatology* 4:260–5.

Karjalainen, J., Knip, M., Hyoty, H., Leinikki, P., Ilonen, J., Kaar, M. L., and Akerblom, H. K. 1988. Relationship between serum insulin autoantibodies, islet cell antibodies and coxsackie-B4 and mumps virus-specific antibodies at the clinical manifestation of type 1 (insulin-dependent) diabetes. *Diabetologia* 31:146-52.

Karol, M. H. 1995. Assays to evaluate pulmonary hypersensitivity. In *Methods in Immu-*

notoxicology, Volume 2 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 401–9.

Karras, J. G., and Holsapple, M. P. 1994. Mechanisms of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced disruption of B lymphocyte signaling in the mouse: a current perspective. *Experimental and Clinical Immunogenetics* 11:110-8.

Karzai, A. W., and McMacken, R. 1996. A bipartite signaling mechanism involved in DnaJ-mediated activation of the Escherichia coli DnaK protein. *Journal of Biological Chemistry* 271:11236-46.

Kasuga, A., Falorni, A., Maruyama, T., Ozawa, Y., Grubin, C. E., Matsubara, K., Takei, I., Saruta, T., Scheynius, A., and Lernmark, A. 1996. HLA class II is associated with the frequency of glutamic acid decarboxylase M(r) 65,000 autoantibodies in Japanese patients with insulin-dependent diabetes mellitus. *Acta Diabetologica* 33:108-13.

Katunuma, N., Kakegawa, H., Matsunaga, Y., and Saibara, T. 1994. Immunological significances of invariant chain from the aspect of its structural homology with the cystatin family. *FEBS Letters* 349:265–9.

Katz, D. R., and Sunshine, G. H. 1986. Comparative accessory cell function of Langerhans cells isolated from mouse skin. *British Journal of Experimental Pathology* 67:157–66.

Katz, S. I. 1986. The role of Langerhans cells in allergic contact dermatitis. *American Journal of Dermatopathology* 8:232–3.

Katz, S. I., Tamaki, K., and Sachs, D. H. 1979. Epidermal Langerhans cells are derived from cells originating in bone marrow. *Nature* 282:324–6.

Kaufmann, S. H. 1987. Lymphokines, interleukins, cytokines: function and action. *Immunitat und Infektion* 15:127-34.

Kaufmann, S. H. 1994. Heat shock proteins and autoimmunity: a critical appraisal. *International Archives of Allergy and Applied Immunology* 103:317-22.

Kawakami, M., Ihara, I., Suzuki, A., and Harada, Y. 1982. Properties of a new complement dependent bactericidal factor specific for Ra chemotype salmonella in sera of conventional and germ free mice. *Journal of Immunology* 129:2198–2201.

Kaye, M. D. 1987. Immunological aspects of gastritis and pernicious anemia. *Baillieres Clinical Gastroenterology* 1:487-506.

Kayser, D., and Schlede, E. 1995. *Chemicals and Contact Allergy — A Summary Evaluation,* Bundesinstitut for gesundheitlichen Verbraucherschutz und Veterinarmedizin, Berlin, pp. 22–4.

Kazatchkine, M. D., and Fearon, D. T. 1990. Deficiencies of human C3 complement receptors type 1 (CR1, CD35) and type 2 (CR2, CD21). *Immunodeficiency Reviews* 2:17–41.

Keegan, A. D., Nelms, K., Wang, L. M., Pierce, J. H., and Paul, W. E. 1994. Interleukin 4 receptor: signaling mechanisms. *Immunology Today* 15:423–32.

Kehry, M. R. 1996. CD40-mediated signaling in B cells. *Journal of Immunology* 156:2345–8.

Keightley, R. G., Cooper, M. D., and Lawton, A. 1976. The T cell dependence on B cell differentiation induced by pokeweed mitogen. *Journal of Immunology* 117:1538-43.

Kelley, W. L., and Georgopoulos, C. 1997. The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the Escherichia coli DnaJ molecular chaperone. *Proceedings of the National Academy of Sciences (USA)* 94:3679–84.

Kemp, M., Kurtzhals, J. A., Kharazmi, A., and Theander, T. G. 1993. Interferon-gamma and interleukin-4 in human Leishmania donovani infections. *Immunology & Cell Biology* 71:583-7.

Kennedy, J., Rossi, D. L., Zurawski, S. M., Vega, F., Jr., Kastelein, R. A., Wagner, J. L., Hannum, C. H., and Zlotnik, A. 1996. Mouse IL-17: a cytokine preferentially expressed by alpha beta TCR+ CD4−CD8− cells. *Journal of Interferon & Cytokine Research* 16:611−7.

Ken, B. M., Kienzle, N., Burrows, J. M., Cross, S., Silins, S. L., Buck, M., Benson, E. M., Coupar, B., Moss, D. J., and Sculley, T. B. 1996. Identification of type B-specific and cross-reactive cytotoxic T-lymphocyte responses to Epstein−Barr virus. *Journal of Virology* 70:8858-64.

Keskinen, H., Alanko, K., and Saarinen, L. 1978. Experience with anhydride specific IgE in asthma due to diisocyanates. *Clinical Allergy* 18:597-604.

Kimber, I. 1989. Aspects of the immune response to contact allergens: opportunities for the development and modification of predictive test methods. *Food and Chemical Toxicology* 27:755-62.

Kimber, I., and Basketter, D. A. 1992. The murine local lymph node assay: a commentary on collaborative studies and new directions. *Food and Chemical Toxicology* 30: 165−9.

Kimber, I., and Dearman, R. J. 1993. Approaches to the identification and classification of chemical allergens in mice. *Journal of Pharmacological and Toxicological Methods* 29:11-6.

Kimber, I., and Weisenberger, C. 1989. A murine local lymph node assay for the identification of contact allergens. Assay development and results from an initial validation study. *Archives of Toxicology* 63:274−82.

Kimber, I., Hilton, J., and Botham, P. A. 1990. Identification of contact allergens using the murine local lymph node assay: comparisons with the Buehler occluded patch test in guinea pigs. *Journal of Applied Toxicology* 10: 173−80.

Kimber, I., Dearman, R. J., Scholes, E. W., and Basketter, D. A. 1994. The local lymph node assay: developments and applications. *Toxicology* 93:13-31.

Kimber, I., Hilton, J., Dearman, R. J., Gerberick, G. F., Ryan, C. A., Basketter, D. A., Scholes, E. W., Ladics, G. S., Loveless, S. E., House, R. V., and Guy, A. 1995. An international evaluation of the murine local lymph node assay and comparison of modified procedures. *Toxicology* 103:63−73.

Kimmel, C., and Gaylor, D. 1988. Issues in qualitative and quantitative risk analyses for developmental toxicology. *Risk Analyses* 8:15-21.

Kincade, P. W. 1992. Cell interaction molecules and cytokines which participate in B cell lymphopoiesis. *Baillieres Clinical Haemtology* 5:575-98.

Kinet, J. P. 1992. The gamma-zeta dimers of Fc receptors as connectors to signal transduction. *Current Opinion in Immunology* 4:43−8.

Kinet, J. P., Jouvin, M. H., Paolini, R., Numerof, R., and Scharenberg, A. 1996. IgE receptor (Fc epsilon RI) and signal transduction. *European Respiratory Journal Supplement* 22:116s-118s.

Kingsley, G., and Panayi, G. 1992. Antigenic responses in reactive arthritis. *Rheumatic Diseases Clinics of North America* 18:49−66.

Kirton, V. 1978. Contact urticaria and cinnamic aldehyde. *Contact Dermatitis* 4:374−5.

Kishimoto, T. 1992. Interleukin-6 and its receptor in autoimmunity. *Journal of Autoimmunity* 5:123–32.

Kishore, U., Sontheimer, R. D., Sastry, K. N., Zappi, E. G., Hughes, G. R., Khamashta, M. A., Reid, K. B., and Eggleton, P. 1997. The systemic lupus erythematosus (SLE) disease autoantigen-calreticulin can inhibit C 1q association with immune complexes. *Clinical & Experimental Immunology* 108:181-90.

Kita, H., Hiroishi, K., Moriyama, T., Okamoto, H., Kaneko, T., Ohnishi, S., Yazaki, Y., and Imawari, M. 1995. A minimal and optimal cytotoxic T cell epitope within hepatitis C virus nucleoprotein. *Journal of General Virology* 76:3189-93.

Klaus, G. G. B., and Humphreys, J. H. 1977. The generation of memory cells. I. The role of C3 in the generation of B memory cells. *Immunology* 33:31–40.

Klaus, S. J., Berberich, I., Shu, G., and Clark, E. A. 1994. CD40 and its ligand in the regulation of humoral immunity. *Seminars in Immunology* 6:279–86.

Klein, G. 1996. EBV–Bcell interactions: immortalization, rescue from apoptosis, tumorigenicity. *Acta Microbiologica et Immunologica Hungarica* 43:97–105.

Kleine-Tebbe, J. 1993. Basophil histamine release by freshly prepared food extracts. *Allergy Proceedings* 14:255–8.

Kleinhans, D., Schach, A., Ruther, U., and Jipp, P. 1986. Immunocytoma with acquired C 1-esterase inhibitor deficiency and recurrent angioneurotic edema. *Deutsche Medizinische Wochenschrift* 111:742-4.

Kligman, A. M. The identification of contact allergens by human assay. **III.** The maximization test: a procedure for screening and rating contact sensitizers. 1966. *Journal of Investigative Dermatology* 92:151S- 152S.

Kligman, A. M., and Basketter, D. A. 1995. A critical commentary and updating of the guinea pig maximization test. *Contact Dermatitis* 32:129-34.

Klinman, N. R. 1996. In vitro analysis of the generation and propagation of memory B cells. *Immunological Reviews* 150:91–111.

Klinman, N. R., and Linton, P. J. 1988. The clonotype repertoire of B cell subpopulations. *Advances in Immunology* 42:1-93.

Klinman, N. R., and Linton, P. J. 1990. The generation of B-cell memory: a working hypothesis. *Current Topics in Microbiology and Immunology* 159:19-35.

Klinman, N. R., and Thorbecke, G. J. 1995. Aging associated changes in the generation and development of B cell memory. *International Reviews of Immunology* 12:5-12.

Klopman, G., Wang, S., and Balthasar, D. M. 1992. Estimation of aqueous solubility of organic molecules by the group contribution approach to the study of biodegradation. *Journal of Chemical Information and Computer Science* 32:474–82.

Knight, S. C., Macatonia, S. E., Compels, M., Pinching, A. J., and Patterson, S. 1992a. Zidovudine reverses the defect in dendritic cells in HIV infection. *AIDS* 6:231–2.

Knight, S. C., Stagg, A., Hill, S., Fryer, P., and Griffiths, S. 1992b. Development and function of dendritic cells in health and disease. *Journal of Investigative Dermatology* 99:33S-38S.

Ko, Y. H., Delannoy, M., and Pedersen, P. L. 1997. Cystic fibrosis, lung infections and human tracheal antimicrobial peptide (hTAP). *FEBS Letters* 405:200–8.

Koers, W. J., Houben, G. F., and Berrens, L. 1989. Blood groups AB0 and grass-pollen hayfever. *Allergie und Immunologie* 35:167-72.

Kogure, A,, Miyata, M., Nishimaki, T., and Kasukawa, R. 1994. Proliferative response of

synovial fluid mononuclear cells of patients with rheumatoid arthritis to mycobacterial 65 kDa heat shock protein and its association with HLA-DR+gamma delta+ T cells. *Journal of Rheumatology* 21:1403-8.

Kohl, S., Sigaroudinia, M., Charlebois, E. D., and Jacobson, M. A. 1996. Interleukin-I2 administered in vivo decreases human NK cell cytotoxicity and antibody-dependent cellular cytotoxicity to human immunodeficiency virus-infected cells. *Journal of Infectious Diseases* 174:1105-8.

Kohler, G., and Milstein, C. 1975. Continuous cultures of fused cells secreting antibodies of predefined specificity. *Nature (London)* 256:495–7.

Kohsaka, H., Nanki, T., Ollier, W. E., Miyasaka, N., and Carson, D. A. 1996. Influence of the rheumatoid arthritis-associated shared epitope on T-cell receptor repertoire formation. *Proceedings of the Association of American Physicians* 108:323–8.

Koike, M., and Takatsu, K. 1994. IL-5 and its receptor: which role do they play in the immune response? *International Archives of Allergy and Applied Immunology* 104:1-9.

Kokai, Y., Kimura, H., Matsuura, A., and Fujimoto, J. 1996. Selective inhibition of resistance to hemopoietic allografts but not rejection to a natural killer cell sensitive tumor in transgenic mice for granulocyte colony stimulating factor. *Artificial Organs* 20:883–9.

Kolb, J. P., Abadie, A., Proschnicka-Chalufour, A., de Gramont, A.. and Poggioli, J. 1994. CD23-mediated cell signalling. *Journal ofLipid Mediators & Cell Signaling* 9:27–35.

Kolb, J. P., Paul-Eugene Dugas, N., Yamaoka, K., Mossalayi, M. D., and Dugas, B. 1995. Role of CD23 in NO production by human monocytic cells. *Research in Immunology* 146:684–9.

Koller, L. D. 1985. Effect of chemical sensitivity on the immune system. *Immunology and Allergy Practice* 7:13-25.

Koller, M. R., Oxender, M., Brott, D. A., and Palsson, B. 0. 1996. flt-3 ligand is more potent than c-kit ligand for the synergistic stimulation of ex vivo hematopoietic cell expansion. *Journal of Hematotherapy* 5:449-59.

Kolls, J. K., Nelson, S., and Summer, W. R. 1993. Recombinant cytokines and pulmonary host defense. *American Journal of the Medical Sciences* 306:330–5.

Kool, J., Gerrits-Boeye, M. Y., Severijnen, A. J., and Hazenberg, M. P. 1992. Immunohistology of joint inflammation induced in rats by cell wall fragments of Eubacterium aerofaciens. *Scandinavian Journal of Immunology* 36:497–506.

Kopp, E., and Ghosh, S. 1994. Inhibition of NF-KB by sodium salicylate and aspirin. *Science* 265:956–8.

Korner, H., and Sedgwick, J. D. 1996. Tumour necrosis factor and lymphotoxin: molecular aspects and role in tissue-specific autoimmunity. *Immunology & Cell Biology* 74:465-72.

Kosco, M. H., Burton, G. F., Kapasi, Z. F., Szakal, A. K., and Tew, J. G. 1989. Antibody-forming cell induction during an early phase of germinal centre development and its delay with aging. *Immunology* 68:312–8.

Kosco, M. H., Pflugfelder, E., and Gray, D. 1992. Follicular dendritic cell-dependent adhesion and proliferation of B cells in vitro. *Journal of Immunology* 148:2331–9.

Kosco-Vilbois, M. H., and Scheidegger, D. 1995. Follicular dendritic cells induce B cell proliferation. *Current Topics in Microbiology and Immunology* 201:69-82.

Kosco-Vilbois, M. H., Zentgraf, H., Gerdes, J., and Bonnefy, J.-Y. 1997. To "B" or not to "B" a germinal center. *Immunology Today* 18:225–9.

Kradin, R. L., Xia, W., Pike, M., Byers, H. R., and Pinto, C. 1996. Interleukin-2 promotes the motility of dendritic cells and their accumulation in lung and skin. *Pathobiology* 64:180-6.

Krakauer, T. 1986. Human interleukin 1. Critical Reviews in Immunology 6:213–44.

Kroemer, G., and Wick, G. 1989. The role of interleukin 2 in autoimmunity. *Immunology Today* 10:246–51.

Kroese, F. G. M., Timens, W., and Nieuwenhuis, P. 1990. *Germinal Center Reaction and B Lymphocytes: Morphology and Function,* Springer-Verlag, Berlin, pp. 103–48.

Kroncke, R., Loppnow, H., Flad, H. D., and Gerdes, J. 1996. Human follicular dendritic cells and vascular cells produce interleukin-7: a potential role for interleukin-7 in the germinal center reaction. *European Journal of Immunology* 26:2541–4.

Kropshofer, H., Hammerling, G. J., and Vogt, A. B. 1997. How DM edits the MHC class II peptide repertoire: survival of the fittest. *Immunology Today* 18:77–81.

Krystek, S. R., Dias, J. A., Reichert, L. E., and Anderson, T. T. 1985. Prediction of antigenic sites in follicle stimulating hormones: different profiles enhance antigenicity. *Endocrinology* 11:1125–31.

Krystyniak, K., Brouland, J. P., Patriarca, C., Verdier, F., Revillard, J. P., and Descotes, J. 1992. Activation of CD4+ and CD8+ lymphocyte subsets by streptozotocin in the murine popliteal lymph node test. *Journal of Autoimmunity* 5:183-97.

Kubota, A., Nagafuji, K., Harada, M., Otsuka, T., Harada, N., Eto, T., Takamatsu, Y., Fukuda, T., Shimoda, K., Inaba, S., Okamura, S., and Niho, Y. 1996. Immunophenotyping of peripheral blood mononuclear cells collected by cytotoxic and cytotoxic/G-CSF mobilization. *Clinical and Laboratory Haematology* 18:181-5.

Kuby, J. 1991. *Immunology,* Freeman, New York, pp. 487–8.

Kuby, J. 1994. *Immunology,* Freeman, New York, pp. 362–7.

Kundig, T. M., Bachmann, M. F., Ohashi, P. S., Pircher, H., Hengartner, H., and Zinkernagel, R. M. 1996. On T cell memory: arguments for antigen dependence. *Immunological Reviews* 150:63–90.

Kunkel, S. L., Lukacs, N., and Strieter, R. M. 1995. Chemokines and their role in human disease. *Agents & Actions Supplement* 46:11-22.

Kupper, T. S. 1988. Interleukin 1 and other human keratinocyte cytokines: molecular and functional characterization. *Advances in Dermatology* 3:293–307.

Kuppers, R. C., Neu, N., and Rose, N. R. 1988. Animal models of autoimmune thyroid disease. In *Immunogenetics of Endocrine Disorders* (N. R. Farid, ed.), Liss, New York, pp. 111–31.

Kuroki, K., Roy, S., Laerum, F., Mollnes, T. E., Solheim, B. G., and Videm, V. 1995. Complement activation by angiographic catheters in vitro. *Journal of Vascular and Interventional Radiology* 6:819–26.

Kutemeier, G., Hohne, W., Werner, T., Schuh, R., and Mocikat, R. 1994. Assembly of humanized antibody genes from synthetic oligonucleotides using a single round-PCR. *Biotechniques* 17:242–6.

Kwok, W. W., Nepon, G. T., and Raymond, F. C. 1995. HLA-DQ polymorphisms are highly selective for peptide binding interactions. *Journal of Immunology* 155:2468–76.

Labrecque, N., Thibodeau, J., and Sekaly, R. P. 1993. T-cell receptor recognition of superantigens: another view. *Research in Immunology* 144:175-80.

Lachmann, P. J. 1974. Genetic deficiencies of the complement system. *Bollettino dell Istituto Sieroterapico Milanese* Supplement 53:195-207.

Lacki, J. K., and Wiktorowicz, K. E. 1994. Biological properties of interleukin 10. *Postepy Higieny i Medycyny Doswiadczalnej* 48:363–70.

LaGasse, J., Jelinek, L., Sexson, S., Lofton-Day, C., Breininger, J., Sheppard, P., Kindsvogel, W., and Hagopian, W. A. 1997. An islet-cell protein tyrosine phosphatase is a likely precursor to the 37-kDa autoantigen in type. 1 diabetes: human and macaque sequences, tissue distribution, unique and shared epitopes, and predictive autoantibodies. *Molecular Medicine* 3:163–73.

Laivoranta, S., Ilonen, J., Tuokko, J., Luukkainen, R., and Toivanen, A. 1995. HLA frequencies in HLA-B27 negative patients with reactive arthritis. *Clinical and Experimental Rheumatology* 13:637-40.

Laman, J. D., Kors, N., Van Rooijen, N., and Claassen, E. 1990. Mechanism of follicular trapping: localization of immune complexes and cell remnants after elimination and repopulation of different spleen cell populations. *Immunology* 71:57-62.

Laman, J. D., Van den Eertwegh, A. J. M., Claassen, E., and Van Rooijen, N. 1992. Cell–cell interactions: in situ studies of splenic humoral responses. In *Immune System Accessory Cells* (L. Fornusek and V. Veivicka, eds.), CRC Press, Boca Raton, pp. 201–31.

Lando, P. A., Olsson, C., Kalland, T., Newton, D., Kotb, M., and Dohlsten, M. 1996. Regulation of superantigen in induced T cell activation in the absence and the presence of MHC class II. *Journal of Immunology* 157:2857–63.

Landry, S. J. 1997. Local protein instability predictive of helper T-cell epitopes. *Immunology Today* 18:527-32.

Lane, P. 1996. Development of B-cell memory and effector function. *Current Opinion in Immunology* 8:331–5.

Lanier, L. L., and Phillips, J. H. 1996. Inhibitory MHC class I receptors on NK cells and T cells. *Immunology Today* 17:86–91.

Lanier, L. L., Kipps, T. J., and Phillips, J. H. 1985. Functional properties of a unique subset of cytotoxic T lymphocytes that express Fc receptors for IgG (CD16/Leu11). *Journal of Experimental Medicine* 162:2089–106.

Lanier, L. L., Corliss, B., and Phillips, J. H. 1997. Arousal and inhibition of human NK cells. *Immunological Reviews* 155:145–54.

Larose, L., Gish, G., Shoelson, S., and Pawson, T. 1993. Identification of residues in the B-platelet-derived growth factor receptor that confer specficity for binding to phospholipase CgI. *Oncogene* 8:2493–9.

Larsen, B., Birkelund, S., Mordhorst, C. H., Ejstrup, L., Andersen, L. S., and Christiansen, G. 1994. The humoral immune response to Chlamydia trachomatis in patients with acute reactive arthritis. *British Journal of Rheumatology* 33:534-40.

Larsen, G. L. 1985. Late-phase reactions: observations on pathogenesis and prevention. *Journal of Allergy and Clinical Immunology* 76:665-9.

Launois, P., Swihart, K. G., Milon, G., and Louis, J. A. 1997. Early production of IL-4 in susceptible mice infected with Leishmania major rapidly induces IL-12 unresponsiveness. *Journal of Immunology* 158:3317-24.

Law, C.-L., Sidorenko, S. V., and Clark, E. A. 1994. Regulation of lymphocyte activation by the cell-surface molecule CD22. *Immunology Today* 15:442-8.

