Cell, Vol. 100, 129–138, January 7, 2000, Copyright ©2000 by Cell Press

Immunology: Improving on Nature in the Twenty-First Century

Review

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Introduction

Immunology is the study of the body's defenses against infection. The birth of immunology as an experimental science dates to Edward Jenner's successful vaccination against smallpox in 1796 (Jenner, 1798). The worldwide acceptance of vaccination led to mankind's greatest achievements in preventing disease, and smallpox is the first and only human disease that has been eradicated. During the twentieth century, the impact of immunology has moved beyond defense against infections. Our growing understanding of the immune system has influenced a variety of different biomedical disciplines and has played an important role in the study and treatment of many human diseases (Abbas et al., 1997; Janeway et al., 1999; Paul, 1999). However, it is still clear that the primary role of the immune system is resistance to infection. In this review we survey the evolution of immunology as a scientific discipline and speculate on some of the directions in which the field is likely to move in the future. Among these, we hope that it will become possible to use manipulation of the adaptive immune response to overcome many human diseases. These are currently treated only with drugs that do not exploit the specificity of adaptive immunity. We also point out some of the areas in which studying the immune system has led to the discovery of principles with broad implications for diverse biological processes.

The Basis for Specificity and Memory in Adaptive Immune Responses

The earliest studies of protective immunity against infections established the two defining characteristics of adaptive immunity, namely, specificity for the antigenic determinants of pathogens, and the memory of having been exposed to the same pathogen previously, called immunological memory. Implicit in the phenomenon of specificity is the concept of the enormous diversity of the receptor repertoires of lymphocytes.

Specificity and Diversity

The specificity of immune responses was initially inferred from studies of protective immunity and vaccination against microbes, which was by definition pathogen specific. These early studies culminated in the formulation of the clonal selection hypothesis in the 1950s, which proposed that clones of immunocompetent cells with unique receptors exist prior to exposure to antigens, and only cells with specific receptors are selected by antigen for subsequent activation (Figure 1) (Burnet, 1959). The idea that specificity for diverse antigens exists prior to encounter with these antigens was a radical notion at the time, one that challenged existing concepts of how proteins conformed to the shapes of other interacting proteins. Nevertheless, the clonal selection theory became the foundation for our understanding of the specificity and development of immune responses. The molecular understanding of how the diverse repertoire of antigen receptors is generated came with the studies of Susumu Tonegawa in the 1970s (Tonegawa et al., 1977). Based on this work, and its many subsequent refinements, it is now known that the antigen receptors of B and T lymphocytes are encoded by genes that are produced by somatic recombination of gene segments during maturation of the cells. The recombination process is initiated by the RAG proteins, and presence of *RAG* genes during phylogeny identifies the evolutionary time of appearance of the adaptive immune system, which is just past the appearance of vertebrates (Agrawal et al., 1998; Hiom et al., 1998). The jawless fish lack *RAG* genes and lymphoid organs, while cartilaginous fish have *RAG* genes and a quite well-developed adaptive immune system. Recombination of antigen receptor gene segments serves three critical functions: it allows a vast number of receptors to be generated from a small number of genes, it maximizes the diversity of the receptors by introducing sequence variations at the sites of recombination, and it regulates the development of individual lymphocytes, which is tightly linked to the status of gene rearrangement. Somatic recombination of antigen receptor genes is the paradigm for studying gene rearrangement during cell maturation.

Immunological Memory

Immunological memory refers to the ability of the immune system to respond more strongly to successive exposures to the same antigen. The classical definition of memory came from the realization that infection with a particular microbe or vaccination rendered an individual resistant to subsequent exposures to that microbe; that is, they were immune to the specific pathogen. Much effort has been devoted to defining the phenotypic and functional characteristics of memory cells, and much has been learned about what these cells do. However, fundamental questions about the signals that are required to generate memory cells, and the mechanisms responsible for the survival of these cells in the apparent absence of antigen, remain unsolved. This uncertainty is mainly a reflection of the fact that immunological memory has to be studied in vivo, and only recently have methods been developed to identify numerically rare antigen-specific populations in the midst of large numbers of lymphocytes with diverse specificities (Altman et al., 1996; Murali-Krishna et al., 1998; Ogg et al., 1998).