Lawyer, C., Pai, S., Watabe, M., Bakir, H., Eagleton, L., and Watabe, K. 1996. Effects of the synthetic form of tracheal antimicrobial peptide on respiratory pathogens. *Journal of Antimicrobial Chemotherapy* 37:599-604.

Leahy, D. J. 1995. A structural view of CD4 and CD8. *FASEB Journal* 9:17–25.

Lechler, R., Aichinger, G., and Lightstone, L. 1996. The endogenous pathway of MHC class II antigen presentation. *Immunological Reviews* 15:51-79.

Lee, C., and McConnell, H. M. 1995. A general model of invariant chain association with class II major histocompatibility complex proteins. *Proceedings of the National Academy of Sciences (USA)* 92:8269–73.

Lee, J. M., Kay, C. M., and Watts, T. H. 1992. Conformational changes in mouse MHC class II proteins at acidic pH. *International Immunology* 4:889–97.

Lee, N., Malacko, A. R., Ishitani, A., Chen, M. C., Bajorath, J., Marquardt, H., and Geraghty, D. E. 1995. The membrane-bound and soluble forms of HLA-G bind identical sets of endogenous peptides but differ with respect to TAP association. *Immunity* 3:591–600.

Legge, K. L., Min, B., Potter, N. T., and Zaghouani, H. 1997. Presentation of a T cell receptor antagonist peptide by immunoglobulins ablates activation of T cells by a synthetic peptide or proteins requiring endocytic processing. *Journal of Experimental Medicine* 185:1043–53.

Leiter, E. H., and Serreze, D. V. 1992. Antigen presenting cells and the immunogenetics of autoimmune diabetes in NOD mice. *Regional Immunology* 4:263–73.

Lernpertz, U., Kuhn, U., Knop, J., and Becker, D. 1996. An approach to predictive testing of contact sensitizers in vitro by monitoring their influence on endocytotic mechanisms. *International Archives of Allergy and Immunology* 111:64-70.

Lenert, P., and Zanetti, M. 1991. CD4/immunoglobulin interaction: implications for immune physiology and autoimmunity. *International Reviews of Immunology* 7:237–44.

Leong, K. H., Ramshaw, I. A., and Ramsay, A. J. 1997. Interleukin-7 enhances cell-mediated immune responses in vivo in an interleukin-2-dependent manner. *Viral Immunology* 10:1-9.

Lernmark, A., Barmeier, H., Dube, S., Hagopian, W., Karlsen, A., and Wassmuth, R. 1991. Autoimmunity of diabetes. *Endocrinology and Metabolism Clinics of North America* 20:589–617.

Leung, D. Y. M. 1993. Mechanisms controlling the human immunoglobulin E response: new directions in the therapy of allergic diseases. *Pediatric Research* 33:S56–62.

Leutwyler. K. 1995. An inside job. IL-12 attacks tumors on two fronts, but can it win the battle? *Scientific American* 273:24.

Levine, B. B., Stember, R. H., and Fotino, M. 1972. Ragweed hay fever: genetic control and linkage to HL-A haplotypes. *Science* 178:1201–3.

Levitz, S. M., Mathews, H. L., and Murphy, J. W. 1995. Direct antimicrobial activity of T cells. *Immunology Today* 16:387–91.

Levy, J. A., Mackewicz, C. E., and Barker, E. 1996. Controlling HIV pathogenesis: the role of the non-cytotoxic anti-HIV response of CD8+ T cells. *Immunology Today* 17:217–24.

Lew, P. D., Southwick, F. S., Stossel, F. S. 1900. A variant of chronic granulomatous disease: deficient oxidative metabolism due to low affinity NADPH oxidase. *New England Journal of Medicine* 305:1329-1333.

Lewis, J. G. 1995. State of macrophage activation: tumor cell cytolysis. In *Methods in Immunotoxicology,* Volume 1 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 59–65.

Lewis, S. C., Lynch, J. R., and Nikiforov, A. I. 1990. A new approach to deriving community exposure guidelines from "no observed-adverse-effect levels." *Regulatory Toxicology and Pharmacology* 11:314-30.

Li, Q., Wang, Z. Y., Inagaki, H., Li, Y. J., and Minami, M. 1995. Evaluation of contact sensitivity to formaldehyde and tetramethylthiuram monosulfide using a modified lymphocyte transformation test. *Toxicology* 104:17-23.

Liang, M. N., Lee, C., Xia, Y., and McConnell, H. M. 1996. Molecular modeling and design of invariant chain peptides with altered dissociation kinetics from class II MHC. *Biochemistry* 35:14734-42.

Licastro, F., Davis, L. J., and Morini, M. C. 1993. Lectins and superantigens: membrane interactions of these compounds with T lymphocytes affect immune responses. *International Journal of Biochemistry* 25:845-52.

Lichtman, A. H., and Abbas, A. K. 1997. T-cell subsets: recruiting the right kind of help. *Current Biology* 7:R242–4.

Liles, W. C., and Van Voorhis, W. C. 1995. Review: nomenclature and biologic significance of cytokines involved in inflammation and the host immune response. *Journal of Infectious Diseases* 172:1573–80.

Limpens, J., Van Meijer, M., Van Santen, H. M., Germeraad, W. T., Hoeben-Schornagel, K., Breel, M., Scheper, R. J., and Kraal, G. 1991. Alterations in dendritic cell phenotype and function associated with immunoenhancing effects of a subcutaneously administered cyclophosphamide derivative. *Immunology* 73:255–63.

Lin, T. J., Enciso, A., Bissonnette, E. Y., Szczepek, A., and Befus, A. D. 1996. Cytokine and drug modulation of TNF alpha in mast cells. *Advances in Experimental Medicine and Biology* 409:279–85.

Lin, W. Q., and White, K. L. 1995. Complement assays to assess immunotoxicity. In *Methods in Immunotoxicology,* Volume 1 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 357–75.

Lin, Y. L., Huang, Y. L., Ma, S. H., Yeh, C. T., Chiou, C. T., Chen, L. K., and Liao, C. L. 1997. Inhibition of Japanese encephalitis virus infection by nitric oxide: antiviral effect of nitric oxide on RNA virus replication. *Journal of Virology* 71:5227-35.

Lindstrom, J. 1979. Autoimmune response to acetylcholine receptors in myasthenia gravis and its animal model. *Advances in Immunology* 27: 1–50.

Lipes, M. A., and Eisenbarth, G. S. 1990. Transgenic mouse models of type I diabetes. *Diabetes* 39:79-84.

Lipscombe, R. J., Sumiya, M., Summerfield, J. A., and Turner, M. W. 1995. Distinct physiochemical characteristics of human mannose binding protein expressed by individuals of differing phenotype. *Immunology* 85:660–7.

Lisak, R. P. 1975. Immunologic aspects of myasthenia gravis. *Annals of Clinical & Laboratory Science* 5:288–93.

Lisak, R. P., Levinson, A. I., and Zweiman, B. 1985. Autoimmune aspects of myasthenia gravis. *Concepts in Immunopathology* 2:65–101.

Lite, H. S., and Braley-Mullen, H. 1981. Induction of IgG memory responses with polyvinylpyrrolidine (PVP) is antigen dose dependent. *Journal of Immunology* 126:928–33.

Liu, C., and Zhang, Y. 1991a. The enhancing effect of calcium ionophore A23187 on the accessory function of mouse dendritic cells. *Chung-Kuo i Hsueh K'o Hsueh Yuan Hsueh Pao Acta Academiae Medicinae Sinicae* 13:176-80.

Liu, C., and Zhang, Y. 1991b. Phorbol myristate acetate and calcium ionophore A23187 modulate the accessory cell function of mouse dendritic cells. *Chinese Medical Sciences Journal* 6:18–23.

Liu, C., and Zhang, Y. 1992. Effect of the tumor promoter phorbol myristate acetate (PMA) on cell-mediated immunity. IV. PMA-induced modulation of mouse dendritic cell function. *Chung-Kuo i Hsueh K'o Hsueh Yuan Hsueh Pao Acta Academiae Medicinae Sinicae* 14:456–60.

Liu, C.-C., Walsh, C. M., and Young, J. D.-E. 1995. Perforin: structure and function. *Immunology Today* 16: 194–9.

Liu, F.-T. 1990. Molecular biology of IgE binding protein, IgE binding factors and IgE receptors. *Critical Reviews in Immunology* 10:289–306.

Liu, J. 1993. K506 and cyclosporin, molecular probes for studying intracellular signal transduction. *Immunology Today* 14:290–5.

Liu, Q., Du, X. X., Schindel, D. T., Yang, Z. X., Rescorla, F. J., Williams, D. A., and Grosfeld, J. L. 1996. Trophic effects of interleukin-11 in rats with experimental short bowel syndrome. *Journal of Pediatric Surgery* 31:1047-50.

Loegering, D. J. 1986. Kupffer cell complement receptor clearance function and host defense. *Circulatory Shock* 20:321–33.

Lopez-Botet, M., Moretta, L., and Strominger, J. 1996. NK-cell receptors and recognition of MHC class I molecules. *Immunology Today* 17:212–4.

Lopez-Botet, M., Perez-Villar, J. J., Carretero, A., Melero, I., Bellon, T., Llano, M., and Navarro, F. 1997. Structure and function of CD94 C-lectin receptor complex involved in the recognition of HLA class I molecules. *Immunological Reviews* 155:165–74.

Lopez De Castro, J. A. 1996. HLA-B27 and HLA-B73 polymorphism and its role on antigenicity, peptide presentation, and disease susceptibility. *Clinical Rheumatology* 15:67–71.

Loveless, S. E., and Munson, A. E. 1981. Maleic vinyl ether activation of murine macrophages against lung metastasizing tumors. *Cancer Research* 41:3901–6.

Lovett, E. J., Schnitzer, B., Keren, D. F., Flint, A., Hudson, J. L., McClatchey, K. D., 1994. Application of flow cytometry to diagnostic pathology laboratory investigation. 50:115-140.

Lu, J. 1997. Collectins: collectors of microorganisms for the innate immune system. *Bioessays* 19:509–18.

Ludmerer, S. W., Benincasa, D., and Mark, G. E. 1996. Two amino acid residues confer type specificity to a neutralizing, conformationally dependent epitope on human papillomavirus type 11. *Journal of Virology* 70:4791–4.

Ludvigsson, J. 1989. Immunological aspects of IDDM in children. *Indian Journal of Pediatrics* 56:S7-14.

Lundkvist, A., Kallio-Kokko, H., Sjolander, K. B., Lankinen, H., Niklasson, B., Vaheri, A., and Vapalahti, 0. 1996. Characterization of Puumala virus nucleocapsid protein: identification of B-cell epitopes and domains involved in protective immunity. *Virology* 216:397–406.

Luster, M. I., Tucker, A. N., Germolec, D. R., Silver, M. T., Thomas, P. T., Vore, S. J., and Bucher, J. R. 1986. Immunotoxicity studies in mice exposed to methyl isocyanate. *Toxicology and Applied Pharmacology* 86:140-4.

Luster, M. I., Munson, A. E., Thomas, P. T., Holsapple, M. P., Fenters, J. D., White, K. L., Lauder, L. D., Germolec, D. R., Rosenthal, G. J., and Dean, J. H. 1988. Methods evaluation, development of a testing guideline to assess chemical-induced immunotoxicity: National Toxicology Program's guidelines for immunotoxicity evaluation in mice. *Fundamental and Applied Toxicology* 10:2–19.

Luster, M. I., Portier, C., Pait, D. G., White, K. L., Gennings, C., Munson, A. E., and Rosenthal, G. J. 1992a. Risk assessment in immunotoxicology I. Sensitivity and predictability of immune tests. *Fundamental and Applied Toxicology* 18:200–10.

Luster, M. I., Pait, D. G., Portier, C., Rosenthal, G. J., Germolec, D. R., Comment, C. E., Munson, A. E., White, K., and Pollock, P. 1992b. Qualitative and quantitative experimental models to aid in risk assessment for immunotoxicology. *Toxicology Letters* 64/65:71–8.

Luster, M. I., Portier, C., Pait, D. G., Rosenthal, G. J., Germolec, D. R., Corsini, E., Blaylock, B. L., Pollock, P., Kouchi, Y., Craig, W., White, K., Munson, A. E., and Comment, C. E. 1993. Risk assessment in immunotoxicology II. Relationships between immune and host resistance tests. *Fundamental and Applied Toxicology* 21:71–82.

Lynch, D. H. 1996. The role of FasL and TNF in the homeostatic regulation of immune responses. *Advances in Experimental Medicine and Biology* 406:135-8.

Lynch, R. G., Sandor, M., Nunez, R., Mathur, A., Hagen, M., Waldschmidt, T., Van Ness, B., Nelms, K., Noben, N., Ibraghimov, A., Mordue, D., Sacco, R., Teeraratkul, P., Schaiff, W. T., and Iakoubov, L. 1992. Lymphocyte Fc receptors: the immunobiology and pathology of CD23. *Immunobiology* 185:235–67.

Lyons, D. S., Lieberman, S. A., Hampl, J., Boniface, J. J., Chien, Y., Berg, L. J., and Davis, M. M. 1996. A TCR binds to antagonist ligands with lower affinities and faster dissociation rates than to agonists. *Immunity* 5:53-61.

Macatonia, S. E., Lau, R., Patterson, S., Pinching, A. J., and Knight, S. C. 1990. Dendritic cell infection, depletion and dysfunction in HIV-infected individuals. *Immunology* 71:38-45.

MacDonald, H. R., Budd, R. C., and Cerottini, J. C. 1990. Pgp-1 (Ly 24) as a marker of murine memory T lymphocytes. *Current Topics in Microbiology and Immunology* 159:97–109.

MacDonald, H. R., Lees, R. K., Baschieri, S., Herrmann, T., and Lussow, A. R. 1993. Peripheral T-cell reactivity to bacterial superantigens in vivo: the response/anergy paradox. *Immunological Reviews* 133:105-17.

MacLennan, I. C., and Gray, D. 1986. Antigen-driven selection of virgin and memory B cells. *Immunological Reviews* 91:61-85.

Maeda, T., and Nomoto, K. 1995. Anti-T cell receptor antibody treatment of mice with lupus-like graft versus host disease: suppression of glomerulonephritis without reduction in anti-DNA antibody levels. *Journal of Rheumatology* 22:2259–65.

Magee, P. S., Hostynek, J. J., and Maibach, H. I. 1994. A classification model for allergic contact dermatitis. *Quantitative Structure Activity Relationships* 13:22–33.

Maguire, H. C., Jr. 1996. Cyclophosphamide and interleukin-12 synergistically upregulate the acquisition of allergic contact dermatitis in the mouse. *Acta Dermaro-Venereologica* 76:277–9.

Mahapatro, D., and Mahapatro, R. C. 1984. Cutaneous basophil hypersensitivity. *American Journal of Dermatopathology* 6:483–9.

Mahzoon, S., Yamamoto, S., and Greaves, M. W. 1977. Response of skin to ammonium persulphate. *Acta Dermato-Venereologica* 57:125-6.

Maillet, F., Weiss, L., Chibani, J., and Kazatchkine, M. 1990. Factor I deficiency, a regulator protein of complement. *Presse Medicale* 19:762.

Maini, R. N., Elliott, M. J., Charles, P. J., and Feldmann, M. 1994. Immunological intervention reveals reciprocal roles for tumor necrosis factor-alpha and interleukin-10 in rheumatoid arthritis and systemic lupus erythematosus. *Springer Seminars in Immunopathology* 16:327–36.

Maki-Ikola, O., Leirisalo-Repo, M., Kantele, A., Toivanen, P., and Granfors, K. 1991. Salmonella-specific antibodies in reactive arthritis. *Journal of Infectious Diseases* 164:1141-8.

Maki-Ikola, O., Hill, J: L., Lahesmaa, R., Toivanen, A., and Granfors, K. 1992a. IgG and IgA antibody responses against porins in Yersinia-triggered reactive arthritis. *British Journal of Rheumatology* 31:315–8.

Maki-Ikola, O., Pulz, M., Heesemann, J., Lahesmaa, R., Saario, R., Toivanen, A., and Granfors, K. 1992b. Antibody response against 26 and 46 kilodalton released proteins of yersinia in yersinia triggered reactive arthritis. *Annals of Rheumatic Diseases* 51:1247-9.

Maki-Ikola, O., Yli-Kerttula, U., Saario, R., Toivanen, P., and Granfors, K. 1992c. Salmonella specific antibodies in serum and synovial fluid in patients with reactive arthritis. *British Journal of Rheumatology* 31:25-9.

Maki-Ikola, O., Lehtinen, K., Nissila, M., and Granfors, K. 1994. IgM, IgA and IgG class serum antibodies against Klebsiella pneumoniae and Escherichia coli lipopolysaccharides in patients with ankylosing spondylitis. *British Journal of Rheumatology* 33:1025-9.

Maki-Ikola, O., Nissila, M., Lehtinen, K., Leirisalo-Repo, M., and Granfors, K. 1995a. IgA1 and IgA2 subclass antibodies against Klebsiella pneumoniae in the sera of patients with peripheral and axial types of ankylosing spondylitis. *Annals of the Rheumatic Diseases* 54:631-5.

Maki-Ikola, O., Lehtinen, K., Toivanen, P., and Granfors, K. 1995b. Antibodies to Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis in the sera of ankylosing spondylitis patients with/without iritis and enthesitis. *British Journal of Rheumatology* 34:418-20.

Makovic, S. N., and Murasko, D. M. 1991. The role of natural killer and T cells in interferon induced inhibition of spontaneous metastases of the B16F10L murine melanoma. *Cancer Research* 51:1124-28.

Malhotra, R., Theil, S., Reid, K. B. M., and Sim, R. B. 1990. Human leukocyte C1q receptor binds other soluble proteins with collagen domains. *Journal of Experimental Medicine* 172:955–9.

Malhotra, R., Sim, R. B., and Jensenius, J. C. 1994. Collectins: collagenous C-lectins of the innate immune defense system. *Immunology Today* 15:67-74.

Manca, F., De Berardinis, P., Fenoglio, D., Ombra, M., Li Pira, G., Saverino, D., Autiero, M., Lozzi, L., Bracci, L., and Guardiolo, J. 1996. Antigenicity of HIV derived T helper determinants in the context of carrier recombinant proteins: effect on helper cell repertoire. *European Journal of Immunology* 26:2461–9.

Manns, M. P. 1997. Hepatotropic viruses and autoimmunity. *Journal of Viral Hepatitis* 4:7–10.

Marchal, T., Dezutter-Dambuyant, C., Bourdoiseau, G., Magnol, J. P., and Schmitt, D. 1995. Evidence that Langerhans cells migrate to regional lymph nodes during experimental contact sensitization in dogs. *Advances in Experimental Medicine & Biology* 378:219-21.

Markowicz, S., and Engleman, E. G. 1990. Granulocyte–macrophagecolony-stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells in vitro. *Journal of Clinical Investigation* 85:955–61.

Markus, N. R., Rosenberg, S. A., and Topalian, S. L. 1995. Analysis of cytokine secretion by melanoma-specific CD4+ T lymphocytes. *Journal of Interferon and Cytokine Research* 15:739–46.

Marletta, M. A. 1988. Mammalian synthesis of nitrite, nitrate, nitric oxide and N-nitrosating agents. *Chemical Research in Toxicology* 1:249–57.

Martensson, U., Thiel, S., Jensenius, J. C., and Sjoholm, A. G. 1996. Human autoantibodies against C1q: lack of cross reactivity with the collectins mannan-binding protein, lung surfactant protein A and bovine conglutinin. *Scandinavian Journal of Immunology* 43:314–20.

Martin, S., Kardorf, J., Schulte, B., Lampeter, E. F., Gries, F. A., Melchers, I., Wagner, R., Bertrams, J., Roep, B. O., and Pfutzner, A. 1995. Autoantibodies to the islet antigen ICA69 occur in IDDM and in rheumatoid arthritis *Diabetologia* 38:351-5.

Martinez-Valdez, H., Doherty, P. J., Thompson, E., Benedict, S. H., Gelfand, E. W., and Cohen, A. 1988. Antagonistic effects of calcium ionophores and phorbol esters on T cell receptor mRNA levels in human thymocytes. *Journal of Immunology* 140:361–6.

Marzulli, F. J., and McGuire, J. 1982. Usefulness and limitations of various guinea pig test methods in detecting human sensitizers — validation of guinea pig tests for skin hypersensitivity. *Fundamental Chemical Toxicology* 20:67–74.

Masera, R. G., Muscettola, M., Tanganelli, C., Grasso, G., Bateman, A., Solomo, P., and Angeli, A. 1995. In vitro effect of peptides of the corticosteroid/defensin family on production of mitogen induced cytokines. *Annali ltaliani di Medicina Interna* 10:113-8.

Mason, D., and Fowell, D. 1992. T-cell subsets in autoimmunity. *Current Opinion in Immunology* 4:728-32.

Mathews, K. P. 1990. Urticaria and conjunctivitis. *Current Opinion in Immunology* 2:535–41.

Mathias, C. G. 1983. *Clinical and Experimental Aspects of Cutaneous Irritation,* 2nd ed., McGraw–Hill, New York, pp. 167–8.

Matsushita, M., and Fujita, T. 1992. Activation of the classical complement pathway by mannose binding protein in association with a novel C1s-serine protease. *Journal of Experimental Medicine* 176:1497-502.

Mattner, F., Magram, J., Ferrante, J., Launois, P., Di Padova, K., Behin, R., Gately, M. K., Louis, J. A., and Alber, G. 1996. Genetically resistant mice lacking interleukin-12 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response. *European Journal of Immunology* 26:1553-9.

Maurer, T., and Hess, R. 1989. The maximization test for skin sensitization potential — updating the standard protocol and validation of a modified protocol. *Food and Chemical Toxicology* 27:807-11.

Maurer, T., and Kimber, I. 1991. Draining lymph node cell activation in guinea pigs: comparisons with the murine local lymph node assay. *Toxicology* 69:209–18.

Mayani, H., Guilbert, L. J., and Janowaska-Wieczorek, A. 1992. Biology of the hemopoietic microenvironment. *European Journal of Hematology* 49:225–33.

Mayer, R. J., and Doherty, F. J. 1992. Ubiquitin. *Essays in Biochemistry* 27:37–48.

Mayer, R. J., and Doherty, F. J. 1996. Selective proteolysis: 70-kDa heat-shock protein and ubiquitin-dependent mechanisms? *Sub-Cellular Biochemistry* 27: 137–58.

Mazur, G., and Frydecka, I. 1993. Interleukin 2 (IL-2) and its receptor (IL-2R) in healthy individuals and individuals with various disease states. *Acta Haematologica Polonica* 24:307-13.

Mazzanti, G., Paladini, G., Damiani, D., Bianchini, D., Florean, M., Mesesnel, E., and Cataldo, R. 1986. Side effects of d-penicillamine in the treatment of rheumatoid arthritis. *Minerva Medica* 17:1009–16.

McCormack, J. E., Wade, T., Morales, H., Kappler, J., and Marrack, P. 1991. Analysis of class II MHC structure in thymic nurse cells. *Cellular Immunology* 138:413–22.

McCune, J. M., Namikawa, R., Kaneshima, H., Shultz, L. D., Lieberman, M., and Weissman, I. L. 1988. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 241 : 1632–9.

McGhee, J. R., Fujihashi, K., Beagley, K. W., and Kiyono, H. 1991. Role of interleukin-6 in human and mouse mucosal IgA plasma cell responses. *Immunologic Research* 10:418-22.

McGrath, L. F., Georgopoulos, P., and Gallo, M. A. 1996. Application of a biologically based RFD estimation method to tetrachlorobenzo-p-dioxin (TCCD) mediated immune suppression and enzyme induction. *Risk Analysis* 16:539-48.

McLennan, I. C., Liu, Y. J., and Johnson, G. D. 1992. Maturation and dispersal of B-cell clones during T cell-dependent antibody responses. *Immunological Reviews* 126: 143–61.

Medenica, M., and Rostenberg, A. 1971. A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic contact dermatitis. *Journal of Investigative Dermatology* 56:259-7 1.

Medof, M. E., Prince, G. M., and Mold, C. 1982. Release of soluble immune complexes from immune adherence receptors on human erythrocytes is mediated by C3b inactivator independently of beta 1H and is accompanied by generation of C3c. *Proceedings of the National Academy of Sciences (USA)* 79:5047-51.

Medzhitov, R., and Janeway, C. A., Jr. 1997. Innate immunity: impact on the adaptive immune response. *Current Opinion in Immunology* 9:4–9.

Melamed, J., Alper, C. A., Cicardi, M., and Rosen, F. S. 1986. The metabolism of C1 inhibitor and C1q in patients with acquired C1-inhibitor deficiency. *Journal of Allergy & Clinical Immunology* 77:322–6.

Melian, A., Beckman,E. M., Porcelli, S. A., and Brenner, M. B. 1996. Antigen presentation by CD1 and MHC-encoded class I-like molecules. *Current Opinion in Immunology* 8:82–8.

Mendell, N. R., Blumenthal, M., Amos, D. B., Yunis, E. J., and Elston, R. C. 1978. Ragweed sensitivity: segregation analysis and linkage to HLA-B. *Cytogenetics and Cell Genetics* 22:330–4.

Mendlovic, S., Fricks, H., Shoenfeld, Y., and Mozes, T. 1989. The role of anti-idiotypic antibodies in the induction of experimental SLE in mice. *European Journal of Immunology* 19:729–34.

Menendez-Arias, L., and Rodriquez, R. 1990. A basic microcomputer program for prediction of BRT cell epitopes in proteins. *Computer Applications in the Biosciences* 6:101-10.

Menne, S., Maschke, J., Tolle, T. K., Lu, M., and Roggendorf, M. 1997. Characterization of T-cell response to woodchuck hepatitis virus core protein and protection of woodchucks from infection by immunization with peptides containing a T-cell epitope. *Journal of Virology* 71:65–74.

Men, S., and Pangburn, M. K. 1994. Regulation of the alternative pathway of complement activation by glycosaminoglycans: specificity of the polyanion binding site on factor H. *Biochemical and Biophysical Research Communications* 198:52-9.

Metcalfe, D. D., Astwood, J. D., Townsend, R., and Sampson, H. A. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. In *Criticial Reviews in Food Science and Nutrition,* Volume 36 (F. M. Clydesdale, ed.), CRC Press, Pearl River, NJ, pp. S165–S86.

Metzger, H. 1992. The receptor with high affinity for IgE. *Immunological Reviews* 125:37–48.

Michelsen, B., Dyrberg, T., Vissing, H., Serup, P., and Lermmark, A. 1990. HLA-DQ and -DX genes in insulin-dependent diabetes mellitus. *Current Topics in Microbiology & Immunology* 164:57-68.

Miedema, F. 1995. Immunobiology of HIV infection. *The Immunologist* 3:228–31.

Miescher, P. A., and Graf, J. 1980. Drug-induced thrombocytopenia. *Clinics in Haematology* 9:505-19.

Mietens, C. 1983. Selective IgA deficiency. *Klinische Pädiatrie* 195:385–7.

Miller, J. F. 1996. Influence and fate of T lymphocytes in autoimmunity. *Journal of Autoimmunity* 9:301–4.

Miller, J. F., Allison, J., and Morahan, G. 1990. Tolerance and diabetes in transgenic mice over-expressing class I histocompatibility molecules in pancreatic beta cells. *Journal of Autoimmunity* 3:87–90.

Miller, S. D., Vanderlugt, C. L., Lenschow, D. J., Pope, J. G., Karandikar, N. J., Dal Canto, M. C., and Bluestone, J. A. 1995. Blockade of CD28/B7–1 interaction prevents epitope spreading and clinical relapses of murine EAE. *Immunity* 3:739–45.

Mills, C. D. 1991. Molecular basis of "suppressor" macrophages. Arginine metabolism via the nitric oxide synthetase pathway. *Journal of Immunology* 146:2719–23.

Minchin, S. A., Leitenberg, D., Stunz, L. L., and Feldbush, T. L. 1990. Polyclonal activa-

tion of rat B cells. II Dextran sulfate as a cofactor in mitogen-induced and antigen-induced differentiation of rat B lymphocytes. *Journal of Immunology* 145:2427–33.

Mingari, M. C., Vitale, C., Cambiaggi, A., Schiavetti, F., Melioli, G., Ferrini, S., and Poggi, A. 1995. Cytolytic T lymphocytes displaying natural killer (NK)-like activity: expression of NK-related functional receptors for HLA class I molecules (p58 and CD94) and inhibitory effect on the TCR-mediated target cell lysis or lymphokine production. *International Immunology* 7:697–703.

Mitreiter, R., and Epplen, C. 1992. Self-reactive and antigen-specific T cell clones derived from a HLA-DR4+/DR5+ donor: T cell receptors and MHC-restriction patterns. *Immunobiology* 186:315–26.

Mittal, A., Woodward, B., and Chandra, R. K. 1988. Involution of thymic epithelium and low thymulin bioactivity in weanling mice subjected to severe food intake restriction or severe protein deficiency. *Experimental and Molecular Pathology* 48:226–35.

Miyashita, K., and Mori, M. 1994. Comparison of the immunogenic regions in the TSH receptor for Graves' IgG and Hashimoto's IgG. *Japanese Journal of Clinical Medicine* 52:1057–62.

Mjares, A., Verdot, L., Peineau, N., Vray, B., Hoebeke, J., and Argibay, J. 1996. Antibodies from Trypanosoma cruzi infected mice recognize the second extracellular loop of the beta 1-adrenergic and M2-muscarinic receptors and regulate calcium channels in isolated myocytes. *Molecular and Cellular Biochemistry* 163–164107–12.

Mocci, S., and Coffman, R. L. 1997. The mechanism of in vitro T helper cell type 1 to T helper cell type 2 switching in highly polarized Leishmania major-specific T cell populations. *Journal of Immunology* 158:1559-64.

Mond, J. J., Farrar, J., Paul, W. E., Fuller-Farrar, J., Schaefer, M., and Howard, M. 1983. T cell dependence and factor reconstitution of in vitro antibody responses to TNP-B. abortus and TNP-Ficoll: restoration of depleted responses with chromatographed fractions of a T cell-derived factor. *Journal of Immunology* 131:633–7.

Montelius, J., Wahlkvist, H., Boman, A., Fernstrom, P., Grabergs, L., and Wahlberg, J. E. 1994. Experience with the murine local lymph node assay: inability to discriminate between allergens and irritants. *Acta Dermato-Venereologica* 74:22–7.

Montelius, J., Boman, A., Wahlkvist, H., and Wahlberg, J. E. 1996. The murine local lymph node assay: search for an alternative, more adequate, vehicle than acetone/olive oil (4:1). *Contact Dermatitis* 34:428–30.

Moodycliffe, A. M., Kimber, I., and Norval, M. 1992. The effect of ultraviolet B irradiation and urocanic acid isomers on dendritic cell migration. *Immunology* 77:394–9.

Moore, K. W., Rousset, F., and Banchereau, J. 1991. Evolving principles in immunopathology: interleukin 10 and its relationship to Epstein–Barr virus protein BCRF1. *Springer Seminars in Immunopathology* 13:157-66.

Moore, K. W., O'Garra, A., de Waal Malefyt, R., Vieira, P., and Mosmann, T. R. 1993. Interleukin- 10. *Annual Review of Immunology* 11:165-90.

Morahan, P. S., Klykken, P. C., Smith, S. H., Harris, L. S., and Munson, A. E. 1979. Effects of cannabinoids on host resistance to Listeria monocytogenes and herpes simplex virus. *Infection and Immunity* 23:670–4.

Moreau, J.-F., Le Savre, P., and de Luca, H. 1988. General toxicity of water-soluble iodinated contrast media. Pathogenic concepts. *Investigative Radiology* 23(Supplement1):S75-8.

Moretta, L., Mingari, M. C., Pende, D., Bottino, C., Biassoni, R., and Moretta, A. 1996. The molecular basis of natural killer (NK) cell recognition and function. *Journal of Clinical Immunology* 16:243–53.

Morimoto, C., and Schlossman, S. F. 1993. P. Rambotti Lecture. Human naive and memory T cells revisited: new markers (CD31 and CD27) that help define CD4+ T cell subsets. *Clinical & Experimental Rheumatology* 11:241-7.

Morschella, S. L., and Cropley, T. G. 1990. Mononuclear phagocytic and dendritic cell systems. *Journal of the American Academy of Dermatology* 22:1091–7.

Mossman, K., Upton, C., and McFadden, G. 1995. The myxoma virus soluble interferon-gamma homolog M-T7 inhibits interferon gamma in a species dependent manner. *Journal of Biological Chemistry* 270:3031-8.

Mossman, T. R., and Sad, S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunology Today* 17:138-45.

Moticka, E. J., and Streilein, J. W. 1978. Hypothesis: nonspecific polyclonal activation of memory B cells by antigen as a mechanism for the preservation of long term immunologic anamnesis. *Cellular Immunology* 41:406-13.

Moulon, C., Vollmer, J., and Weltzein, H. U. 1995. Characterization of processing requirements and metal cross-reactivities in T cell clones from patients with allergic contact dermatitis to nickel. *European Journal of Immunology* 25:3308–15.

Moxon, E. R. 1981. Experimental infections of animals in the study of Streptococcus pneumoniae. *Review of Infectious Disease* 3:354–7.

Mudde, G. C., Bheekha, R., and Bruijnzeel-Koomen, C. A. F. M. 1995. Consequences of IgEKD23-mediated antigen presentation in allergy. *Immunology Today* 16:380–2.

Mullbacher, A., and Flynn, K. 1996. Aspects of cytotoxic T cell memory. *Immunological Reviews* 150:113–27.

Muller, C. P., Ammerlaan, W., Fleckenstein, B., Krauss, S., Kalbacher, H., Schneider, F., Jung, G., and Wiesmuller, K. H. 1996. Activation of T cells by the ragged tail of MHC class II-presented peptides of the measles virus fusion protein. *International Immunology* 8:445-56.

Muller, G., Saloga, J., Germann, T., Schuler, G., Knop, J., and Enk, A. H. 1995. IL-12 as mediator and adjuvant for the induction of contact sensitivity in vivo. *Journal of Immunology* 155:4661–8.

Muller, G., Knop, J., and Enk, A. H. 1996. Is cytokine expression responsible for differences between allergens and irritants? *American Journal of Contact Dermatitis* 7: 177–84.

Muller, W. D., Karamfilov, T., Bufe, A., Fahlbush, B., Wolf, I., and Jager, L. 1996. Group 5 allergens of timothy grass (Phl p5) bear cross-reacting T cell epitopes with group 1 allergens of rye grass (Lol p1). *International Archives of Allergy & Immunology* 109:352-5.

Muller-Eberhard, H. J. 1968. Chemistry and reaction mechanisms of complement. *Advances in Immunology* 8:1-80.

Muller-Eberhard, H. J. 1969. Complement. *Annual Review of Biochemistry* 38:389–414.

Muller-Eberhard, H. J. 1971. The molecular basis of the biological activities of complement. *Harvey Lectures* 66:75–104.

Muller-Eberhard, H. J. 1975. Complement. *Annual Review of Biochemistry* 44:697–724.

Munson, A. E., and White, K. L. 1990. *Immunotoxicology,* Elsevier, Amsterdam, pp. 285–313.

Munson, A. E., McCay, J. A., and Cao, W. 1991. Approaches to immunotoxicologic studies with emphasis on chemical-induced immunomodulation. *Annals of Allergy* 66:505-18.

Murray, M. J., Lauer, L. D., Luster, M. I., Luebke, R. W., Adams, D. O., and Dean, J. H. 1985. Correlation of murine susceptibility to tumor, parasite and bacterial challenge with altered cell mediated immunity following systemic exposure to the tumor promoter phorbol myristate acetate. *International Journal of Immunopharmacology* 7:491-500.

Mustafa, A. S. 1995. Recognition of mycobacterial HSP65 in association with HLA-DR4 is not sufficient for autoreactivity. *Nutrition* 11:661-4.

Nacy, C. A., Meierovics, A. I., Belosevic, M., and Green, S. J. 1991. Tumor necrosis factor-alpha: central regulatory cytokine in the induction of macrophage antimicrobial activities. *Pathobiology* 59:182-4.

Nakagawa, K., and Harrison, L. C. 1996. The potential roles of endogenous retroviruses in autoimmunity. *Immunological Reviews* 152:193-236.

Nakamoto, Y., Kaneko, S., Ohno, H., Honda, M., Unoura, M., Murakami, S., and Kobayashi, K. 1996. B-cell epitopes in the hypervariable region 1 of hepatits C virus obtained from patients with chronic persistent hepatitis. *Journal* of *Medical Virology* 50:35–41.

Nakamura, A., Momma, J., Sekiguchi, H., Noda, T., Yamano, T., Kaniwa, M., Kojima, S., Tsuda, M., and Kurokawa, Y. 1994. A new protocol and criteria for the quantitative determination of sensitization potencies of chemicals by guinea pig maximization test. *Contact Dermatitis* 31:72-85.

Nakayama, M., Arai, K., Hasegawa, K., Sato, K., Otsuka, K., Watanabe, H., Sakai, K., Rikiishi, H., and Abo, T. 1995. Adhesion molecules on intermediate TCR cells. II. Hepatoprotective effects of hyaluronic acid on acute liver injury. *Cellular Immunology* 166:275–85.

Nakayama, M., Otsuka, K., Sato, K., Hasegawa, K., Osman, Y., Kawamura, T., and Abo, T. 1996. Activation by estrogen of the number and function of forbidden T-cell clones in intermediate T-cell receptor cells. *Cellular Immunology* 172:163-71.

Namba, T., Nakata, Y., and Grob, D. 1976. The role of humoral and cellular immune factors in neuromuscular block in myasthenia gravis. *Annals of the New York Academy of Sciences* 274:493–515.

Namikawa, R., Weilbaecher, H., Kaneshima, H., Yee, E. J., and McCune, J. M. 1990. Long term hematopoiesis in the SCID-hu mouse. *Journal of Experimental Medicine* 175:1055-63.

Naucler, C. S., Larsson, S., and Moller, E. 1996. A novel mechanism for virus-induced autoimmunity in humans. *Immunological Reviews* 152:175-92.

Negus, R. P. 1996. The chemokines: cytokines that direct leukocyte migration. *Journal of the Royal Society of Medicine* 89:312-4.

Negus, R. P., and Balkwill, F. R. 1996. Cytokines in tumour growth, migration and metastasis. *World Journal of Urology* 14:157–65.

Nelson, H. S. 1985. The atopic diseases. *Annals of Allergy* 55:441–7.

Nepom, G. T. 1990. A unified hypothesis for the complex genetics of HLA associations with IDDM. *Diabetes* 39:1153–7.

Nesheim, M. C., Austic, R. E., and Wang, S.-H. 1971. Genetic factors in lysine and arginine metabolism of chicks. *Federation Proceedings* 30:121-6.

Neta, R., Sayers, T. J., and Oppenheim, J. J. 1992. Relationship of TNF to interleukins. *Immunology Series* 56:499–566.

Newberg, M. H., Smith, D. H., Haertel, S. B., Vining, D. R., Lacy, E., and Engelhard, V. H. 1996. Importance of MHC class 1 alpha2 and alpha3 domains in the recognition of self and non-self MHC molecules. *Journal of Immunology* 156:2473–80.

Newcomb, J. R., Carboy-Newcomb, C., and Cresswell, P. 1996. Trimeric interactions of the invariant chain and its association with major histocompatibility complex class II alpha beta dimers. *Journal of Biological Chemistry* 271:24249-56.

Newman Taylor, A. J. 1988. Occupational asthma. *Postgraduate Medicine* 64:505.

Nguyen, Q. V., Reyes, V. E., and Humphreys, R. E. 1995. Structural analysis of invariant chain subsets as a function of their association with MHC class II chains. *Archives of Biochemistry & Biophysics* 317:128-32.

Ni, H.-T., Deeths, M. J., and Mescher, M. F. 1996. Phosphatidylinositol 3 kinase activity is not essential for B7–1 mediated costimulation of proliferation or development of cytotoxicity in murine mast cells. *Journal of Immunology* 157:2243–6.

Nicholson-Weller, A., March, J. P., Rosenfeld, S. I., and Austen, K. F. 1983. Affected erythrocytes of patients with paroxysmal nocturnal hemoglobinuria are deficient in the complement regulatory protein, decay accelerating factor. *Proceedings of the National Academy of Sciences (USA)* 80:5066-70.

Nickoloff, B. J., and Turka, L. A. 1994. Immunological function of non-professional antigen presenting cells: new insights from studies with keratinocytes. *Immunology Today* 15:464–8.

Nicolas, P., and Mor, A. 1995. Peptides as weapons against microorganisms in the chemical defense system of vertebrates. *Annual Review of Microbiology* 49:277–304.

Nielsen, H. E., Christensen, K. C., Koch, C., Thomsen, B. S., Heegaard, N. H., and Tranum-Jensen, J. 1989. Hereditary, complete deficiency of complement factor H associated with recurrent meningococcal disease. Scandinavian *Journal of Immunology* 30:711–8.

Ninomiya, H., Muraki, Y., Shibuya, K., Nagasawa, T., and Abe, T. 1993. Induction of Fc gamma R-III (CD16) expression on neutrophils affected by paroxysmal nocturnal haemoglobinuria by administration of granulocyte colony-stimulating factor. *British Journal of Haematology* 84:497–503.

Nishimura, T., Kuge, S., Watanabe, K., Lee, U., Yahata, T., and Habu, S. 1994. An efficient method for the induction of human antitumor effector CD4+ and CD8+ T cells: their application to tumor immunotherapy. *Human Cell* 7:131-7.

Nisini, R., Paroli, M., Accapezzato, D., Bonino, F., Rosina, F., Santantonio, T., Sallusto, F., Amoroso, A., Houghton, M., and Barnaba, V. 1997. Human CD4+ T-cell response to hepatitis delta virus: identification of multiple epitopes and characterization of T-helper cytokine profiles. *Journal of Virology* 71:2241-51.

Noelle, R. J., Ledbetter, J. A., and Aruffo, A. 1992. CD40 and its ligand, an essential ligand–receptor pair for thymus dependent B cell activation. *Immunology Today* 13:431-5.

Noelle, R. J., Mackey, M., Foy, T., Buhlmann, J., and Bums, C. 1997. CD40 and its ligand in autoimmunity. *Annals of the New York Academy of Sciences* 815:384–91.

Nordeng, T. W., and Bakke, O. *1994. The biological role of invariant chain (Ii) in MHC class II antigen presentation. Immunology Letters* 43:47–55.

Notarangelo, L. D., Duse, M., and Ugazio, A. G. 1992. Immunodeficiency with hyper-IgM (HIM). *Immunodeficiency Reviews* 3:101-21.

Nutman, J., Berger, M., Chase, P. A., Dearborn, D. G., Miller, K. M., Waller, R. L., and Sorensen, R. U. 1987. Studies on the mechanism of T cell inhibition by the Pseudomonas aeruginosa phenazine pigment pyocyanine. *Journal of Immunology* 138:3481-7.

Obeid, 0. E., Stanley, C. M., and Steward, M. W. 1996. Immunological analysis of the protective responses to the chimeric synthetic peptide representing T- and B-cell epitopes from the fusion protein of measles virus. *Virus Research* 42:173–80.

O'Byrne, P. M., Dolovich, J., and Hargreave, F. E. 1987. Late asthmatic responses. *American Review of Respiratory Disease* 136:740–5l.

Oda, A., Miyata, M., Kodama, E., Satoh, H., Sato, Y., Nishimaki, T., Nomaguchi, H., and Kasukawa, R. 1994. Antibodies to 65Kd heat-shock protein were elevated in rheumatoid arthritis. *Clinical Rheumatology* 13:261–4.

Odom, R. B., and Maibach, H. I. 1976. Contact urticaria: a different contact dermatitis. *Citus* 18:672–6.

Oettigen, H. C., Martin, T. R., Wynshaw-Boris, A., Deng, C., Drazen, J. M., and Leder, P. 1994. Active anaphylaxis in IgE deficient mice. *Nature* 370:367–70.

Ofek, I., Goldhar, J., Keisari, Y., and Sharon, N. 1995. Non opsonic phagocytosis of microorganisms. *Annual Review of Microbiology* 49:239–76.

Offenstadt, G., Guidet, B., and Staikowsky, F. 1993. Cytokines and severe infections. *Pathologie Biologie* 41:820–31.

O'Garra, A. 1989a. Interleukins and the immune system 1. *Lancet* 1:943–7.

O'Garra, A. 1989b. Interleukins and the immune system 2. *Lancet* 1:1003–5.