The Initiation of Immune Responses: What Lymphocytes See

The key breakthrough that opened the way for our modern understanding of the immune system was the identification by Jim Gowans of small lymphocytes as the cellular units of clonal selection in adaptive immunity (Gowans et al., 1962). We now know that there are two

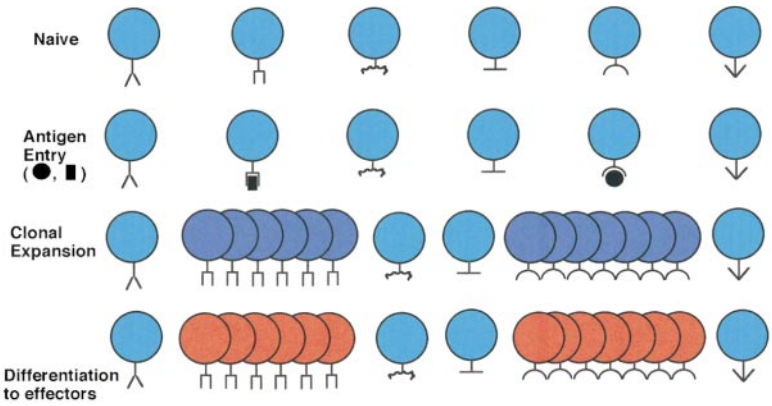

Figure 1. The Clonal Selection Theory of Lymphocytes

Schematic of clonal selection hypothesis illustrating the idea that each naïve lymphocyte has a different receptor specificity, each of which can bind a different antigenic determinant. When a pathogen is recognized by the cells, in this case by two different antigenic determinants, then the cells that bind to these determinants are selected to proliferate or undergo clonal expansion, and then differentiate into effector cells that either secrete antibody or mediate various effector mechanisms of cell-mediated immunity.

classes of antigen-specific lymphocytes with receptors for antigens, B and T cells, which function as the mediators of humoral immunity and cell-mediated immunity, respectively. The use of monoclonal antibodies (Kohler and Milstein, 1975) to identify different populations of lymphocytes and to isolate these populations for functional and biochemical analyses has been instrumental in understanding lymphocyte biology. In fact, the ease of producing fairly homogeneous populations of lymphocytes with defined specificities—by purification, cell cloning, and transgenic technology—is the principal reason why lymphocytes have taught us a great deal about fundamental biological phenomena.

One of the most impressive accomplishments of immunology is the elucidation of antigen recognition by lymphocytes. We now know that B cell antigen receptors bind a wide variety of macromolecules and small chemicals in different conformations. However, the greatest surprises have come from studies of T cell antigen recognition. This story began with the discovery, in the 1960s and 1970s, that different inbred strains of animals did or did not respond to simple polypeptide antigens, and this immune responsiveness mapped to a highly polymorphic genetic locus that had been discovered as the target of graft rejection and was therefore called the major histocompatibility complex (MHC) (Benacerraf and McDevitt, 1972). These phenomena eluded explanation until, in the mid-1970s, Zinkernagel and Doherty found rather serendipitously that virus-specific CTLs generated in one inbred mouse strain would kill virus-infected target cells only from strains with the same MHC (Zinkernagel and Doherty, 1974). This observation, and the results of experiments examining the interactions of T cells with either B lymphocytes (Katz et al., 1973) or macrophages (Rosenthal and Shevach, 1973), ultimately led to the conclusion that in each individual or inbred strain of mouse, T lymphocytes are limited to recognizing foreign protein antigens on the surface of that individual's or strain's own cells, and the key element in antigen recognition by T cells is the MHC. This ability to recognize antigen only in the context of self-MHC molecules is called MHC restricted antigen recognition, or MHC restriction for short. It is now known that MHC molecules expressed on antigen-presenting cells function to display peptides derived from complex proteins to T lymphocytes. Each individual T cell is selected to recognize one or a small number of related peptides bound to one of that individual's 5–15 MHC allelic products (Babbitt et al., 1985). As there are over 100 alleles at several loci within the MHC, this means that the MHC is the most polymorphic set of loci within the human genome.