O'Garra, A., and Murphy, K. 1993. T-cell subsets in autoimmunity. *Current Opinion in Immunology* 5:880–6.

Ogasawara, T., Murakami, M., Suzuki-Nishimura, T., Uchida, M. K., and Kudo, I. 1997. Mouse bone marrow-derived mast cells undergo exocytosis, prostanoid generation, and cytokine expression in response to G protein-activating polybasic compounds after coculture with fibroblasts in the presence of c-kit ligand. *Journal ofImmunology* 158:393-404.

Ohashi, P. S. 1996. T cell selection and autoimmunity: flexibility and tuning. *Current Opinion in Immunology* 8:808–14.

Oldfield, S., Lortan, J. E., Hyatt, M. A., and MacLennan, I. C. 1988. Marginal zone B cells and the localization of antigen on follicular dendritic cells. *Advances in Experimental Medicine & Biology* 237:99–104.

Orvieto, R., and Ben-Rafael, Z. 1996. The immune system in severe ovarian hyperstimulation syndrome. *Israel Journal of Medical Sciences* 32:1180-2.

Osler, A. G., and Sandberg, A. L. 1973. Alternate complement pathways. *Progress in Allergy* 17:51-92.

Ouellette, A. J., and Selsted, M. E. 1996. Paneth cell defensins: endogenous peptide components of intestinal host defense. *FASEB Journal* 10:1280-9.

Pabst, R. 1988. The spleen in lymphocyte migration. *Immunology Today* 9:43–5.

Pabst, R., and Binns, R. M. 1989. Heterogeneity of lymphocyte homing physiology:

several mechanisms operate in the control of migration to lymphoid and non-lymphoid organs in vivo. *Immunological Reviews* 198:83-109.

Palacios, R., and Moller, G. 1981. Cyclosporin A blocks receptors for HLA-DR antigens on T cells. *Nature* 290:792–4.

Palanivel, V., Posey, C., Horauf, A. M., Solbach, W., Piessens, W. F., and Harn, D. A. 1996. B-cell outgrowth and ligand-specific production of IL-10 correlate with Th2 dominance in certain parasitic diseases. *Experimental Parasitology* 84:168-77.

Palker, T. J., Muir, A. J., Spragion, D. E., Staats, H. F., Langlois, A., and Montefiori, D. C. 1996. The V3 domain of SIV mac251 gp120 contains a linear neutralizing epitope. *Virology* 224:415–26.

Pallares-Trujillo, J., Lopez-Soriano, F. J., and Argiles, J. M. 1995. TNF and AIDS: two sides of the same coin? *Medicinal Research Reviews* 15:533-46.

Pangburn, M. K., Schreiber, R. D., Trombold, J. S., and Muller-Eberhard, H. J. 1983. Paroxysmal nocturnal hemoglobinuria: deficiency in factor H-like functions of the abnormal erythrocytes. *Journal of Experimental Medicine* 157:1971-80.

Paolini, R., Numerof, R., and Kinet, J. P. 1994. Kinase activation through the high-affinity receptor for immunoglobulin E. *Immunomethods* 4:35–40.

Papadopoulos, G., and Lernmark, A. 1984. The immune response in individuals with HLA-DR specificities conferring susceptibility to insulin-dependent diabetes—hypothesis. *Diabetes Research* 1:3–11.

Parhar, R. S., and Lala, P. K. 1987. Amelioration of B16F10 melanoma lung metastasis in mice by a combination therapy with indomethacin and interleukin. *Journal of Experimental Medicine* 165:14-28.

Parker, C. W. 1977. Immune responses to environmental antigens absorbed through the gastrointestinal tract. *Federation Proceedings* 36:1732-5.

Parkkonen, P., Hyoty, H., Ilonen, J., Reijonen, H., Yla-Herttuala, S., and Leinikki, P. 1994. Antibody reactivity to an Epstein–Barrvirus BERF4-encoded epitope occurring also in the Asp-57 region of HLA-DQ8 beta chain. Childhood Diabetes in Finland Study Group. *Clinical and Experimental Immunology* 95:287–93.

Parmentier, H. K., van der Linden, J. A., Krijnen, J., van Wichen, D. F., Rademakers, L. H., Bloem, A. C., and Schuurman, H. J. 1991. Human follicular dendritic cells: isolation and characteristics in situ and in suspension. *Scandinavian Journal of Immunology* 33:441–52.

Para-Campos, V., Escobar-Gutierrez, A., Dominguez-Lopez, M. L., Cancino-Diaz, M., Burgos-Vargas, R., Granados-Arreola, J., Jimenez-Zamudio, L., and Garcia-Latorre, E. 1996. Antibody response to nitrogenase-positive and -negative Klebsiella pneumoniae strains in juvenile-onset ankylosing spondylitis patients and their first degree relatives: lack of differential recognition of the bacterial nitrogenase. *Revista Latinoamericana de Microbiologia* 38:121–7.

Parren, P. W., Fisicaro, P., Labrijn, A. F., Binley, J. M., Yang, W. P., Ditzel, H. J., Barbas, C. F. R., and Burton, D. R. 1996. In vitro antigen challenge of human antibody libraries for vaccine evaluation: the human immunodeficiency virus type 1 envelope. *Journal of Virology* 70:9046–50.

Pascual, M., Plastre, O., Montdargent, B., Labarre, D., and Schifferli, J. A. 1993. Specific interactions of polystyrene biomaterials with factor D of human complement. *Biomaterials* 14:665–70.

Pasparakis, M., Alexopoulou, L., Douni, E., and Kollias, G. 1996. Tumour necrosis factors in immune regulation: everything that's interesting is . . . new. *Cytokine and Growth Factor Reviews* 7:223–9.

Patterson, S., Helbert, M., English, N. R., Pinching, A. J., and Knight, S. C. 1996. The effect of AZT on dendritic cell number and provirus load in the peripheral blood of AIDS patients: a preliminary study. *Research in Virology* 147:109-14.

Paul, N. L., and Ruddle, N. H. 1988. Lymphotoxin. *Annual Review of Immunology* 6:407–38.

Pawlowski, T. J., and Staerz, U. E. 1994. Thymic education—T cells do it for themselves. *Immunology Today* 15:205-8.

Paxton, W. A., Dragic, T., Koup, R. A., and Moore, J. P. 1996. The beta-chemokines, HIV type 1 second receptors, and exposed uninfected persons. *AIDS Research & Human Retroviruses* 12:1203-7.

Payne, N. R., and Horowitz, M. A. 1987. Phagocytosis of Legionella pneumophila is mediated by human monocyte complement receptors. *Journal of Experimental Medicine* 166:1377-89.

Peacock, J. S., Bush, J., Jr., Krakauer, H., Hiernaux, J., DeLisi, C., and Barisas, B. G. 1983. Thymus-independent immunogenicity in vitro of the divalent antigen DNP-polyethylene oxide. *Cellular Immunology* 79:220-3 1.

Pearce, E. J., Vasconcelos, J. P., Brunet, L. R., and Sabin, E. A. 1996. IL-4 in schistosomiasis. *Experimental Parasitology* 84:295–9.

Pearl, E. R. 1997. Food allergy. *Lippincotts Primary Care Practice* 1:154–67.

Pearson, W., and Lipman, D. 1988. Improved tools for biological sequence comparison. *Proceedings of the National Academy of Sciences (USA)* 85:2444–8.

Pellequer, J. L., and Westhof, E. 1993. Preditop: A program for antigenicity prediction. *Journal of Molecular Graphics* 11:204-10.

Penn, I. 1985. *Neoplastic Consequences of Immunosuppression,* Raven Press, New York, pp. 79–98.

Penn, I. 1988. Cancer is a long-term hazard of immunosuppressive therapy. *Journal of Autoimmunity* 1:545-58.

Pepys, M. B. 1976. The role of complement in the induction of immunological responses. *Transplantation* 32:93–120.

Pereboev, A. V., Razumov, I. A., Svyatchenko, V. A., and Loktev, V. B. 1996. Glycoproteins E2 of the Venezuelan and eastern equine encephalomyelitis viruses contain multiple cross-reactive epitopes. *Archives of Virology* 141:2191-205.

Perry, H. M., Jr. 1985. The evolution of antihypertensive therapy. *American Journal of Cardiology* 56:75H–80H.

Petit-Frere, C., Dugas, B., Braquet, P., and Mencia-Huerta, J. M. 1993. Interleukin-9 potentiates the interleukin-4-induced IgE and IgG1 release from murine B lymphocytes. *Immunology* 79:146-51.

Petrek, M., Du Bois, R. M., Sirova, M., and Weigl, E. 1995. Chemotactic cytokines (chemokines) and their role in physiological and immunopathological reactions. *Folia Biologica* 41:263-83.

Petz, L. D., and Fundenberg, H. H. 1975. Immunologic mechanisms in drug-induced cytopenias. *Progress in Hematology* 9:185-206.

Picado, C. 1992. Early and late-phase asthmatic reactions: a hypothesis. *Allergy* 47:331–3.

Picker, L. J., Treer, J. R., Ferguson-Damell, B., Collins, P. A., Bergstresser, P. R., and Terstappen, L. W. M. M. 1993. Control of lymphocyte recirculation in man II. Differential regulation of the cutaneous lymphocyte associated antigen, a tissue selective homing receptor for skin-homing T cells. *Journal of Immunology* 150:1122–36.

Piemonti, L., Bernasconi, S., Luini, W., Trobonjaca, Z., Minty, A., Allavena, P., and Mantovani, A. 1995. IL-13 supports differentiation of dendritic cells from circulating precursors in concert with GM-CSF. *European Cytokine Network* 6:245–52.

Pikaar, J. C., Voorhout, W. F., van Golde, L. M., Verhoef, J., Van Strijp, J. A., and van Iwaarden, J. F. 1995. Opsonic activities of surfactant proteins A and D in phagocytosis of gram-negative bacteria by alveolar macrophages. *Journal of Infectious Diseases* 172:481-9.

Pinching, A. J. 1985. Laboratory investigations of secondary immunodeficiencies. *Clinics in Immunology and Allergy* 5:469–91.

Pistoia, V. 1997. Production of cytokines by human B cells in health and disease. *Immunology Today* 18:343–8.

Plank, C., Mechtler, K., Szoka, F. C. J., and Wagner, E. 1996. Activation of the complement system by synthetic DNA complexes: a potential barrier for intravenous drug delivery. *Human Gene Therapy* 7:1437-46.

Plebani, A., Monafo, V., Cattaneo, R., Carella, G., Brugnoni, D., Facchetti, F., Battocchio, S., Meini, A., Notarangelo, L. D., Duse, M., and Ugazio, A. G. 1996. Defective expression of HLA class I and CD1a molecules in a boy with Marfan-like phenotype and deep skin ulcers. *Journal of the American Academy of Dermatology* 35:814-8.

Ploegh, H. L. 1995. Trafficking and assemby of MHC molecules: how viruses elude the immune system. *The Immunologist* 3:226–8.

Pober, J. S. 1988. Warner-Lambert/Parke-Davis award lecture. Cytokine-mediated activation of vascular endothelium. Physiology and pathology. *American Journal of Pathology* 133:426–33.

Podack, E. R. 1995. Execution and suicide: cytotoxic lymphocytes enforce Draconian laws through separate molecular pathways. *Current Opinion in Immunology* 7:11-6.

Poplack, D. G., Bonnard, G. D., Holman, B. J., and Blaese, R. M. 1976. Monocyte mediated antibody dependent cellular cytotoxicity: a clinical test of monocyte function. *Blood* 48:809-16.

Porcelli, S. A., and Modlin, R. L. 1995. CD1 and the expanding universe of T cell antigens. *Journal of Immunology* 155:3709–10.

Porteu, F., Fischer, A., Descamps-Latscha, B., and Halbwachs-Mecarelli, L. 1986. Defective complement receptors (CR1 and CR3) on erythrocytes and leukocytes of factor I (C3b-inactivator) deficient patients. *Clinical & Experimental Immunology* 66:463–71.

Preiksaitis, J. K. 1989. Cytomegalovirus infection in transplant recipients. *Immunology Allergy Clinics of North America* 9:137-51.

Pretolani, M., and Vargaftig, B. B. 1996. Role of eosinophil mobilization and activation in experimental airway inflammation and bronchopulmonary hyperreactivity. *Annals of the New York Academy of Sciences* 796:72–81.

Probst, P., Hermann, E., Meyer zum Buschenfelde, K. H., and Fleischer, B. 1993. Multiclonal synovial T cell response to Yersinia enterocolitica in reactive arthritis: the Yersinia 61-kDa heat-shock protein is not the major target antigen. *Journal of Infectious Diseases* 167:385–91.

Probst, P., Hermann, E., and Fleischer, B. 1994. Role of bacteria-specific T cells in the immunopathogenesis of reactive arthritis. *Trends in Microbiology* 2:329–32.

Proost, P., Wuyts, A., and van Damme, J. 1996a. The role of chemokines in inflammation. International *Journal of Clinical and Laboratory Research* 26:211–23.

Proost, P., Wuyts, A., and Van Damme, J. 1996b. Human monocyte chemotactic proteins-2 and 3: structural and functional comparison with MCP-1. *Journal of Leukocyte Biology* 59:67–74.

Proudfoot, L., Nikolaev, A. V., Feng, G. J., Wei, W. Q., Ferguson, M. A., Brimacombe, J. S., and Liew, F. Y. 1996. Regulation of the expression of nitric oxide synthase and leishmanicidal activity by glycoconjugates of Leishmania lipophosphoglycan in murine macrophages. *Proceedings of the National Academy of Sciences (USA)* 93:10984-9.

Pryhuber, K. G., Murray, K. J., Donnelly, P., Passo, M. H., Maksymowych, W. P., Glass, D. N., Giannini, E. H., and Colbert, R. A. 1996. Polymorphism in the LMP2 gene influences disease susceptibility and severity in HLA-B27 associated juvenile arthritis. *Journal of Rheumatology* 23:747–52.

Pugliese, A., and Eisenbarth, G. S. 1992. Type I diabetes mellitus: lessons for human autoimmunity. *Journal of Laboratory & Clinical Medicine* 120:363–6.

Pujol-Borrell, R., Todd, I., Londei, M., Foulis, A., Feldmann, M., and Bottazzo, G. F. 1986. Inappropriate major histocompatibility complex class II expression by thyroid follicular cells in thyroid autoimmune disease and by pancreatic beta cells in type I diabetes. *Molecular Biology & Medicine* 3:159-65.

Qoronfleh, M. W., Weraarchakul, W., and Wilkinson, B. J. 1993. Antibodies to a range of Staphylococcus aureus and Escherichia coli heat shock proteins in sera from patients with S. aureus endocarditis. *Infection & Immunity* 61:1567-70.

Quinti, I., Papetti, C., D'Offizi, G., Cavagni, G., Panchor, M. L., Lunardi, C., and Paganelli, R. 1988. IgG subclasses to food antigens. *Allergie et Immunologie* 20:41, 43–4.

Raab, M., da Silva, A. J., and Rudd, C. E. 1997. Regulation of Vav-SLP-76 binding to ZAP-70 and its relevance to TCR zeta/CD3 induction of interleukin-2. *Immunity* 6:155-64.

Rabinowe, S. L., and Eisenbarth, G. S. 1984. Type I diabetes mellitus: a chronic autoimmune disease? *Pediatric Clinics of North America* 31:531-43.

Radvanyi, L. G., Shi, Y., Vaziri, H., Sharma, A., Dhala, R., Mills, G. B., and Miller, R. G. 1996. CD28 costimulation inhibits TCR-induced apoptosis during a primary T cell response. *Journal of Immunology* 156:1788-98.

Ragg, S. J., Dandie, G. W., Woods, G. M., and Muller, H. K. 1995. Abrogation of afferent lymph dendritic cell function after cutaneously applied chemical carcinogens. *Cellular Immunology* 162:80–8.

Rammensee, H. G. 1995. Chemistry of peptides associated with MHC class I and class II molecules. *Current Opinion in Immunology* 7:85–96.

Ramos, O. F., Patarroyo, M., Yefenof, E., and Klein, E. 1989. Requirement of leukocytic cell adhesion molecules (CDl 1a-cKD18) in the enhanced NK lysis of iC3b-opsonized targets. *Journal of Immunology* 142:4100–4.

Ranson, J. T., Moms, L. K., and Cambier, J. C. 1986. Anti-IgG induces release of inositol 1,4,5-triphosphate which mediates mobilization of intracellular Ca++ stores in B cells. *Journal of Immunology* 137:708–14.

Rao, A. 1991. Signaling mechanisms in T cells. *Critical Reviews in Immunology* 10:495–519.

Raport, C. J., Schweickart, V. L., Chantry, D., Eddy, R. L., Jr., Shows, T. B., Godiska, R., and Gray, P. W. 1996. New members of the chemokine receptor gene family. *Journal of Leukocyte Biology* 59:18–23.

Rappaport, E., Misiura, K., Agrawal, S., and Zamecnik, P. C. 1992. Antimalarial activities of oligodeoxynucleotide phosphorothioates in chloroquine resistant Plasmodium falciparum. *Proceedings of the National Academy of Sciences (USA)* 89:857740.

Rasmussen, J. M., Teisner, B., Brandt, J., Brandslund, I., and Gry, H. 1990. Metabolism of C3 and factor B in patients with congenital factor I deficiency. *Journal of Clinical & Laboratory Immunology* 31:59-67.

Ratajczak, M., Kant, J., Luger, S., Alliya, N., Zhang, J., Zon, G., and Gerwirtz, A. 1991. Treatment of human leukemia in a SCID mouse model with c-myb antisense oligodeoxynucleotide. *Proceedings of the National Academy of Sciences (USA)* 89:11823-7.

Rath, S., Lin, R. H., Rudensky, A., and Janeway, C. A., Jr. 1992. T and B cell receptors discriminate major histocompatibility complex class II conformations influenced by the invariant chain. *European Journal of Immunology* 22:2121–7.

Raybould, T. J. G., and Takahashi, M. 1988. Production of stable rabbit mouse hybridomas that secrete rabbit Mab of defined specificity. *Science* 240:1788-90.

Razin, E., Pecht, I., and Rivera, J. 1995. Signal transduction in the activation of mast cells and basophils. *Immunology Today* 16:370–4.

Razis, P. A., Coulson, I. H., Gould, T. R., and Findley, I. L. 1986. Acquired C1 esterase inhibitor deficiency. *Anaesthesia* 41:838-40.

Rechsteiner, M. 1987. Ubiquitin-mediated pathways for intracellular proteolysis. *Annual Review of Cell Biology* 3:1-30.

Reich, Z., Altman, J. D., Boniface, J. J., Lyons, D. S., Kozono, H., Ogg, G., Morgan, C., and Davis, M. M. 1997. Stability of empty and peptide-loaded class II major histocompatibility complex molecules at neutral and endosomal pH: comparison to class I proteins. *Proceedings of the National Academy of Sciences (USA)* 94:2495–500.

Reid, C. D., Stackpoole, A., Meager, A., and Tikerpae, J. 1992. Interactions of tumor necrosis factor with granulocyte–macrophage colony-stimulating factor and other cytokines in the regulation of dendritic cell growth in vitro from early bipotent CD34+ progenitors in human bone marrow. *Journal of Immunology* 149:2681–8.

Reiner, S. L., Zheng, S., Wang, Z. E., Stowring, L., and Locksley, R. M. 1994. Leishmania promastigotes evade interleukin 12 (IL-12) induction by macrophages and stimulate a broad range of cytokines from CD4+ T cells during initiation of infection. *Journal of Experimental Medicine* 179:447–56.

Rennick, D. M., Fort, M. M., and Davidson, N. J. 1997. Studies with IL-10-/- mice: an overview. *Journal of Leukocyte Biology* 61:389-96.

Renwick, A. G. 1991. Safety factors and establishment of acceptable daily intakes. *Food Additives and Contaminants* 8:135-50.

Renwick, A. G. 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. *Food Additives and Contaminants* 10:275–305.

Res, P., Thole, J., and de Vries, R. 1991. Heat-shock proteins and autoimmunity in humans, *Springer Seminars in Immunopathology* 13:81–98.

Reth, M. 1995. The B-cell antigen receptor complex and co-receptors. *Immunology Today* 16:3 10–2.

Revillard, J. P. 1990. Iatrogenic immunodeficiencies. *Current Opinions in Immunology* 2:445–50.

Revillard, J. P., Robinet, E., Goldman, M., Bazin, H., Latinne, D., and Chatenoud, L. 1995. In vitro correlates of the acute toxic syndrome induced by some monoclonal antibodies: a rationale for the design of predictive tests. *Toxicology* 96:51-8.

Reynaud, C.-A., Garcia, C., Hein, W. R., and Weill, J.-C. 1988. Somatic hyperconversion diversifies the single Vh gene of the chicken with a high incidence in the D region. *Immunology* 64:539-44.

Reynolds, H. Y., and Thomson, R. E. 1973. Pulmonary host defenses II. Interaction of respiratory antibodies with Pseudomonas aeruginosa and alveolar macrophages. *Journal of Immunology* 111:369-80.

Reynolds, J. D., and Morris, B. 1983. The evolution of Peyer's patches in fetal and post natal sheep. *European Journal of Immunology* 13:627–35.

Reynolds, J. D., Kennedy, L., Peppard, J., and Pabst, R. 1991. Ileal Peyer's patch emigrants are predominantly B cells and travel to all lymphoid tissue in sheep. *European Journal of Immunology* 21:283-289.

Ricevuti, G., Mazzone, A., Pasotti, D., Fioravanti, A., Uccelli, E., and Notario, A. 1989. Granulocyte disease caused by glycoprotein complex deficiency correlated with leukocyte adhesion. *Recenti Progressi in Medicina* 80:520-5.

Richardson, J., Fossati, I., Moraillon, A., Castelot, S., Sonigo, P., and Pancino, G. 1996. Neutralization sensitivity and accessibility of continuous B cell epitopes of the feline immunodeficiency virus envelope. *Journal of General Virology* 77:759–71.

Richter, W., Mertens, T., Schoel, B., Muir, P., Ritzkowsky, A., Scherbaum, W. A., and Boehm, B. 0. 1994. Sequence homology of the diabetes-associated autoantigen glutamate decarboxylase with Coxsackie B4–2C protein and heat shock protein 60 mediates no molecular mimicry of autoantibodies. *Journal ofExperimental Medicine* 180:721–6.

Riggs, M. W., Yount, P. A., Stone, A. L., and Langer, R. C. 1996. Protective monoclonal antibodies define a distinct, conserved epitope on an apical complex exoantigen of Cryptosporidium parvum sporozoites. *Journal of Eukaryotic Microbiology* 43:74S–75S.

Ringrose, J. H., Yard, B. A., Muijsers, A., Boog, C. J., and Feltkamp, T. E. 1996. Comparison of peptides eluted from the groove of HLA-B27 from Salmonella infected and non-infected cells. *Clinical Rheumatology* 1:74-8.

Ritz, H., Evans, B., Bruce, R., Fletcher, G., and Sarlo, K. 1993. Respiratory and immunological responses of guinea pigs to enzyme containing detergents: A comparison of intratracheal and inhalation models of exposure. *Fundamental and Applied Toxicology* 21:31-7.

Roake, J. A., Rao, A. S., Morris, P. J., Larsen, C. P., Hankins, D. F., and Austyn, J. M. 1995. Dendritic cell loss from nonlymphoid tissues after systemic administration of lipopolysaccharide, tumor necrosis factor, and interleukin 1. *Journal ofExperimental Medicine* 181:2231-47.

Roberts, C. G., Meister, G. E., Jesdale, B. M., Lieberman, J., Berzofsky, J. A., and De

Groot, A. S. 1996. Prediction of HIV peptide epitopes by a novel algorithm. *AIDS Research & Human Retroviruses* 12:593-610.

Roberts, D. W., and Basketter, D. A. 1990. A quantitative structure activity/dose relationship for contact allergenic potential of alkyl group transfer agents. *Toxicology in Vitro* 4:686–7.

Robertson, B. 1980. Interaction of pulmonary surfactant and alveolar macrophages in the non-specific defence system of the lung. *European Journal of Respiratory Diseases Supplement* 108:16–8.

Robinson, M. 1995. Cutaneous basophil hypersensitivity assay for distinguishing allergic contact sensitization from primary irritant responses. In *Methods in Immunotoxicology,* Volume 2 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 309–25.

Robinson, M. K., and Cruze, C. A. 1996. Preclinical skin sensitization testing of antihistamines: guinea pig and local lymph node assay responses. *Food and Chemical Toxicology* 34:495-506.

Robinson, M. K., Stotts, J., Danneman, P. J., Nusair, T. L., and Bay, P. H. 1989. A risk assessment process for allergic contact sensitization. *Food & Chemical Toxicology* 27:479–89.

Robinson, M. K., Nusair, T. L., Fletcher, E. R., and Ritz, H. L. 1990a. A review of the Buehler guinea pig skin sensitization test and its use in a risk assessment process for human skin sensitization. *Toxicology* 61:9 1–107.

Robinson, M. K., Fletcher, E. R., Johnson, G. R., Wyder, W. E., and Maurer, J. K. 1990b. Value of the cutaneous basophil hypersensitivity (CBH) response for distinguishing weak contact sensitization from irritation reactions in the guinea pig. *Journal of Investigative Dermatology* 94:636–43.

Rocha, B., Vassalli, P., and Guy-Grand, D. 1992. The extrathymic T-cell development pathway. *Immunology Today* 13:449–58.

Rocha, G., Deschenes, J., and Baines, M. G. 1994. Immunologic responses of the anterior chamber of the eye. *Allergologia et Immunopathologia* 22:23–7.

Roche, P. A., Marks, M. S., and Cresswell, P. 1991. Formation of a nine-subunit complex by HLA class II glycoproteins and the invariant chain. *Nature* 354:392–4.

Rock, K. L. 1996. A new foreign policy: MHC class I molecules monitor the outside world. *Immunology Today* 17:131-5.

Rocken, M., Racke, M., and Shevach, E. M. 1996. IL-4-induced immune deviation as antigen-specific therapy for inflammatory autoimmune disease. *Immunology Today* 17:225–31.

Rockland, R. E., Myers, 0. L., and David, J. R. 1970. An in vitro assay for cellular hypersensitivity in man. *Journal of Immunology* 194:95–102.

Roder, J. C., Todd, R. F., and Haliotis, T. 1983. The Chediak–Higashi gene in humans: III Studies on the mechanics of NK impairment. *Clinical and Experimental Immunology* 51 :359–68.

Rogala, B., and Grzanka, A. 1995. CD23/sCD23—receptor/cytokine in IgE-dependent reactions. *Postepy Higieny i Medycyny Doswiadczalnej* 49:425–32.

Rohold, A. E., Nielsen, G. D., and Andersen, K. E. 1991a. Colorimetric quantification of erythema in the guinea pig maximization test. *Contact Dermatitis* 24:373–4.

Rohold, A. E., Nielsen, G. D., and Andersen, K. E. 1991b. Nickel-sulphate-induced

contact dermatitis in the guinea pig maximization test: a dose–responsestudy. *Contact Dermatitis* 24:35–9.

Rohren, E. M., McCormick, D. J., and Pease, L. R. 1994. Peptide-induced conformational changes in class I molecules. Direct detection by flow cytometry. *Journal of Immunology* 152:5337-43.

Rollins, B. J. 1996. Monocyte chemoattractant protein 1: a potential regulator of monocyte recruitment in inflammatory disease. *Molecular Medicine Today* 2:198-204.

Romagnani, S. 1989. Induction of Th1 and Th2 subsets: regulation of differentiation and role in protection and immunopathology. *International Archives of Allergy and Immunology* 98:279–85.

Romagnoli, P., and Germain, R. N. 1994. The CLIP region of the invariant chain plays a critical role in regulating major histocompatibility complex class II folding, transport, and peptide occupancy. *Journal of Experimental Medicine* 180:1107-13.

Romani, L., Bistoni, F., Mencacci, A., Cenci, E., Spaccapelo, R., and Puccetti, P. 1995. IL12 in Candida albicans infections. *Research in Immunology* 146:532-8.

Ronnenberger, H. 1990. Toxicological testing of monoclonal antibodies. *Development of Biological Standards* 71:185-90.

Root, R. K., and Chem, M. S. 1981. The microbial mechanisms of human neutrophils and eosinophils. *Review of Infectious Diseases* 3:565-98.

Rose, N., and Bhatia, S. 1995. Autommunity: Animal models of human autoimmune disease. In *Methods in Immunotoxicology,* Volume 2 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 427–42.

Rose, N., and Friedman, H. 1986. *Manual of Clinical Immunology,* American Society for Microbiology, Washington, DC.

Rosenthal, G. J., and Snyder, C. A. 1987. Inhaled benzene reduces aspects of cell mediated tumor surveillance in mice. *Toxicology and Applied Pharmacology* 88:35–43.

Ross, G. D. 1980. Analysis of the different types of leukocyte membrane complement receptors and their interaction with the complement system. *Journal of Immunological Methods* 37:197–211.

Rossini, A. A., Mordes, J. P., and Like, A. A. 1985. Immunology of insulin-dependent diabetes mellitus. *Annual Review of Immunology* 3:289–320.

Rot, A., Hub, E., Middleton, J., Pons, F., Rabeck, C., Thierer, K., Wintle, J., Wolff, B., Zsak, M., and Dukor, P. 1996. Some aspects of IL-8 pathophysiology. III: Chemokine interaction with endothelial cells. *Journal of Leukocyte Biology* 59:39–44.

Rothbard, J. B. 1987. Recognition of peptide epitopes. *Modern Trends in Human Leukemia* 7:44–66.

Rothbard, J. B. 1990. The ternary complex: T cell receptor, MHC protein, and immunogenic peptide. *Seminars in Immunology* 2:283–95.

Rothbard, J. B., and Gefter, M. L. 1991. Interactions between immunogenic peptides and MHC proteins. *Annual Review of Immunology* 9:527-65.

Rothstein, J. L., and Schreiber, H. 1992. Tissue damage caused by TNF and complement. *Immunology Series* 56:453–81.

Rowden, G. 1980. Expression of Ia antigens on Langerhans cells in mice, guinea pigs, and man. *Journal of Investigative Dermatology* 75:22–31.

Rubin, B. Y. 1992. TNF and viruses: multiple interrelationships. *Immunology Series* 56:331–40.

Rubin, D. M., and Finley, D. 1995. Proteolysis. The proteasome: a protein-degrading organelle? *Current Biology* 5:854–8.

Rubin, R. H. 1990. Impact of cytomegalovirus infection in organ transplant recipients. *Review of Infectious Diseases* 12:S754–66.

Rubin, R. H., Cosimi, A. B., Hirsch, M. S., Herrin, J. T., Russell, P. S., and Tolkoff-Rubin, N. E. 1981. Effects of antithymocyte globulin on CMV infection in renal transplant recipients. *Transplantation* 31:143-5.

Rudd, E. R., Janssen, O., Cai, Y.-C., da Silva, A. J., Raab, M., and Prasad, K. V. S. 1994. Two-step TCR z/CD-3-CD-4 and CD28 signaling in T cells: SH2/SH3 domains, protein tyrosine and lipid kinases. *Immunology Today* 15:225–34.

Ruddle, N. H., and Homer, R. 1988. The role of lymphotoxin in inflammation. *Progress in Allergy* 40:162-82.

Ruddy, S. 1975. Complement and properdin: biologic and clinical importance. *Orthopedic Clinics of North America* 6:609–17.

Ruddy, S., and Austen, K. F. 1970. Inherited abnormalities of the complement system in man. *Progress in Medical Genetics* 7:69–95.

Ruddy, S., Gigli, I., and Austen, K. F. 1972a. The complement system of man. I. *New England Journal of Medicine* 287:489-95,

Ruddy, S., Gigli, I., and Austen, K. F. 1972b. The complement system of man. 4. *New England Journal of Medicine* 287:642-6.

Rudin, A., and Svennerholm, A. M. 1996. Identification of a cross-reactive continuous B-cell epitope in enterotoxigenic Escherichia coli colonization factor antigen I. *Infection & Immunity* 64:4508–13.

Rudy, G., and Lew, A. M. 1993. Images of self a model for tonic peripheral tolerance. *Immunology & Cell Biology* 71:327-36.

Ruitenberg, E. J., Buys, J., Teppema, J. S., and Elggersma, A. 1983. Rat mononuclear cells and neutrophils are more effective than eosinophils in antibody mediated stage specific killing of Trichinella spiralis. *Zeitschrift fuer Parasitenkunde* 69:807-15.

Runnels, H. A., Moore, J. C., and Jensen, P. E. 1996. A structural transition in class II major histocompatibility complex proteins at mildly acidic pH. *Journal of Experimental Medicine* 183:127–36.

Ruocco, V., Satriano, R. A., and Guerrera, V. 1992. "Two-step" pemphigus induction by ACE-inhibitors. *International Journal of Dermatology* 31:33-6.

Ruppert, J., Franco, A., Alexander, J., Snoke, K., Ishioka, G. Y., Page, D. M., Hedrick, S. M., Adorini, L., Grey, H. M., and Sette, A. 1995. MHC blocking peptides and T-cell receptor antagonists: novel paths to selective immunosuppression? *Chemical Immunology* 60:61–78.

Russell, M. W., and Wu, H. Y. 1990. Streptococcus mutans and the problem of heart cross-reactivity, *Critical Reviews in Oral Biology and Medicine* 1:191-205.

Ryan, J. C., and Seaman, W. E. 1997. Divergent functions of lectin-like receptors on NK cells. *Immunology Reviews* 155:79–88.

Ryan, J. J., Huang, H., McReynolds, L. J., Shelburne, C., Hu-Li, J., Huff, T. F., and Paul, W. E. 1997. Stem cell factor activates STAT-5 DNA binding in IL-3-derived bone marrow mast cells. *Experimental Hematology* 25:357–62.

Saavedra-Delgado, A. M., and Metcalfe, D. D. 1985. Interactions between food antigens

and the immune system in the pathogenesis of gastrointestinal diseases. *Annals of Allergy* 55:694-702.

Sadegh-Nasseri, S., and Germain, R. N. 1991. A role for peptide in determining MHC class II structure. *Nature* 353:167-70.

Saha, B., Das, G., Vohra, H., Ganguly, N. K., and Mishra, G. C. 1994. Macrophage T cell interactions in experimental mycobacterial infection. *European Journal of Immunology* 24:2618–24.

Saikh, K. U., Martin, J. D., Nishikawa, A. H., and Dillon, S. B. 1995. Influenza A virus-specific H-2d restricted cross-reactive cytotoxic T lymphocyte epitope(s) detected in the hemagglutinin HA2 subunit of AlUdorn/72. *Virology* 214:445–52.

Sailstad, D. M., Krishnan, S. D., Tepper, J. S., Doerfler, D. L., and Selgrade, M. K. 1995. Dietary vitamin A enhances sensitivity of the local lymph node assay. *Toxicology* 96:157–63.

Salk, P. L., and Salk, J. 1994. Cell-mediated immunologic memory in prevention and treatment of HIV disease. *Research in Immunology* 145:629-33.

Salmi, M., and Jalkanen, S. 1992.A 90 kilodalton endothelial cell molecule mediating lymphocyte binding in humans. *Science* 257:1407.

Saltini, C., Winestock, K., Kirby, M., Pinkston, P., and Crystal, R. G. 1989. Maintenance of the alveolitis in patients with chronic beryllium disease by beryllium specific helper T cells. *New England Journal of Medicine* 320:1103-9.

Sanchez, M. E., Maki, D. V., McLaren, L. C., and Bankhurst, A. D. 1979. Modulation of the adherence of human lymphocytes to measles-infected cells by prostaglandin El : a differential effect on lymphocyte subpopulations. *Prostaglandins* 18:35–41.

Sanchez-Madrid, F., Szklut, P., and Springer, T. A. 1983. Stable hamster mouse hybridomas producing IgG and IgM hamster monoclonal antibodies of defined specificity. *Journal of Immunology* 130:309–17.

Sanford-Driscoll, M., and Knodel, L. C. 1986. Induction of hemolytic anemia by nonsteroidal antiinflammatory drugs. *Drug Intelligence & Clinical Pharmacy* 20:925–34.

Sant, A. J. 1994. Endogenous antigen presentation by MHC class II molecules. *Immunologic Research* 13:253–67.

Sant, A. J., and Miller, J. 1994. MHC class II antigen processing: biology of the invariant chain. *Current Opinion in Immunology* 6:57-63.

Saraiva, S. 1970. Autoimmune aspects of myasthenia gravis. *Revista do Hospital das Clinicas, Faculdade de Medicina da Universidade de Sao Paulo* 25:181–8.

Sarlo, *K.,* and Clark, E. D. 1992. A tier approach for evaluating the respiratory allergenicity of low molecular weight compounds. *Fundamental and Applied Toxicology* 18:107-14.

Sartori, A., Scott, P., and Trinchieri, G. 1996. Leishmania major metacyclogenesis modulates the ability to induce IL-12. *Annals of the New York Academy of Sciences* 795:400–2.

Sarvetnick, N. 1989. Transgenic models of diabetes. *Current Opinion in Immunology* 2:604–6.

Sato, *S.,* Zhang, L., Kim, J., Jakob, J., Grant, R. A., Wollmann, R., and Roos, R. P. 1996. A neutralization site of DA strain of Theiler's murine encephalomyelitis virus important for disease phenotype. *Virology* 226:327–37.

Satoh, T., Kramarick, J. A., Tollerud, D. J., and Karol, M. H. 1995. A murine model for assessing the respiratory potential of chemical allergens. *Toxicology Letters* 78:57-66.

Sattler, M., Salgia, R., Shrikhande, G., Verma, S., Pisick, E., Prasad, K. V., and Griffin, J. D. 1997. Steel factor induces tyrosine phosphorylation of CRKL and binding of CRKL to a complex containing c-kit, phosphatidylinositol 3-kinase, and p120(CBL). *Journal of Biological Chemistry* 272:10248–53.

Sauzet, J. P., Gras-Masse, H., Guillet, J. G., and Gomard, E. 1996. Influence of a strong CD4 epitope on long-term virus-specific cytotoxic T cell responses induced in vivo with peptides. *International Immunology* 8:457–65.

Savelkoul, H. F., and van Ommen, R. 1996. Role of IL-4 in persistent IgE formation. *European Respiratory Journal Supplement* 22:67s–71s.

Sayles, P. C., and Wassom, D. L. 1988. Immunoregulation in murine malaria. Susceptibility of inbred mice to infection with Plasmodium yoelii depends on the dynamic interplay of host and parasite genes. *Immunology* 141:241–8.

Scala, R. 1991. *Risk Assessment,* 3 ed., Pergamon Press, New York, pp. 895–996.

Schafer, P. H., Pierce, S. K., and Jardetzky, T. S. 1995. The structure of MHC class 11: a role for dimer of dimers. *Seminars in Immunology* 7:389–98.

Schaffer, F. M., Monteiro, R. C., Volanakis, J. E., and Cooper, M. D. 1991. IgA deficiency. *Immunodeficiency Reviews* 3:15-44.

Schall, T. J. 1991. Biology of the Rantes/SIS cytokine family. *Cytokine* 3:165–83.

Schernthaner, G., and Mayr, W. R. 1984. Clinical and immunologic studies of HLA-DR typed insulin-dependent diabetics—a mini-review. *Experimental & Clinical Endocrinology* 83:184-91.

Schifferli, J. A., Ng, Y. C., and Peters, D. K. 1986. The role of complement and its receptor in the elimination of immune complexes. *New England Journal of Medicine* 315:488-95.

Schiffman, G. 1982. Immune response to pneumococcal vaccine: two uses. Protection against infection and evaluation of B cell function of the immune system. *Clinical Immunology Newsletter* 3:33–4.

Schiffman, G. 1983. Pneumococcal vaccine: a tool for the evaluation of the B-cell function of the immune system. *Proceedings of the Society for Experimental Biology and Medicine* 174:309–15.

Schlede, E., Maurer, T., Potokar, M., Schmidt, W. M., Schulz, K. H., Roll, R., and Kayser, D. 1989. A differentiated approach to testing skin sensitization. Proposal for a new test guideline for skin sensitization. *Archives of Toxicology* 63:81–4.

Schlievert, P. M. 1993. Role of superantigens in human disease. *Journal of Infectious Diseases* 167:997–1002.

Schloot, N. C., Roep, B. O., Wegmann, D. R., Yu, L., Wang, T. B., and Eisenbarth, G. S. 1997. T-cell reactivity to GAD65 peptide sequences shared with coxsackie virus protein in recent-onset IDDM, post-onset IDDM patients and control subjects. *Diabetologia* 40:332–8.

Schlumberger, H. D. 1983. Pseudo-allergic reactions to drugs and chemicals. *Annals of Allergy* 51:317-24.

Schmidt, B., Gyapay, G., Valay, M., and Fust, G. 1991. Human recombinant macrophage colony-stimulating factor (M-CSF) increases Cl-esterase inhibitor (Cl-INH) synthesis by human monocytes. *Immunology* 74:677–9.

Schoenfeld, A., Segal, J., Friedman, S., Hirsch, M., and Ovadia, J. 1986. Adverse reactions to antihypertensive drugs in pregnancy. *Obstetrical & Gynecological Survey* 41:67-73.

Scholes, E. W., Basketter, D. A., Sarll, A. E., Kimber, I., Evans, C. D., Miller, K., Robbins, M. C., Harrison, P. T., and Waite, S. J. 1992. The local lymph node assay: results of a final inter-laboratory validation under field conditions. *Journal ofApplied Toxicology* 12:217–22.

Schriever, F., Freedman, A. S., Freeman, G., Messner, E., Lee, G., Daley, J., and Nadler, L. M. 1989. Isolated human follicular dendritic cells display a unique antigenic phenotype. *Journal of Experimental Medicine* 169:2043–58.

Schroder, J. M., Noso, N., Sticherling, M., and Christophers, E. 1996. Role of eosinophil-chemotactic C-C chemokines in cutaneous inflammation. *Journal of Leukocyte Biology* 59:1-5.

Schubothe, H., Maas, D., and Weber, S. 1983. Clinical significance and pathogenesis of drug or microbe induced autoimmune hemolytic anemias. *Immunitat und Infektion* 11:3–15.

Schultz, D. R. 1989. Erythrocyte membrane protein deficiencies in paroxysmal nocturnal hemoglobinuria. *American Journal of Medicine* 87:22N–29N.

Schur, P. H., and Austen, K. F. 1968. Complement in human disease. *Annual Review of Medicine* 19:–24.

Schuurman, H.-J., Nagelkerken, L., De Weger, R. A., and Rozing, J. 1991. Age-associated involution: significance of a physiological process. *Thymus* 18:1-16.

Schuurman, H.-J., Van Loveren, H., Rozing, J., and Vos, J. G. 1992. Chemicals trophic for the thymus: risk for immunodeficiency and autoimmunity. *International Journal of Immunopharmacology* 14:369–75.

Schuurman, H.-J., Kuper, C. F., and Vos, J. G. 1994. Histopathology of the immune system as a tool to assess immunotoxicity. *Toxicology* 86:187-212.

Schwartz, A. L., and Ciechanover, A. 1992. Ubiquitin-mediated protein modification and degradation. *American Journal of Respiratory Cell and Molecular Biology* 7:463–8.

Scott, D. W., Grdina, T., and Shi, Y. 1996. T cells commit suicide, but B cells are murdered. *Journal of Immunology* 156:2352–6.

Scott, J. 1990. The spontaneously diabetic BB rat: sites of the defects leading to autoimmunity and diabetes mellitus. A review. *Current Topics in Microbiology & Immunology* 156:1–14.

Segal, B. M., Klinman, D. M., and Shevach, E. M. 1997. Microbial products induce autoimmune disease by an IL-12 dependent pathway. *Journal of Immunology* 158:5087–90.

Seiberler, S., Scheiner, O., Kraft, D., Lonsdale, D., and Valenta, R. 1994. Characterization of birch pollen allergen Bet v. III representing a novel class of Ca^{2+} binding proteins; specific expression in mature pollen and dependence of patients IgE binding on protein bound Ca^{2+}. *EMBO Journal* 13:3481–6.

Selgrade, M. K., and Daniels, M. J. 1995. Host resistance models: murine cytomegalovirus. In *Methods in Immunotoxicology,* Volume 2 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 203–20.

Selgrade, M. K., Daniles, M. J., Hu, P. C., Miller, F. J., and Graham, J. A. 1982a. Effects of immunosuppression with cyclophosphamide on acute murine cytomegalovirus

infection and virus-augmented natural killer cell activity. *Infection and Immunity* 38:1046-55.