The dual specificity of T cells, for peptide antigens and for MHC molecules, led to many theories about how T cells recognized antigens. The great controversy was whether T cells expressed a single receptor that saw both peptide and MHC, or one receptor for each. Early studies showed that fusing two distinct T cell lines did not generate four different specificities, as predicted by the dual specificity model, but rather the two original specificities of the parent clones, as would be predicted from the single receptor hypothesis (Kappler et al., 1981). Biochemical analyses of the T cell antigen receptor also showed a single receptor molecule, but the identification of the T cell receptor (TCR) for antigen came from molecular cloning (Hedrick et al., 1984). The definitive results that established the structural basis of MHC restriction came with the solution of the crystal structure of MHC molecules, which showed that these molecules contained bound peptides (Bjorkman et al., 1987) (Figure 2), and ultimately in the solution of the crystal structures of T cell receptors binding to MHC: peptide complexes (Figure 2) (Garboczi et al., 1996; Garcia et al., 1996). These remain among the most informative crystal structures ever defined because they revealed in fine detail the basis for one of the puzzling features of antigen recognition. A great surprise from these structural results, which confirm previous data based on mutational analyses of peptides, is that each TCR recognizes very few residues of the MHC-associated peptide. Taken together with the established idea that even in complex microbes, only a few peptides are recognized by the immune system, this means that T cells are able to distinguish between different microbes on the basis of very few differences in amino acid sequences. How this remarkable specificity is maintained despite the enormous structural diversity of microbes remains a fascinating question.

In parallel with these structural studies of the MHC, cell biologists dissected the mechanisms of antigen uptake, processing, and presentation. The key conclusion of these studies is that the two classes of MHC molecules function to sample protein antigens from different cellular compartments and display these antigens for

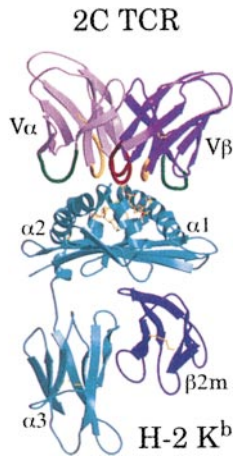

Figure 2. The Outline Structure to the T Cell Receptor Recognizing a Cognate Peptide:MHC Class I Ligand

The T cell receptor (TCR) binds to MHC:peptide complexes in a diagonal fashion as shown in the figure (Bjorkman, 1997). This has been shown directly for several MHC class I:TCR cocrystals, and indirectly for an MHC class II recognized by a TCR, in which all 6 CDRs can be positioned diagonally over a MHC class II:peptide complex (Sant'Angelo et al., 1996).

recognition by different classes of T cells. Protein antigens located in the cytosol are processed by proteasomes, translocated into the endoplasmic reticulum, and displayed by MHC class I molecules for recognition by CD8 T cells. Thus, MHC class I molecules can alert CD8 T cells to the presence of foreign antigens, such as viral and tumor antigens, that are synthesized within infected or transformed cells, leading to the elimination of these cells. By contrast, extracellular pathogens and proteins that are internalized into the vesicles of phagocytes are processed by vesicular proteases and displayed bound to MHC class II molecules for recognition by CD4 T cells (Nakagawa et al., 1999). The elucidation of the peptide display function of MHC molecules has solved two fundamental problems in immunology. The first is how the immune system ensures that T cells, designed to combat intracellular microbes, are the cells of cell-mediated immunity. The answer to this is that because the MHC molecules that display peptides are integral membrane proteins, T cells can only see antigens bound to other cells, and these are the antigens of intracellular or phagocytosed microbes. The second is how the immune system knows that it should make antibodies against extracellular microbes but trigger the effector mechanisms of cell-mediated immunity, such as T cell–mediated killing of infected cells, after microbes have found a haven inside host cells. This problem was especially puzzling because humoral and cell-mediated immune responses can be generated against the same microbe, such as a virus, at different stages of its life, i.e., extracellular and intracellular, respectively. The answer is that MHC molecules instruct the immune system on how to respond by segregating the antigens of vesicular and cytosolic antigens in such a way that they are recognized by different classes of T cells—helper cells that stimulate antibody production and CTLs that kill infected cells, respectively.

The realizations that very few lymphocytes out of the total population recognize any one antigen, very few peptides derived from any microbe are recognized by the immune system, and very few MHC molecules on APCs display any one peptide have highlighted what appears to be an insurmountable logistical problem—how do we ever get effective immune responses against microbes? This problem is largely handled by a specialized population of professional antigen-presenting cells (APCs), the dendritic cells (DCs), which are strategically located under the skin and the mucosal epithelia—common sites of contact with the external environment (Banchereau and Steinman, 1998). Immature DCs are highly phagocytic and are designed to capture microbial antigens in the periphery. They then migrate to the T cell–rich areas of local lymph nodes, where, as mature DCs, they express high levels of MHC-associated peptides derived from the pathogen. The migration is not a random process and is tightly regulated by chemokines produced in the lymph nodes, for which dendritic cells express specific receptors (Melchers et al., 1999). Once in the lymph node, DCs also begin to express molecules that are necessary to activate the naïve T cells that continuously flow through the same areas of the lymph node. Thus, the rare antigen-specific T cells come in contact with antigen-loaded dendritic cells, leading to the initiation of lymphocyte responses. Overall, our present understanding of anitgen presentation to T lymphocytes provides one of the best examples of how immunologists, biochemists, cell biologists, and morphologists can come together to attack a complex problem (Cresswell, 1994; Germain, 1994).

Innate Immunity

Innate immunity provides early host defense against infections, before the development of an adaptive immune response. Innate immune responses of marine starfish were discovered by Elie Metchnikoff in the late 1800s, and this work remains a cornerstone in the study of various aspects of innate immunity (Metchnikoff, 1893). Innate immunity is mediated by cells, such as phagocytes and natural killer cells, circulating proteins, such as the complement system, and numerous antimicrobial peptides. These components use germline-encoded "pattern recognition receptors" to recognize molecular structures, or "patterns," present on various classes of microbes. Many of these receptors, such as the Toll family of proteins, are conserved throughout evolution and serve the same function of anti-microbial defense in all multicellular organisms, including plants. Innate immunity also stimulates adaptive immune responses (Medzhitov et al., 1997). This realization has explained some fundamental features of adaptive immunity and is discussed in more detail below.

The Development of Immune Responses: Lymphocyte Activation

The survival and functional responses of lymphocytes are regulated by cell–cell interactions and by an orchestrated interplay of positive and negative signals. All adaptive immune responses are dependent on the activation of antigen-specific lymphocytes, and this process is triggered by binding of antigen to the lymphocyte

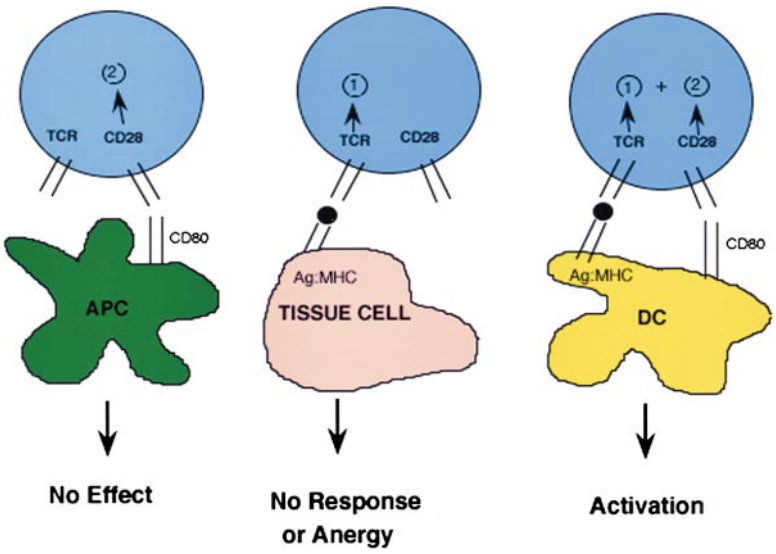

Figure 3. The Two-Signal Theory of T Cell Activation

When a T cell encounters an antigen presenting cell (APC) that expresses costimulatory molecules but no foreign antigens, there is no apparent signal (left panel); when a T cell encounters a tissue cell or an APC that expresses antigens recognized by the TCR in the absence of costimulatory signals, the result is either no response or inactivation of the cell (anergy). However, when a T cell encounters an activated dendritic cell or other APC expressing both antigen and costimulatory molecules, then the T cell is activated to proliferate and undergo differentiation to an effector cell. Thus, T cells only become activated by their ligands when they are presented by a cell that expresses costimulatory molecules.