Selgrade, M. K., Huang, Y. S., Graham, J. A., Huang, C. H., and Hu, P. C. 1982b. Humoral antibody response to individual viral proteins after murine cytomegalovirus infection. *Journal of Immunology* 131:3032-35.

Selgrade, M. K., Daniels, M. J., Burleson, G. R., Lauer, L. D., and Dean, J. H. 1988. Effects of 7,12 dimethylbenz[a]anthracene, benzo[a]pyrene and cyclosporin A on murine cytomegalovirus infection: studies on resistance mechanisms. *International Journal of Immunopharmacology* 10:811–8.

Selgrade, M. K., Starnes, D. M., Illing, J. W., Daniels, M. J., and Graham, J. A. 1989. Effects of phosgene exposure on bacterial, viral and neoplastic lung disease. *Inhalation Toxicology* 1:243-59.

Selgrade, M. K., Cooper, K. D., Devlin, R. B., Van Loveren, H., Biagini, R. E., and Luster, M. **I.** 1995. Immunotoxicity—bridging the gap between animal research and human health effects. *Fundamental and Applied Toxicology* 24:13-21.

Senpuku, H., Iizima, T., Yamaguchi, Y., Nagata, S., Ueno, Y., Saito, M., Hanada, N., and Nisizawa, T. 1996. Immunogenicity of peptides coupled with multiple T-cell epitopes of a surface protein antigen of Streptococcus mutans. *Immunology* 88:275–83.

Serreze, D. V., and Leiter, E. H. 1994. Genetic and pathogenic basis of autoimmune diabetes in NOD mice. *Current Opinion in Immunology* 6:900–6.

Serushago, B. A., Yoshikai, Y., Handa, T., Mitsuyama, M., Muramori, K., and Nomoto, K. 1992. Effect of recombinant human granulocyte colony-stimulating factor (rh G-CSF) on murine resistance against Listeria monocytogenes. *Immunology* 75:475–80.

Sette, A., Southwood, S., O'Sullivan, D., Gaeta, F. C., Sidney, J., and Grey, H. M. 1992. Effect of pH on MHC class 11–peptideinteractions. *Journal of Immunology* 148:844–51.

Sette, A., Alexander, J., Ruppert, J., Snoke, K., Franco, A., Ishioka, G., and Grey, H. M. 1994. Antigen analogs/MHC complexes as specific T cell receptor antagonists. *Annual Review of Immunology* 12:413–31.

Sette, A., Alexander, J., and Grey, H. M. 1995. Interaction of antigenic peptides with MHC and TCR molecules. *Clinical Immunology and Immunopathology* 76:S168-71.

Seya, T., Nakamura, K., Masaki, T., Ichihara-Itoh, C., Matsumoto, M., and Nagasawa, S. 1995. Human factor H and C4b-binding protein serve as factor I-cofactors both encompassing inactivation of C3b and C4b. *Molecular Immunology* 32:355–60.

Shamoto, M., Hosokswa, S., Shinzato, M., and Kaneko, C. 1993. Comparison of Langerhans cells and interdigitating reticulum cells. *Advances in Experimental Medicine and Biology* 329:311-4.

Shapiro, F., Pytowski, B., Rafii, S., Witte, L., Hicklin, D. J., Yao, T. J., and Moore, M. A. 1996. The effects of Flk-2/flt3 ligand as compared with c-kit ligand on short-term and long-term proliferation of CD34+ hematopoietic progenitors elicited from human fetal liver, umbilical cord blood, bone marrow, and mobilized peripheral blood. *Journal of Hematotherapy* 5:655-62.

Sharon, R., and Naor, D. 1989. Experimental model of autoimmune hemolytic anemia induced in mice with levadopa. *Clinical Immunology and Immunopathology* 52:160-72.

de Shazo, R. D., Lopez, M., and Salvaggio, J. E., 1987. Use and interpretations of diagnostic immunological laboratory tests. *JAMA* 258:30 11-3031.

She, J.-X. 1996. Susceptibility to type I diabetes: HLA-DQ and DR revisited. *Immunology Today* 17:323-9.

Shearman, C. W., Pollock, D., White, G., Hehir, K., Moore, G. P., Kanzy, E. J., and Kurrle, R. 1991. Construction, expression and characterization of humanized antibodies directed against the alpha/beta T cell receptor. *Journal of Immunology* 147:4366–73.

Shephard, R. J., Rhind, S., and Shek, P. N. 1994. Exercise and training: influences on cytotoxicity, interleukin-1, interleukin-2 and receptor structures. *International Journal of Sports Medicine* 15:S154–66.

Shi, J., Ross, C. R., Chengappa, M. M., Sylte, M. J., McVey, D. S., and Blecha, F. 1996. Antibacterial activity of a synthetic peptide PR-26 derived from PR-39 proline-arginine rich neutrophil antimicrobial peptide. *Antimicrobial Agents and Chemotherapy* 40:115–21.

Shibaki, A., Meunier, L., Ra, C., Shimada, S., Ohkawara, A., and Cooper, K. D. 1995. Differential responsiveness of Langerhans cell subsets of varying phenotypic states in normal human epidermis. *Journal of Investigative Dermatology* 104:42–6.

Shimada, A., Kasatani, T., Takei, I., Maruyama, T., Nomaguchi, H., Ozawa, Y., Ishii, M., Kasuga, A., Tashiro, F., Miyazaki, J., Yamamura, K., and Saruta, T. 1996. Immune response to heat-shock protein correlates with induction of insulitis in I-E alpha d transgenic NOD mice. *Diabetes* 45:165–9.

Shimozato, T., and Kincade, P. W. 1997. Indirect suppression of IL-7-responsive B cell precursors by vasoactive intestinal peptide. *Journal of Immunology* 158:5178-84.

Shivnan, E., Clayton, L., Allridge, L., Keating, K. E., Gullberg, M., and Alexander, D. R. 1996. CD45 monoclonal antibodies inhibit TCR-mediated calcium signals, calmodulin-kinase IV/Gr activation, and oncoprotein 18 phosphorylation. *Journal of Immunology* 157:101-9.

Siekevitz, M., Kocks, C., Rajewsky, K., and Dildrop, R. 1987. Analysis of somatic mutation and class switching in naive and memory B cells generating adoptive primary and secondary responses. *Cell* 48:757.

Sieling, P. A., Chaterjeen, D., Porcelli, S. A., Progizy, T. I., Mazzaccaro, R. J., Soriano, T., Bloom, B. R., Brenner, M. B., Kronenberg, M., Brennan, P. J., and Modlin, R. L. 1995. CD-1 restricted T cell recognition of microbial lipoglycan products. *Science* 269:227-30.

Sieper, J., Kingsley, G. H., and Marker-Hermann, E. 1996. Aetiological agents and immune mechanisms in enterogenic reactive arthritis. *Baillieres Clinical Rheumatology* 10:105-21.

Sikorski, E. E., McCay, J. A., White, K. L., Bradley, S. G., and Munson, A. E. 1989. Immunotoxicity of the semiconductor gallium arsenide in female B6C3F1 mice. *Fundamental and Applied Toxicology* 13:843–58.

Sikorski, E. E., Gerberick, G. F., Ryan, C. A., Miller, C. M., and Ridder, G. M. 1996. Phenotypic analysis of lymphocyte subpopulations in lymph nodes draining the ear following exposure to contact allergens and irritants. *Fundamental and Applied Toxicology* 34:25–35.

Silberberg, I., Baer, R. L., Rosenthal, S. A., Thorbecke, G. J., and Berezowsky, V. 1975.

Dermal and intravascular Langerhans cells at sites of passively induced allergic contact sensitivity. *Cellular Immunology* 18:435–53.

Silberberg, I., Baer, R. L., and Rosenthal, S. A. 1976. The role of Langerhans cells in allergic contact hypersensitivity. A review of findings in man and guinea pigs. *Journal of Investigative Dermatology* 66:210–7.

Sim, R. B., Malhotra, V., Ripoche, J., Day, A. J., Micklem, K. J., and Sim, E. 1986. Complement receptors and related complement control proteins. *Biochemical Society Symposia* 51:83-96.

Simon, C., Mercader, A., Portoles, E., Frances, A., and Pellicer, A. 1995. The interleukin-l system during human implantation. *Contraception, Fertilite, Sexualite* 23:626–30.

Sinclair, A. J., Barnett, A. H., and Lunec, J. 1990. Free radicals and antioxidant systems in health and disease. *British Journal of Hospital Medicine* 43:334–44.

Singer, L., Schlienger, J. L., Kammerer, F., and Stephan, F. 1979. Lithium and thyroid function. Significance of the TRH test in the diagnosis of lithium-induced thyroid dysfunction. *Encephale* 5:171-88.

Sinigaglia, F., Sturniolo, T., Raddrizzani, L., and Hammer, J. 1997. Antigenic peptides and autoimmunity. *APMIS* 105:81–8.

Siraganian, R. P. 1975. Automated histamine release: a method for in vitro allergy diagnosis. *International Archives of Applied Immunology* 49:108-10.

Sirchia, G., and Lewis, S. M. 1975. Paroxysmal nocturnal haemoglobinuria. *Clinics in Haematology* 4:199-229.

Skoner, D. P., Whiteside, T. L., Wilson, J. W., Doyle, W. J., Herberman, R. B., and Fireman, P. 1996. Effect of influenza A virus infection on natural and adaptive cellular immunity. *Clinical Immunology and Immunopathology* 79:294–302.

Smerdou, C., Anton, I. M., Plana, J., Curtiss, R. R., and Enjuanes, L. 1996. A continuous epitope from transmissible gastroenteritis virus S protein fused to E. coli heat-labile toxin B subunit expressed by attenuated Salmonella induces serum and secretory immunity. *Virus Research* 41:1-9.

Smialowicz, R. J. 1995. In vitro lymphocyte proliferation assays: the mitogen stimulated response and the mixed lymphocyte reaction in immunotoxicity testing. In *Methods in Immunotoxicology,* Volume 1 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp. 197–210.

Smialowicz, R. J., Rogers, R. R., Rowe, D. G., Riddle, M. M., and Luebke, R. W. 1987. The effects of nickel on immune function in the rat. *Toxicology* 44:271–81.

Smialowicz, R., Riddle, M. M., Luebke, R. W., Copeland, C. B., Andrews, D., Rogers, R. R., Gray, L. E., and Laskey, J. W. 1991. Immunotoxicity of 2-methoxyethanol following oral administration in Fischer rats. *Toxicology and Applied Pharmacology* 109:494–506.

Smith, G. L. 1996. Virus proteins that bind cytokines, chemokines or interferons. *Current Opinion in Immunology* 8:467–71.

Smith, J. D., Myers, N. B., Gorka, J., and Hansen, T. H. 1993. Model for the in vivo assembly of nascent Ld class I molecules and for the expression of unfolded Ld molecules at the cell surface. *Journal of Experimental Medicine* 178:2035–46.

Smith, M. E., and Ford, W. L. 1983. The migration of lymphocytes across specialized vascular epithelium. VI. The migratory behaviour of thoracic duct lymphocytes re-

transferred from the lymph node, spleen, blood or lymph of a primary recipient *Cellular Immunology* 78: 161–73.

Snapper, C. M., and Mond, J. J. 1996. A model for induction of T cell independent humoral immunity in response to polysaccharide antigens. *Journal of Immunology* 157:2229–33.

So, E. L., and Penry, J. K. 1981. Adverse effects of phenytoin on peripheral nerves and neuromuscular junction: a review. *Epilepsia* 22:467–73.

Sobieszczanska, B., and Przondo-Mordarska, A. 1996. Cross-reactivity between Borrelia burgdorferi and "arthrogenic" bacteria in sera from patients with reactive arthritis. *Roczniki Akademii Medycznej w Bialymstoku* 41:90-5.

Soldheim, J. C., Cook, J. R., and Hansen, T. H. 1995. Conformational changes induced in the MHC class I molecule by peptide and beta 2-microglobulin. *Immunologic Research* 14:200–17.

Soldheim, J. C., Carreno, B. M., and Hansen, T. H. 1997a. Are transporter associated with antigen processing (TAP) and tapasin class I MHC chaperones? *Journal ofImmunology* 158:541-3.

Soldheim, J. C., Hams, M. R., Kindle, C. S., and Hansen, T. H. 1997b. Prominence of beta 2-microglobulin, class I heavy chain conformation, and tapasin in the interactions of class I heavy chain with calreticulin and the transporter associated with antigen processing. *Journal of Immunology* 158:2236–41.

Song, Y.-H., Li, Y., and Maclaren, N. K. 1996. The nature of autoantigens targeted in autoimmune and endocrine diseases. *Immunology Today* 17:232–8.

Sontheimer, R. D., Stastny, P., and Nunez, *G.* 1986. HLA-D region antigen expression by human epidermal Langerhans cells. *Journal of Investigative Dermatology* 87:707–10.

Soulillou, J. P., Cantarovich, D., LeMauff, B., Giral, M., Robillard, N., Hourmant, M., Him, M., and Jacques, Y. 1990. Randomized trial of a monoclonal antibody against the interleukin receptor (33B3.1) as compared with rabbit antithymocyte globulin for prophylaxis against rejection of renal allografts. *New England Journal of Medicine* 17:1175–82.

Sozzani, S., Locati, M., Allavena, P., Van Damme, J., and Mantovani, A. 1996. Chemokines: a superfamily of chemotactic cytokines. *International Journal of Clinical and Laboratory Research* 26:69–82.

Sparer, T. E., Wynn, S. G., Clark, D. J., Kaplan, J. M., Cardoza, L. M., Wadsworth, S. C., Smith, A. E., and Gooding, L. R. 1997. Generation of cytotoxic T lymphocytes against immunorecessive epitopes after multiple immunizations with adenovirus vectors is dependent on haplotype. *Journal of Virology* 71:2277–84.

Spath, P., and Huber, H. 1973. Clinical importance of the complement system. *Wiener Klinische Wochenschrifr* 85:445-50.

Sperber, K. 1991. The role of atopy in asthma. *Mount Sinai Journal of Medicine* 58:494–9.

Spickett, G. P., Webster, A. D., and Farrant, J. 1990. Cellular abnormalities in common variable immunodeficiency. *Immunodeficiency Reviews* 2:199-219.

Spits, H., and de Waal Malefyt, R. 1992. Functional characterization of human IL-10. *International Archives of Allergy and Immunology* 99:8-15.

Spouge, J. L., Guy, H. R., Cornette, J. L., Margalit, H., Cease, K., Barzofsky, J. A., and

DeLisi, C. 1987. Strong conformational propensities enhance T cell antigenicity. *Journal of Immunology* 138:204.

Sprecher, E., and Becker, Y. 1986. Skin Langerhans cells play an essential role in the defense against HSV-1 infection. *Archives of Virology* 91:341–9.

Sprent, J. 1994. T and B memory cells. *Cell* 76:315–22.

Sprent, J. 1995a. Antigen-presenting cells. Professionals and amateurs. *Current Biology* 5:1095–7.

Sprent, J. 1995b. Immunological memory. *The Immunologist* 3:212–5.

Sprent, J., and Tough, D. F. 1994. Lymphocyte life-span and memory. *Science* 265:1395–400.

Stagsted, J., Mapelli, C., Meyers, C., Matthews, B. W., Anfinsen, C. B., Goldstein, A., and Olsson, L. 1993. Amino acid residues essential for biological activity of a peptide derived from a major histocompatibility complex class I antigen. *Proceedings of the National Academy of Sciences (USA)* 90:7686-90.

Stahlmann, R., Korte, M., Van Loveren, H., Vos, J. G., Theil, R., and Neubert, D. 1992. Abnormal thymus development and impaired function of the immune system in rats following prenatal exposure to acyclovir. *Archives of Toxicology* 66:55 1–9.

Standiford, T. J. 1997. Cytokines and pulmonary host defenses. *Current Opinion in Pulmonary Medicine* 3:81–8.

Standiford, T. J., Kunkel, S. L., Greenberger, M. J., Laichalk, L. L., and Strieter, R. M. 1996. Expression and regulation of chemokines in bacterial pneumonia. *Journal of Leukocyte Biology* 59:24–8.

Steiner, B. 1966. Immunoallergic pulmonary hemorrhage and the Goodpasture syndrome. *Acta Paediatrica Academiae Scientiarum Hungaricae* 7:157-73.

Steinman, R. M. 1991. The dendritic cell system and its role in immunogenicity. *Annual Review of Immunology* 9:271–96.

Steinman, R., Hoffman, L., and Pope, M. 1995. Maturation and migration of cutaneous dendritic cells. *Journal of Investigative Toxicology* 105:2S–7S.

Stejskal, V. D. M., Olin, R. G., and Forsbeck, M. 1986. The lymphocyte transformation test for diagnosis of drug induced occupational allergy. *Journal of Allergy and Clinical Immunology* 77:411–26.

Stellato, C., de Crescenzo, G., Patella, V., Mastronardi, P., Mazzarella, B., and Marone, G. 1996. Human basophil/mast cell releasability. XI. Heterogeneity of the effects of contrast media on mediator release. *Journal of Allergy and Clinical Immunology* 97:838-50.

Stem, L. J., and Wiley, D. C. 1994. Antigenic peptide binding by class I and class II histocompatibility proteins. *Behring Institute Mitteilungen* 1994:1-10.

Stevens, M. B. 1986. Connective tissue disease in the elderly. *Clinics in Rheumatic Diseases* 12:11-32.

Stevenson, M. M., Lyanga, J. J., and Skamene, E. 1982. Murine malaria: Genetic control of resistance to Plasmodium chabaudi. *Infection and Immunity* 38:80–8.

Stickle, J. E. 1996. The neutrophil. Function, disorders, and testing. *Veterinary Clinics of North America, Small Animal Practice* 26:1013-21.

Stiehm, E. R. 1993. New and old immunodeficiences. *Pediatric Research* 33:S2–7.

Stingl, G., Wolff-Schreiner, E. C., Pichler, W. J., Gschnait, F., Knapp, W., and Wolff, K. 1977. Epidermal Langerhans cells bear Fc and C3 receptors. *Nature* 268:245–6.

Stingl, G., Katz, S. I., Clement, L., Green, I., and Shevach, E. M. 1978. Immunologic functions of Ia-bearing epidermal Langerhans cells. *Journal of Immunology* 121:2005-13.

Stingl, G., Katz, S. I., Green, I., and Shevach, E. M. 1980. The functional role of Langerhans cells. *Journal of Investigative Dermatology* 74:315-8.

Stolzenberg, E. D., Kari, U. P., Zasloff, M., and Wilson, J. M. 1997. Human-beta-defensin-1 is a salt-sensitive antibiotic in the lung that is inactivated in cystic fibrosis. *Cell* 88:553–60.

Storkus, W. J., and Dawson, J. R. 1991. Target structures involved in natural killing (NK): characteristics, distribution and candidate molecules *Critical Reviews in Immunology* 10:393-417.

Stotts, J. 1980. Planning, conduct and interpretation of human predictive sensitization patch tests. In *Current Concepts in Cutaneous Toxicity* (V. A. Drill and P. Lazar, eds.), Academic Press, New York.

Stratton, M. A. 1985. Drug-induced systemic lupus erythematosus. *Clinical Pharmacy* 4:657–63.

Streilein, J. W. 1990. Anterior chamber associated immune deviation: the privilege of immunity in the eye. *Survey of Ophthalmology* 35:67–73.

Streilein, J. W., and Cousins, S. W. 1990. Aqueous humor factors and their effect on the immune response in the anterior chamber. *Current Eye Research* 9:175–82.

Streilein, J. W., Grammer, S. F., Yoshikawa, T., Demidem, A., and Vermeer, M. 1990a. Functional dichotomy between Langerhans cells that present antigen to naive and to memory/effector T lymphocytes. *Immunological Reviews* 117:159-83.

Streilein, J. W., Cousins, S., and Williamson, J. S. 1990b. Ocular molecules and cells that regulate immune responses in situ. *International Ophthalmology* 14:317-25.

Strieter, R. M., Polverini, P. J., Arenberg, D. A., and Kunkel, S. L. 1995a. The role of CXC chemokines as regulators of angiogenesis. *Shock* 4:155-60.

Strieter, R. M., Polverini, P. J., Arenberg, D. A., Walz, A., Opdenakker, G., Van Damme, J., and Kunkel, S. L. 1995b. Role of C-X-C chemokines as regulators of angiogenesis in lung cancer. *Journal of Leukocyte Biology* 57:752–62.

Strieter, R. M., Standiford, T. J., Huffnagle, G. B., Colletti, L. M., Lukacs, N. W., and Kinkel, S. L. 1996. "The good, the bad and the ugly." The role of cytokines in models of human disease. *Journal of Immunology* 156:3583–6.

Strzelecka, A., Kwiatkowska, K., and Sobota, A. 1997. Tyrosine phosphorylation and Fc gamma receptor-mediated phagocytosis. *FEBS Letters* 400:11-4.

Stuehr, D. J., and Nathan, C. F. 1989. Nitric oxide: a macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *Journal of Experimental Medicine* 169:1543-55.

Su, D. M., Wang, J., Lin, Q., Cooper, M. D., and Watanabe, T. 1997. Interferons alpha/beta inhibit IL-7-induced proliferation of CD4–CD8– CD3– CD44+ CD25+ thymocytes, but do not inhibit that of CD4–CD8– CD3– CD44– CD25– thymocytes. *Immunology* 90543–9.

Su, S., Mukaida, N., Wang, J., Zhang, Y., Takami, A., Nakao, S., and Matsushima, K. 1997. Inhibition of immature erythroid progenitor cell proliferation by macrophage inflammatory protein-1alpha by interacting mainly with a C-C chemokine receptor, CCR1. *Blood* 90:605–11.

Sugamura, K., Asao, H., Kondo, M., Tanaka, N., Ishii, N., Ohbo, K., Nakamura, M., and Takeshita, T. 1996. The interleukin 2 receptor gamma chain: its role in the multiple cytokine receptor complexes and T cell development in XSCID. *Annual Review of Immunology* 14:179-205.

Sumida, T., Namekawa, T., Maeda, T., and Nishioka, K. 1996. New T-cell epitope of Ro/SS-A 52 kDa protein in labial salivary glands from patients with Sjogren's syndrome. *Lancet* 348:1667.

Sung, Y.-J., Hotchkiss, J. H., Austic, R. E., and Dietert, R. R. 1991. L-Arginine dependent production of reactive nitrogen intermediate by macrophages of a uricotelic species. Journal of Leukocyte *Biology* 50:49–56.

Super, M., Thiel, S., Lu, J., Levinsky, R. J., and Turner, M. W. 1989. Association of low levels of mannan-binding protein with a common defect of opsonization, *Lancet* 2:1236-9.

Swain, S. L. 1991. Lymphokines and the immune response: the central role of interleukin-2. *Current Opinion in Immunology* 3:304–10.

Sweet, R. W., Truneh, A., and Hendrickson, W. A. 1991. CD4: its structure, role in immune function and AIDS pathogenesis, and potential as a pharmacological target. *Current Opinion in Biotechnology* 2:622–33.

Symth, M. J., and Trapiani, J. A. 1995. Granzymes: exogenous proteinases that induce target cell apoptosis. *Immunology Today* 16:202–6.

Szakal, A. K., and Tew, J. G. 1991. Significance of iccosomes in the germinal centre reaction. *Research in Immunology* 142:261–3.

Szakal, A. K., Kosco, M. H., and Tew, J. G. 1988a. A novel in vivo follicular dendritic cell-dependent iccosome-mediated mechanism for delivery of antigen to antigen-processing cells. *Journal of Immunology* 140:341–53.

Szakal, A. K., Kosco, M. H., and Tew, J. G. 1988b. FDC-iccosome mediated antigen delivery to germinal center B cells, antigen processing and presentation to T cells. *Advances in Experimental Medicine and Biology* 237:197-202.

Szakal, A. K., Kosco, M. H., and Tew, J. G. 1989. Microanatomy of lymphoid tissue during a humoral immune response: structure function relationships. *Annual Review of Immunology* 7:91–109.

Szakal, A. K., Kapasi, Z. F., Masuda, A., and Tew, J. G. 1992. Follicular dendritic cells in the alternative antigen transport pathway: microenvironment, cellular events, age and retrovirus related alterations. *Seminars in Immunology* 4:257–65.

Szamel, M., Ebel, U., Uciechowski, P., Kaever, V., and Resch, K. 1997. T cell antigen receptor dependent signaling in human lymphocytes: cholera toxin inhibits interleukin-2 receptor expression but not interleukin-2 synthesis by preventing activation of a protein kinase C isotype, PKC-alpha. *Biochimica et Biophysica Acta* 1356:237–48.

Taga, T., and Kishimoto, T. 1997. Gp130 and the interleukin-6 family of cytokines. *Annual Review of Immunology* 15:797–819.

Taga, T., Kariya, Y., Shimada, M., and Uchida, A. 1993. Suppression of natural killer cell activity by granulocytes in patients with aplastic anemia: role of granulocyte colony-stimulating factor. *Immunology Letters* 39:65–70.

Tagboto, S. K. 1995. Interleukin-5, eosinophils and the control of helminth infections in man and laboratory animals. *Journal of Helminthology* 69:27 1–8.

Tagliabue, A., and Boraschi, D. 1993. Cytokines as vaccine adjuvants: interleukin 1 and its synthetic peptide 163–171. *Vaccine* 11:594-5.

Taguchi, T., and Kimoto, Y. 1986. LAK cells and cancer. *Gan to Kagaku Ryoho (Japanese Journal of Cancer & Chemotherapy)* 13:3327–33.

Takatsu, K., Takaki, S., Hitoshi, Y., Mita, S., Katoh, S., Yamaguchi, N., and Tominaga, A. 1992. Cytokine receptors on Ly-1 B cells. IL-5 and its receptor system. *Annals of the New York Academy of Sciences* 651:241-58.

Takeda, K., Okuno, T., Isegawa, Y., and Yamanishi, K. 1996. Identification of a variant A-specific neutralizing epitope on glycoprotein B (gB) of human herpesvirus-6 (HHV-6). *Virology* 222:176–83.

Tamir, H., and Ratner, S. 1963. Enzymes of arginine metabolism in chicks. *Archives* of *Biochemistry and Biophysics* 102:249–58.

Tanaka, K. 1996. Antigen-presenting mechanism of MHC class I molecules. *Nihon Rinsho Meneki Gakkai Kaishi* 19:544-7.

Tangri, S., Holcombe, H. R., Castano, A. R., Miller, J. E., Teitell, M., Huse, W. E., Peterson, P. A., and Kronenberg, M. 1996. Antigen-presenting function of the mouse CD1 molecule. *Annals of the New York Academy of Sciences* 778:288–96.

Tani, Y., Sato, H., Tanaka, N., and Hukuda, S. 1997a. Antibodies against bacterial lipopolysaccharides in Japanese patients with ankylosing spondylitis. *British Journal of Rheumatology* 36:491–3.

Tani, *Y.,* Tiwana, H., Hukuda, S., Nishioka, J., Fielder, M., Wilson, C., Bansal, S., and Ebringer, A. 1997b. Antibodies to Klebsiella, Proteus, and HLA-B27 peptides in Japanese patients with ankylosing spondylitis and rheumatoid arthritis. *Journal of Rheumatology* 24:109-14.

Tanner, J. E., and Alfieri, C. 1996. Interactions involving cyclosporine A, interleukin 6, and Epstein Barr virus lead to the promotion of B-cell lymphoproliferative disease. *Leukemia and Lymphoma* 21:379–90.

Taub, D. D. 1996. Chemokine–leukocyteinteractions. The voodoo that they do so well. *Cytokine and Growth Factor Reviews* 7:355–76.

Taub, D. D., Ortaldo, J. R., Turcovski-Corrales, S. M., Key, M. L., Longo, D. L., and Murphy, W. J. 1996. Beta chemokines costimulate lymphocyte cytolysis, proliferation, and lymphokine production. *Journal of Leukocyte Biology* 59:81–9.

Taylor, S. L. 1995. Evaluation of the allergenicity of foods developed through biotechnology. In *Proceedings of the Third International Symposium on Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms* (D. D. Jones, ed.), University of California Press, Oakland, p. 195–208.

Tedder, T. F., Zhou, L. J., and Engel, P. 1994. The CD19/CD21 signal transduction complex of B lymphocytes. *Immunology Today* 15:437–41.

Teitell, M., Holcombe, H. R., Brossay, L., Hagenbaugh, A., Jackson, M. J., Pond, L., Balk, S. P., Terhorst, C., Peterson, P. A., and Kronenberg, M. 1997. Nonclassical behavior of the mouse CDl class I-like molecule. *Journal of Immunology* 158:2143–9.

Temple, L., Kawabata, T. T., Munson, A. E., and White, K. L. 1993. Comparison of ELISA and plaque forming assays for measuring the humoral response to SRBC in rats and mice treated with benzo[a]pyrene and cyclophosphamide. *Fundamental and Applied Toxicology* 21:412-19.

Tenner, A. J., Robinson, S. L., and Ezekowitz, R. A. B. 1995. Mannose binding protein

(MBP) enhances mononuclear phagocyte function via a receptor that contains 126,000 M(r) component of the C1q receptor. *Immunity* 3:485–93.

Teran, L. M., and Davies, D. E. 1996. The chemokines: their potential role in allergic inflammation. *Clinical and Experimental Allergy* 26:1005-19.

Terashima, K., Dobashi, M., Maeda, K., and Imai, Y. 1992. Follicular dendritic cell and iccosomes in germinal center reactions. *Seminars in Immunology* 4:267–74.

Tew, J. G., and Mandel, T. E. 1978. The maintenance and regulation of serum antibody levels: evidence indicating a role for antigen retained in lymphoid follicles. *Journal of Immunology* 120:1063.

Tew, J. G., Kosco, M. H., Burton, G. F., and Szakal, A. K. 1990. Follicular dendritic cells as accessory cells. *Immunological Reviews* 117:185-211.

Theodore, A. C., Center, D. M., Nicoll, J., Fine, G., Kornfeld, H., and Cruikshank, W. W. 1996. CD4 ligand IL-16 inhibits the mixed lymphocyte reaction. *Journal ofImmunology* 157:1958–64.

Tharp, M. D. 1990. IgE and immediate hypersensitivity. *Dermatological Clinics* 8:619–631.

Theofilopoulos, A. N. 1995. The basis of autoimmunity: Part I. Mechanisms of aberrant self-recognition. *Immunology Today* 16:90–8.

Thijs, L. G., and Hack, C. E. 1995. Time course of cytokine levels in sepsis. *Intensive Care Medicine* 21:S258–63.

Thivolet, C. 1992. Immunologic mechanisms implicated in autoimmune destruction of beta cells in type I diabetes. *Annales di Endocrinologie* 53:78–81.

Thomas, C., Groten, J., Kammuller, M. E., De Bakker, J. M., Seinen, W., and Bloksma, N. 1989. Popliteal lymph node reactions in mice induced by the drug zimelidine. *International Journal of Immunopharmacology* 11:693-702.

Thomas, H. C., Foster, G. R., Sumiya, G. R., Mc Intosh, D., Jack, D. L., Turner, M. W., and Summefield, J. A. 1996. Mutation of gene of mannose-binding protein associated with chronic hepatitis B viral infection. *Lancet* 348: 1417–9.

Thomas, P., Fugmann, R., Aranyi, C., Barbera, P., Gibbons, R., and Fenters, J. 1985. Effect of dimethylnitrosamine in host resistance and immunity. *Toxicology and Applied Pharmacology* 77:219-29.

Thompson, R. A., and Winterborn, M. H. 1981. Hypocomplementaemia due to a genetic deficiency of beta 1H globulin. *Clinical and Experimental Immunology* 46:110-9.

Thornsen, B. S. 1988. Human erythrocyte complement receptor type one (CR1): expression, function and role in the pathogenesis of inflammatory connective tissue diseases. *Danish Medical Bulletin* 35:414–22.

Thomson, G. T., DeRubeis, D. A., Hodge, M. A., Rajanayagam, C., and Inman, R. D. 1995. Post-Salmonella reactive arthritis: late clinical sequelae in a point source cohort. *American Journal of Medicine* 98:13–21.

Thomssen, H., Kahan, M., and Londei, M. 1996. IL-3 in combination with IL-4, induces the expression of functional CD1 molecules on monocytes. *Cytokine* 8:476–81.

Thome, P. S., and Karol, M. H. 1988. Assessment of airway reactivity in guinea pigs: comparison of methods employing whole body plethysmography. *Toxicology* 52:141–63.

Thorne, P. S., Hillibrand, J. A., Lewis, G. R., and Karol, M. H. 1986. Experimental contact sensitization by isocyanates. *The Toxicologist* 6:15.

Thorpe, C. J. 1993. Outsize peptides bulge out of the groove. *Immunology Today* 14:51-2.

Ting, C. C., Wang, J., and Yang, Y. 1996. Interleukin-2 and interleukin-7 augment the cytolytic activity and expand the antitumor killing spectrum of alpha CD3-induced activated killer cells: potential use in the immunotherapy of non-immunogenic tumors. *Cancer Immunology and Immunotherapy* 43:283–92.

Tishler, M., and Shoenfeld, Y. 1996. Anti-heat-shock protein antibodies in rheumatic and autoimmune diseases. *Seminars in Arthritis and Rheumatism* 26:558-63.

Todd, J. A. 1990. Genetic control of autoimmunity in type 1 diabetes. *Immunology Today* 11:122-9.

Toivanen, A., and Toivanen, P. 1996. Reactive arthritis. *Current Opinion in Rheumatology* 8:334–40.

Tough, D. F., and Sprent, J. 1995. Life span of naive and memory T cells. *Stem Cells* 13:242–9.

Trautinger, F., Kokoschka, E. M., and Menzel, E. J. 1991. Antibody formation against human collagen and C1q in response to a bovine collagen implant. *Archives of Dermatological Research* 283:395–9.

Trembleau, S., Germann, T., Gately, M. K., and Adorini, L. 1995. The role of IL-12 in the induction of organ-specific autoimmune diseases. *Immunology Today* 16:383–6.

Trinchieri, G. 1995a. Interkeukin 12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annual Review of Immunology* 13:251-76.

Trinchieri, G. 1995b. The two faces of interleukin 12: a proinflammatory cytokine and a key immunoregulatory molecule produced by antigen-presenting cells. *Ciba Foundation Symposium* 195:203–14; discussion 214–20.

Tsai, W., Strieter, R. M., Zisman, D. A., Wilkowski, J. M., Bucknell, K. A., Chen, G. H., and Standiford, T. J. 1997. Nitric oxide is required for effective innate immunity against Klebsiella pneumoniae. *Infection and Immunity* 65:1870-5.

Tsiagbe, V. K., and Thorbecke, G. J. 1991. Memory B cells and germinal centres. *Research in Immunology* 142:268–72.

Tsokos, G. C. 1996. Lymphocytes, cytokines, inflammation, and immune trafficking. *Current Opinion in Rheumatology* 8:395–402.

Tsukada, S., Saffran, D. C., Rawlings, D. J., Parolino, O., Klinsak, I., Sparkers, R. S., Kubagawa, H., Mohandas, T., and Quan, S. 1993. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 72:279–90.

Tuchscherer, G., Servis, C., Corradin, G., Blum, U., Rivier, J., and Mutter, M. 1992. Total chemical synthesis, characterization, and immunological properties of an MHC class I model using the TASP concept for protein de novo design. *Protein Science* 1:1377-86.

Tun, R. Y., Smith, M. D., Lo, S. S., Rook, G. A., Lydyard, P., and Leslie, R. D. 1994. Antibodies to heat shock protein 65 kD in type 1 diabetes mellitus. *Diabetic Medicine* 11:66-70.

Tuokko, J., Reijonen, H., Ilonen, J., Anttila, K., Nikkari, S., Mottonen, T., Yli-Kerttula, U., and Toivanen, A. 1997. Increase of HLADRB1*0408 and DQB1*0301 in HLA-B27 positive reactive arthritis. *Annuls of the Rheumatic Diseases* 56:37–40.

Turner, M. W. 1996. Mannose binding lectin: the pluripotent molecule of the innate immune system. *Immunology Today* 17:532-9.

Turner, M. W., Seymour, N. D., Kazatchkine, M. D., and Mowbray, J. F. 1985. Suboptimal C3b/C3bi deposition and defective yeast opsonization I. Evidence for the absence of cofactor activity. *Clinical and Experimental Immunology* 62:435–41.

Tutt, A., Stevenson, G. T., and Glennie, M. J. 1991. Trispecific F(ab') derivatives that use cooperative signaling via the TCR/CD3 complex and CD2 to activate and redirect resting cytotoxic T cells. *Journal of Immunology* 147:60–70.

Uittenbogaart, C. H., Schmidc, I., Kiertscher, S., Shau, H., Anisman, D. J., Zsebo, K. M., and Clement, L. T. 1996. Human thymocyte responsiveness to stem cell factor: synergy with interleukin-2 for the generation of NK/LAK cytotoxicity. *Immunology Letters* 52:45–52.

Urban, J. L., Burton, R. C., Holland, J. M., Kripke, M. L., and Schreiber, H. 1982. Mechanisms of syngeneic tumor rejection, susceptibility of host-selected progressor variants to various immunological effector cells. *Journal of Experimental Medicine* 155:557-73.

Urban, J., Qabar, M., Sia, C., Klein, M., and Kahn, M. 1996. Sculpted immunogens; B-cell epitope optimization using constrained secondary structure libraries. *Bioorganic and Medicinal Chemistry* 4:673-6.

Urban, T., Hurbain, I., Urban, M., Clement, A., and Housset, B. 1995. Oxidants and antioxidants. Biological effects and therapeutic perspectives. *Annales de Chirurgie* 49:427–34.

Vaigot, P., Czernielewski, J., and Prunieras, M. 1985. Detection of distinct subpopulations of Langerhans cells by flow cytometry and sorting. *Cytometry* 6:422–7.

Vajdy, M., and Lycke, N. Y. 1992. Cholera toxin adjuvant promotes long-term immunological memory in the gut mucosa to unrelated immunogens after oral immunization. *Immunology* 75:488–92.

Valenzona, H. O., Pointer, R., Ceredig, R., and Osmond, D. G. 1996. Prelymphomatous B cell hyperplasia in the bone marrow of interleukin-7 transgenic mice: precursor B cell dynamics, microenvironmental organization and osteolysis. *Experimental Hematology* 24:1521–9.

Van Baarlen, J., Schuurman, H.-J., and Huber, J. 1988. Acute thymus involution in infancy and childhood: a reliable marker for duration of acute illness. *Human Pathology* 19:1155-68.

van Bleek, G. M., and Nathenson, S. G. 1991. The structure of the antigen-binding groove of major histocompatibility complex class I molecules determines specific selection of self-peptides. *Proceedings of the National Academy of Sciences (USA)* 88:11032–6.

Vandebriel, R. J., Meredith, C., Scott, M. P., Gleichman, E., Bloksma, N., Van't Erve, E. H., Descotes, J., and Loveren, H. 1996. Early indicators of immunotoxicity: Development of molecular biological test batteries. *Human and Experimental Toxicology* 15:S2–9.

Vandekerckhove, B. A. E., Krowka, J. F., McCune, J. M., DeVries, J. E., Spits, H., and Roncarolo, M. G. 1991. Clonal analysis of the peripheral T cell compartment of the SCID-hu mouse. *Journal of Immunology* 146:4173–9.

Van den Eertwegh, A. J. M., Boersma, W. J. A., and Claassen, E. 1992. Immunological functions and in vivo cell–cellinteractions of T cells in the spleen. *Critical Reviews of Immunology* 11:337–80.

van der Burg, S. H., Visseren, M. J., Brandt, R. M., Kast, W. M., and Melief, C. J. 1996. Immunogenicity of peptides bound to MHC class I molecules depends on the MHC–peptide complex stability. *Journal of Immunology* 156:3308–14.

van der Heijden, R. W., Bunschoten, H., Hoek, A., Van Es, J., Punter, M., Osterhaus, A. D., and Uytdehaag, F. G. 1991. A human CD5+ B cell clone that secretes an idiotype-specific high affinity IgM monoclonal antibody. *Journal of Immunology* 146:1503-8.

van der Linden, E. F. H., Kroonenburgh, M. J. P. G., and Pauwels, E. K. J. 1988. Side-effects of monoclonal antibody infusions for the diagnosis and treatment of cancer. International *Journal of Biomarkers* 12:147-53.

Van Dijk, H., Rademacher, P. M., and Willers, J. M. N. 1980. Estimation of the classical pathway of mouse complement activity by use of sensitized rabbit erythrocytes. *Journal of Immunological Methods* 30:257–68.

Van Dyke, T. E., and Hoop, G. A. 1990. Neutrophil function and oral disease. *Critical Reviews in Oral Biology and Medicine* 1:117–33.

Vane, J. R. 1971. Inhibition of prostaglandins synthesis as mechanism of action for aspirin-like drugs. Nature 23 1 :232–4.

van Eden, W. V. 1993. Possible role of bacterial heat-shock proteins in autoimmune diseases. *Zentralblatt fuer Bakteriologie* 278:377–82.

Van Ert, M., and Battugelli, M. C. 1975. Mechanism of respiratory injury by TDI (toluene diisocyanate). *Annals of Allergy* 35:142-7.

van Herwenden, L., Harrap, S. B., Wong, Z. Y., Abramson, M. J., Kutin, J. J., Forbes, A. B., Raven, J., Lanigan, A., and Walters, E. H. 1995. Linkage of a high affinity IgE receptor gene with bronchial hyperreactivity even in the absence of atopy. *Lancet* 346:1262-5.

Van Loveren, H., Postma, G. W., Van Soolingen, D., Kruizinga, W., Groothuis, D., and Vos, J. 1987. Enhanced macrophage activity and deficient acquired resistance to Listeria monocytogenes in nude rats. In *Immune deficient animals in biomedical research.* J. Rygaard, N. Brunner, N. Gream, and M. Spang-Thompson (eds.) Karger, Basel p. 108.

Van Loveren, H., Luebke, R. W., and Vos, J. G. 1995. Assessment of immunotoxicity with the parasitic infection model Trichinella spiralis. In *Methods in Immunotoxicology,* Volume 2 (G. R. Burleson, J. H. Dean, and A. E. Munson, eds.), Wiley–Liss, New York, pp 243–71.

van Noesel, C. J. M., Lankester, A. C., and van Liera, R. A. 1993. Dual antigen recognition by B cells. *Immunology Today* 14:8-12.

Van Regernmortel, M. H., and Daney de Marcillac, G. 1988. An assessment of prediction methods for locating continuous epitopes in proteins. *Immunology Letters* 17:95–107.

van Rooijen, N. 1989. Are bacterial endotoxins involved in autoimmunity by CD5+ (Ly-1 +) B cells? *Immunology Today* 10:334–6.

Varshavsky, A,, Bachmair, A., Finley, D., Gonda, D. K., and Wunning, I. 1989. Targeting of proteins for degradation. *Biotechnology* 13:109-43.

Veerhuis, R., van Es, L. A., and Daha, M. R. 1985. In vivo modulation of rat complement activities by infusion of anti-H antibodies. *Immunobiology* 170:133-45.

Velazquez, C., Valette, I., Cruz, M., Labra, M. L., Montes, J., Stanley, S. L., Jr., and

Calderon, J. 1995. Identification of immunogenic epitopes of the 170-kDa subunit adhesin of Entamoeba histolytica in patients with invasive amebiasis. *Journal of Eukaryotic Microbiology* 42:636–41.

Venza-Teti, D., Misefari, A., Sofo, V., Fimiani, V., and LaVia, M. F. 1980. Interaction between prostaglandins and human T lymphocytes: effect of PGE2 on E-receptor expression. *Immunopharmacology* 2:165-71.

Verjans, G. M., Klaren, V. N., Leirisalo-Repo, M., Ringrose, J. H., Repo, H., Steinle, A., Van Doornik, C. E., and Feltkamp, T. E. 1996. Heterogeneity of rearranged T cell receptor V alpha and V beta gene transcripts in synovial fluid T cells of HLA-B27 positive reactive arthritis patients. *Clinical Rheumatology* 15:91–6.

Vetrie, D., Vorechovysky, I., Sideras, P., Holland, J., Davies, A., Flinter, F., Hammaarstrom, L., Kinnon, C., Levinsky, R., Bobrow, M., Edwards, M., Edvard Smith, C. I., and Bentley, D. R. 1993. The gene involved in X-linked agammaglobulinaemia is a member of the src family of protein kinases. *Nature* 361:226–33.

Vicenzi, E., Biswas, P., Mengozzi, M., and Poli, G. 1997. Role of pro-inflammatory cytokines and beta-chemokines in controlling HIV replication. *Journal of Leukocyte Biology* 62:34–40.

Vieira, L. Q., Hondowicz, B. D., Afonso, L. C., Wysocka, M., Trinchieri, G., and Scott, P. 1994. Infection with Leishmania major induces interleukin-12 production in vivo. *Immunology Letters* 40:157-61.

Vihinen, M., Cooper, M. D., de Saint Basite, G., Fischer, A., Good, R. A., Hendriks, R. W., Kinnon, C., Kwan, S. P., Litman, G. W., and Notarangelo L. D. 1995. BTKbase: a database of XLA-causing mutations — International Study Group. *Immunology Today* 16:460–5.

Villarrubia, V. G., Sanchez, L., and Alvarez de Mon, M. 1996. New vaccines and the immune response (I). Immunologic memory. Humoral response versus cellular response. *Medicina Clinica* 107:146-54.

Vireligier, J. 1975. Host defense against influenza virus: the role of anti-hemagglutinin antibody. *Journal of Immunology* 115:434–9.

Vitetta, E. S., Berton, M. T., Burger, C., Kepron, M., Lee, W. T., and Yin, X. M. 1991. Memory B and T cells. *Annual Review of Immunology* 9:193–217.

Vivier, E., Rochet, N., Kochan, J.-P., Presky, D. H., and Schlossman, S. F. 1991. Structural similarities between Fc receptors and T cell receptors: expression of the γ subunit of FcεRI in human T cells, NK cells and thymocytes. *Journal of Immunology* 147:4263–70.

Vivier, E., Ackerly, M., Rochet, N., and Anderson, P. 1992. Structure and function of CD16:zeta:gamma complex expressed on human natural killer cells. *International Journal of Cancer* 7(supplement):11-4.

Vogt, W. 1974. Activation, activities and pharmacologically active products of complement. *Pharmacological Reviews* 26:125-69.

Voitenok, N. N. 1988. Interleukin 2. *Terapevticheskii Arkhiv* 60:42–6.

Volanakis, J. E. 1975. The human complement system. *Journal of Oral Pathology* 4:195-221.

Vollger, L. W., and Uittenbogaart, C. H. 1993. Interleukin-7 promotes the generation of phenotypically mature CD45RA positive human thymocytes in vitro. *Cytokine* 5:157-68.

Volpe, R. 1994. Immunoregulation in autoimmune thyroid disease. Thyroid 4:373–7.

Vongsakul, M. 1995. Characterization of immunological memory cells. A minireview. *Asian Pacific Journal of Allergy & Immunology* 13:75–9.

Von Willebrand, M., Jascur, T., Bonnefoy-Berard, N., Yano, H., Altman, A., Matsuda, Y., and Mustelin, T. 1996. Inhibition of phosphatidylinositol 3-kinase blocks T cell antigen receptor/CD34nduced activation of the mitogen-activated kinase Erk2. *European Journal of Biochemistry* 235:828–35.

Vos, J. G. 1977. Immune suppression as related to toxicity. *Critical Reviews in Toxicology* 5:67–78.

Vos, J. G., Ruitenberg, E. J., Van Basten, N., Buys, J., Elgersma, A., and Kruizinga, W. 1983. The athymic nude rat. IV. Immunocytochemical study to detect T cells and immunological reactions against Trichinella spiralis. *Parasite Immunology* 5:195-215.

Vos, J. G., deKlerk, A., Krajnc, E. I., Kruizinga, W., van Ommen, B., and Rosing, J. 1984. Toxicity of bis(tri-n-butyltin)oxide in the rat. II Suppression of thymus dependent responses and parameters of nonspecific resistance after short term exposure. *Toxicology and Applied Pharmacology* 75:387-408.

Vos, J. G., deKlerk, A., Krajnc, E. I., Van Loveren, H., and Rozing, J. 1990. Immunotoxicity of bis(tri-n-butyltin)oxide in the rat: effects of thymus dependent-immunity and non-specific resistance following long term exposure in young versus old rats. *Toxicology and Applied Pharmacology* 195:144-55.

Vouldoukis, I., Riveros-Moreno, V., Dugas, B., Ouaaz, F., Becherel, P., Debre, P., Moncada, s., and Mossalayi, M. D. 1995. The killing of Leishmania major by human macrophages is mediated by nitric oxide induced after ligation of the Fc epsilon RII/CD23 surface antigen. *Proceedings of the National Academy of Science (USA)* 92:7804–8.

Vouldoukis, I., Becherel, P. A., Riveros-Moreno, V., Arock, M., da Silva, O., Debre, P., Mazier, D., and Mossalayi, M. D. 1997. Interleukin-10 and interleukin-4 inhibit intracellular killing of Leishmania infantum and Leishmania major by human macrophages by decreasing nitric oxide generation. *European Journal of Immunology* 27:860-5.

Vremec, D., Zorbas, M., Scollay, R., Saunders, D. J., Ardavin, C. F., Wu, L., and Shortman, K. 1992. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *Journal of Experimental Medicine* 176:47–58.

Wacker, H. H., Radsun, H. J., and Parwaresch, M. R. 1990. Accessory cells in normal and rodent lymph nodes: morphology, phenotype and functional implications. *Current Topics in Pathology* 84:193–218.

Wahn, V., Gobel, U., and Day, N. K. 1984. Restoration of complement function by plasma infusion in factor I (C3b inactivator) deficiency [letter]. *Journal of Pediatrics* 105:673-4.

Wainberg, M. A., and Mills, E. L. 1985. Mechanisms of virus-induced immune suppression. *Canadian Medical Association Journal* 132:1261-7.

Wakil, A. E., Wang, Z. E., and Locksley, R. M. 1996. Leishmania major: targeting IL-4 in successful immunomodulation of murine infection. *Experimental Parasitology* 84:214-22.

Waldmann, T. A. 1993. The IL-2/IL-2 receptor system: a target for rational immune intervention. *Immunology Today* 14:264–9.

Waldmann, H., and Munro, A. 1975. B cell activation. *Transplantation Reviews* 23:213–22.

Waldmann, T. A., and Goldman, C. K. 1989. The multichain interleukin-2 receptor: a target for immunotherapy of patients receiving allografts. *American Journal of Kidney Diseases* 14:45–53.

Walker-Smith, J. A., Ford, R. P., and Phillips, A. D. 1984. The spectrum of gastrointestinal allergies to food. *Annals of Allergy* 53:629–36.

Wallengren, M. L., Ericson, D., Forsberg, B., and Johnson, U. 1991. Human leukocyte antigens in relation to colonization by mutant streptococci in the oral cavity. *Oral Microbiology and Immunology* 6:292–4.

Ward, P. A. 1972. Biological activities of the complement system. *Annals of Allergy* 30:307–14.

Ward, P. A. 1996. Role of complement, chemokines, and regulatory cytokines in acute lung injury. *Annals of the New York Academy of Sciences* 796:104–12.

Ward, S. G. 1996. CD28: a signaling perspective. *Biochemical Journal* 318:361–77.

Ward, S. G., June, C. H., and Olive, D. 1996. P13-kinase: a pivotal pathway in T-cell activation. *Immunology Today* 17:187-98.

Weaver, J. E., and Herrman, K. W. 1981. Evaluation of adverse reaction reports for a new laundry product. *Journal of the American Academy of Dermatology* 4:577.

Webster, A. D. B. 1985. Laboratory investigations of primary deficiencies of the lymphoid system. *Clinics in Allergy and Immunology* 5:447-67.

Weeratna, R., Stamler, D. A., Edelstein, P. H., Ripley, M., Marrie, T., Hoskin, D., and Hoffman, P. S. 1994. Human and guinea pig immune responses to Legionella pneumophila protein antigens OmpS and Hsp60. *Infection and Immunity* 62:3454–62.

Weinstein, A. 1980. Drug-induced systemic lupus erythematosus. *Progress in Clinical Immunology* 4:1-21.

Weiss, L., and Geduldig, U. 1991. Barrier cells: stromal regulation of hematopoiesis and blood release in normal and stressed murine bone marrow. *Blood* 78:975–90.

Weitzman, S. A., and Gordon, L. I. 1990. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* 76:655–63.

Wekerle, H. 1993. T-cell autoimmunity in the central nervous system. *Intervirology* 35:95–100.

Wells, T. N., and Peitsch, M. C. 1997. The chemokine information source: identification and characterization of novel chemokines using the WorldWideWeb and expressed sequence tag databases. *Journal of Leukocyte Biology* 61:545-50.

Wells, T. N., Proudfoot, A. E., Power, C. A., and Marsh, M. 1996a. Chemokine receptors — the new frontier for AIDS research. *Chemistry and Biology* 3:603–9.

Wells, T. N., Lusti-Narasimhan, M., Chung, C. W., Cooke, R., Power, C. A., Peitsch, M. C., and Proudfoot, A. E. 1996b. The molecular basis of selectivity between CC and CXC chemokines: the possibility of chemokine antagonists as anti-inflammatory agents. *Annals of the New York Academy of Sciences* 796:245–56.

Wells, T. N., Power, C. A., Lusti-Narasimhan, M., Hoogewerf, A. J., Cooke, R. M., Chung, C. W., Peitsch, M. C., and Proudfoot, A. E. 1996c. Selectivity and antagonism of chemokine receptors. *Journal of Leukocyte Biology* 59:53-60.

West, D. J., and Calandra, G. B. 1996. Vaccine induced immunologic memory for hepatitis B surface antigen: implications for policy on booster vaccination. *Vaccine* 14:1019-27.

Westermann, J., and Pabst, R. 1990. Lymphocyte subsets in the blood: a diagnostic window on the lymphoid tissue. *Immunology Today* 11:406–10.

Whaley, K., and Thompson, R. A. 1978. Requirements for beta1H globulin and C3b inactivator in the control of the alternative complement pathway in human serum. *Immunology* 35:1045-9.

Whaun, J. M., Ritterhaus, C., and Ip, S. H. C. 1983. Rapid identification and detection of parasitized human red cells by flow cytometry. *Cytometry* 4:117–22.

Whelan, J. P., Shearer, W. T., Gilliam, E. B., and Hardy, K. J. 1992. A protein kinase C-activating phorbol ester accelerates the T cell antigen receptor-stimulated phosphatidylinositol cycle in normal human CD4+ T cells. *Journal of Immunology* 148:2872-8.

Wherry, J. C., Pennington, J. E., and Wenzel, R. P. 1993. Tumor necrosis factor and the therapeutic potential of anti-tumor necrosis factor antibodies. *Critical Care Medicine* 21:S436–40.

White, K. L. 1992. Specific immune assays. In *Principles and Practice of Immunotoxicology* (K. Miller, J. Turk, and S. Nicklin, eds.), Blackwell Scientific, London, pp. 304–23.

White, K. L. 1996. *Relevance of Decreased Natural Killer Cell Activity with Altered Host Resistance.* International Life Sciences Institute Health and Environmental Science Institute, Immunotoxicology Technical Committee, Washington, DC.

White, K. L., and Anderson, A. C. 1984. Measurement or modulation of mouse complement levels in vivo utilizing a microtiter hemolytic assay. *Agents and Actions* 15:562–8.

White, K. L., Sander, V. M., Barnes, D. W., Shopp, G. M., and Munson, A. E. 1985. Immunotoxicological investigations in the mouse: general approach and methods. *Drug and Chemical Toxicology* 8:299-33 1.

White, K. L., Lysy, H. H., McCay, J. A., and Anderson, A. C. 1986. Modulation of serum complement levels following exposure to polychlorinated dibenzo-p-dioxins. *Chemical Toxicology* 8:209–19.

White-Owen, C., Hartmann, S., Alexander, J. W., and Babcock, G. F. 1993. Reduced PMN beta 2 integrins after trauma: a possible role for colony-stimulating factors. *Clinical and Experimental Immunology* 92:477–81.

Whiteside, T. L., and Parmiani, G. 1994. Tumor-infiltrating lymphocytes: their phenotype, function and clinical use. *Cancer Immunology* 39:15-21.

Wild, J., Rossmeissl, P., Walter, W. A., and Gross, C. A. 1996. Involvement of the DnaK–DnaJ–GrpE chaperone team in protein secretion in Escherichia coli. *Journal of Bacteriology* 178:3608–13.

Williams, D. B., and Watts, T. H. 1995. Molecular chaperones in antigen presentation. *Current Opinion in Immunology* 7:71-84.

Williams, G. M., and Nossal, G. J. V. 1966. Ontogeny of the immune response. I. The development of the follicular antigen-trapping mechanism. *Journal of Experimental Medicine* 124:47–56.

Williams, W. J. 1989. Beryllium workers — sarcoidosis or chronic beryllium disease. *Sarcoidosis* 6(supplement):34–5.

Wilsher, M. L., Hughes, D. A., and Haslam, P. L. 1988. Immunoregulatory properties of pulmonary surfactant: effect of lung lining fluid on proliferation of human blood lymphocytes. *Thorax* 43:354–9.

Wilson, C., Tiwana, H., Ebringer, A., Cunningham, P., and Ettelaie, C. 1997. HLA-DR4 restriction, molecular mimicry and rheumatoid arthritis. *Immunology Today* 18:96–7.

Wilson, I. A., and Fremont, D. H. 1993. Structural analysis of MHC class I molecules with bound peptide antigens. *Seminars in Immunology* 5:75–80.

Wilson, K., and Eisenbarth, G. S. 1990. Immunopathogenesis and immunotherapy of type 1 diabetes. *Annual Review of Medicine* 41:497–508.

Winkelstein, J. A. 1981. The role of complement in the host defense against Streptococcus pneumoniae. *Review of Infectious Disease* 3:289–98.

Winrow, V. R., Ragno, S., Morris, C. J., Colston, M. J., Mascagni, P., Leoni, F., Gromo, G., Coates, A. R., and Blake, D. R. 1994. Arthritogenic potential of the 65 kDa stress protein — anexperimental model. *Annals of the Rheumatic Diseases* 53:197-201.

Wiseman, K. C. 1993. New insights on Goodpasture's syndrome. *Anna Journal* 20:17-26.

Woessner, J. F. J. 1991. Matrix metalloproteinases and their inhibitors in connective tissue. *FASEB Journal* 5:2145–54.

Wolowczuk, I., Delacre, M., Roye, O., Giannini, S. L., and Auriault, C. 1997. Interleukin-7 in the skin of Schistosoma mansoni-infected mice is associated with a decrease in interferon-gamma production and leads to an aggravation of the disease. *Immunology* 91:35-44.

Worsaae, A., Hejnaes, K., Moody, A., Ludvigsson, J., Pociot, F., Lorenzen, T., and Dyrberg, T. 1995. T cell proliferative responses to glutamic acid decarboxylase-65 in IDDM are negatively associated with HLA DR3/4. *Autoimmunity* 22:183-9.

Wucherpfennig, K. W. 1994. Autoimmunity in the central nervous system: mechanisms of antigen presentation and recognition. *Clinical Immunology and Immunopathology* 72:293–306.

Wyatt, R. J., Forristal, J., Davis, C. A., Coleman, T. H., and West, C. D. 1980. Control of serum C3 levels by beta 1H and C3b inactivator. *Journal ofLaboratory and Clinical Medicine* 95:905–17.

Wynn, T. A. 1996. Development of an antipathology vaccine for schistosomiasis. *Annals of the New York Academy of Sciences* 797:191–5.

Xu, L. L., Warren, M. K., Rose, W. L., Gong, W., and Wang, J. M. 1996. Human recombinant monocyte chemotactic protein and other C-C chemokines bind and induce directional migration of dendritic cells in vitro. *Journal ofLeukocyte Biology* 60:365–71.

Yagi, K., and Uono, M. 1979. Acetylcholine receptors and myasthenia gravis. *Nippon Rinsho (Japanese Journal of Clinical Medicine)* 37:3115–24.

Yamaguchi, H., Yamamoto, T., Konoeda, Y., Taguchi, H., and Ogata, S. 1992. Epitope homology between bacterial heat shock protein and self-proteins in the host cell. *APMIS* 100:957–62.

Yamashino, T., Kakeda, M., Ueguchi, C., and Mizuno, T. 1994. An analogue of the DnaJ molecular chaperone whose expression is controlled by sigma S during the stationary phase and phosphate starvation in Escherichia coli. *Molecular Microbiology* 13:475–83.

Yamashita, N., Natsauki, M., and Sagami, S. 1989. Flare-up reaction on murine contact hypersensitivity. I. Description of an experimental model: rechallenge system. *Immunology* 67:365–9.

Yao, Z., Fanslow, W. C., Seldin, M. F., Rousseau, A. M., Painter, S. L., Comeau, M. R., Cohen, J. I., and Spriggs, M. K. 1995a. Herpesvirus saimiri encodes a new cytokine, IL-17, which binds to a novel cytokine receptor. *Immunity* 3:811-21.

Yao, Z., Painter, S. L., Fanslow, W. C., Ulrich, D., Macduff, B. M., Spriggs, M. K., and Armitage, R. J. 1995b. Human IL-17: a novel cytokine derived from T cells. *Journal of Immunology* 155:5483-6.

Yasumoto, S., Okabe, N., and Mori, R. 1986. Role of epidermal Langerhans cells in resistance to herpes simplex virus infection. *Archives of Virology* 90:261–71.

Yefenof, E., Sanders, V. M., Snow, E. C., Noelle, R. J., Oliver, K. G., Uhr, J. W., and Vitetta, E. S. 1977. Preparation and analyses of antigen specific memory B cells. *Journal of Immunology* 135:3777–84.

Yeo, P. P. 1985. Islet cell autoantibodies and insulin-dependent (type I) diabetes mellitus. *Annals of the Academy of Medicine, Singapore* 14:343–6.

Yi, Y., Zhong, G., and Brunham, R. C. 1993. Continuous B-cell epitopes in Chlamydia trachomatis heat shock protein 60. *Infection and Immunity* 61:1117-20.

Yokoyama, W. M. 1995. Natural killer cell receptors. *Current Opinion in Immunology* 7:110-20.

Yonemura, Y., Ku, H., Lyman, S. D., and Ogawa, M. 1997. In vitro expansion of hematopoietic progenitors and maintenance of stem cells: comparison between FLT3/FLK-2 ligand and KIT ligand. *Blood* 89:1915–21.

Young, A. C., Nathenson, S. G., and Sacchettini, J. C. 1995. Structural studies of class I major histocompatibility complex proteins: insights into antigen presentation. *FASEB Journal* 9:26–36.

Young, D. B. 1990. Chaperonins and the immune response. *Seminars in Cell Biology* 1:27-35.

Yu, D., and Inman, R. 1991. Infectious agents and other nongenetic immunologic factors in spondyloarthropathies. *Current Opinion in Rheumatology* 3:581–5.

Yuan, Q., Austen, K. F., Friend, D. S., Heidtman, M., and Boyce, J. A. 1997. Human peripheral blood eosinophils express a functional c-kit receptor for stem cell factor that stimulates very late antigen 4 (VLA-4)-mediated cell adhesion to fibronectin and vascular cell adhesion molecule 1 (VCAM-1). *Journal of Experimental Medicine* 186:313-23.

Zamorano, P., Wigdorovitz, A., Perez-Filgueira, M., Carrillo, C., Escribano, J. M., Sadir, A. M., and Borca, M. V. 1995. A 10-amino-acid linear sequence of VP1 foot and mouth disease virus containing B- and T-cell epitopes induces protection in mice. *Virology* 212:614-21.

Zeiss, C. R., Kanellaks, T. M., Bellone, T. D., Levitz, D., Pruzansky, J. J., and Patterson, R. 1980. Immunoglobulin E mediated asthma and hypersensitivity pneumonitis with precipitating antihapten antibodies due to diphenylmethane diisocyanate (MDI) exposure. *Journal of Allergy and Clinical Immunology* 65:347-52.

Zerbini, G., Bazzigaluppi, E., Mangili, R., Bosi, E., and Bonifacio, E. 1997. Prevalence of GAD65 and IA-2 autoantibodies in IDDM patients affected by diabetic nephropathy. *Diabetologia* 40:485.

Zhang, P., Bagby, G. J., Stoltz, D. A., Spitzer, J. A., Summer, W. R., and Nelson, S. 1997. Modulation of the lung host response by granulocyte colony-stimulating factor in rats challenged with intrapulmonary endotoxin. *Shock* 7:193-9.

Zhao, Q., and Weiss, A. 1996. Enhancement of lymphocyte responsiveness by a gain of function mutation of ZAP-70. *Molecular and Cellular Biology* 16:6765–74.

Zhou, P., Cao, H., Smart, M., and David, C. 1993. Molecular basis of genetic polymorphism in major histocompatibility complex-linked proteasome gene (Lmp-2). *Proceedings of the National Academy of Sciences (USA)* 90:2681–4.

Ziccardi, R. J., and Cooper, N. R. 1979. Active disassembly of the first complement component, C-1, by C-1 inactivator. *Journal of Immunology* 123:788–92.

Ziegler, B., Schlosser, M., Luhder, F., Strebelow, M., Augstein, P., Northemann, W., Powers, A. C., and Ziegler, M. 1996. Murine monoclonal glutamic acid decarboxylase GAD65 antibodies recognize autoimmune-associated GAD epitope regions targeted in patients with type 1 diabetes mellitus and stiff-man syndrome. *Acta Diabetologica* 33:225–3l.

Ziegler-Heitbrock, H. W. L. 1996. Heterogeneity of human blood monocytes: the CD14+CD16+ subpopulation. Immunology Today 17:424–7.

Zinkemagel, R. M. 1990. Antiviral T-cell memory? *Current Topics in Microbiology & Immunology* 159:65–77.

Zinkemagel, R. M. 1997. Immunology and immunity studies with viruses. *Ciba Foundation Symposium* 204:105–25.

Zola, H. 1992. T cell memory: a role for MHC class II molecules on T cells? *Immunology & Cell Biology* 70:337–41.

Zoller, K. E., MacNeil, I. A., and Brugge, J. S. 1997. Protein tyrosine kinases syk and ZAP-70 display distinct requirements for Src family kinases in immune response receptor signal transduction. *Journal of Immunology* 158:1650-9.

Zumla, A. 1992. Superantigens, T cells, and microbes. *Clinical Infectious Diseases* 15:313–20.

Zurawski, G., and de Vries, J. E. 1994. Interleukin 13 elicits a subset of the activities of its close relative interleukin 4. *Stem Cells* 12:169-74.

Zweiman, B. 1993. The late-phase reaction: role of IgE, its receptor and cytokines. *Current Opinion in Immunology* 5:950–5.

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