receptors. To ensure that immune responses develop only when they are needed, i.e., when there is an infection, naïve lymphocytes need at least two signals to be fully activated to proliferate and differentiate. The first signal is provided by specific antigen recognition. By itself, this signal can fail to stimulate a response or can induce a state of inactivity referred to as clonal anergy, the inability to respond to antigen (Schwartz, 1992) (Figure 3). However, when the antigen is delivered as part of a pathogen, the second signal is provided by innate immune responses to microbes or, in some cases, by the pathogen itself (Medzhitov et al., 1997). This requirement for second signals explains why adaptive immune responses are stimulated by microbes but not by most self-antigens, which are not normally recognized by the innate immune system and therefore do not elicit adaptive immune responses. Adjuvants are required to stimulate adaptive immune responses to protein antigens, and the key constituents in these adjuvants are microbial products, which function by stimulating innate immunity (Janeway, 1989). The best defined second signals for T cells are the costimulatory molecules known as B7-1 (CD80) and B7-2 (CD86) (Harding et al., 1992; Linsley and Ledbetter, 1993). These molecules are only expressed on professional APCs, and their expression peaks after the APCs are activated by microbial products. Activated APCs also produce cytokines during innate immune reactions, which further stimulate T cell responses. The B7 proteins are recognized by the CD28 receptor, which is expressed on most naïve T cells, especially those of the CD4 subset. Together with the antigen receptor signal (signal 1), recognition of B7 proteins by CD28 leads to T cell activation, clonal expansion, and the development of effector T cell function (Figure 3). Activated T cells express a molecule called CD40 ligand (CD40L), which has several functions. One of these is to activate APCs to increase expression of B7 costimulators and to stimulate production of cytokines, such as IL-12, that induce the differentiation of T cells. Thus, CD40L serves to amplify T cell proliferation and differentiation into effector cells. The identification of second signals for lymphocyte activation has led to the development of a new class of agents that inhibit immune responses by blocking these signals. Antagonists against B7 molecules and CD40 ligand are currently in clinical trials for inhibiting graft rejection and for treating some hypersensitivity and autoimmune disorders (Foy et al., 1996; Markees et al., 1998; Kenyon et al., 1999; Kirk et al., 1999). The story is very much an evolving one because new costimulators and their receptors continue to be discovered. What is needed is an integrated picture of when the individual costimulators are active during immune responses, what types of responses they stimulate, and how they signal T cells.

Effector T cells can respond to antigen in the absence of costimulators and thus are able to interact with many cell types that express microbial antigens. They function by altering the behavior of their targets. MHC class I–restricted CD8 effector T cells can attack virtually any cell in the body, as most cells express MHC class I molecules. MHC class II–restricted CD4 T cells can only interact with cells that express MHC class II molecules, such as macrophages carrying pathogens or B cells that bind specific antigens. The functions of effector T cells are described in more detail later.

The molecules that mediate cell–cell interactions are better defined in the immune system than in any other biological system (Dustin et al., 1998). This knowledge has given immunologists valuable tools for analyzing the processes of T cell–APC and T cell–B cell interactions in considerable detail. Recent studies have shown that various surface molecules of T cells undergo rapid reorientation upon antigen recognition, with antigen receptors and their associated signal-transducing subunits aggregating in the central regions of membrane microdomains, and adhesion molecules forming peripheral rings around these clustered receptors (Dustin et al., 1998; Monks et al., 1998). These molecular aggregates have been called supramolecular activation complexes, or immunological synapses, and some of them lie in lipid rafts on the cell surface. Presumably, aggregation of antigen receptors brings sufficient receptor-associated signaling molecules into proximity to achieve the threshold needed to trigger cellular responses. The signaling molecules include a number of adapter proteins,

kinases, and their substrates. How the receptors are aggregated in a particular topology upon binding to their cognate ligand remains a mystery, and this issue is likely to be one of interest to all biologists interested in the problem of how cells sense and respond to other cells and external stimuli. Recent studies using the BIAcore suggest that T cell receptors change their shape upon binding antigen and form aggregates on the cell surface (Alam et al., 1999). The general rules of signal transduction in lymphocytes are probably fundamentally similar to those in other cells, using common response pathways such as increases in intracellular calcium, activation of kinase cascades, and activation of the mitogen activated kinases (Weiss and Littman, 1994). The culmination of these signaling cascades is translocation of activated transcription factors into the cell nucleus, resulting in the expression of genes that encode cytokines, cytokine receptors, and cell cycle regulators, all of which are necessary for clonal expansion.

In response to antigen recognition, second signals, and cytokines, lymphocytes proliferate and differentiate into effector cells. Recent analyses of the numbers of antigen-specific CD8 T cells, performed using tetravalent complexes of MHC molecules loaded with antigenic peptides as staining reagents, have revealed a remarkable and unexpected level of clonal expansion in acute microbial infections (Altman et al., 1996; Murali-Krishna et al., 1998). From a preimmunization level of 1 in 10^5 T cells specific for a particular microbial antigenic peptide, the frequency can increase to almost 1 in 3 within a few days of infection, and the total number of antigen-specific T cells can increase by almost 100,000-fold in this time, with virtually no change in the numbers of lymphocytes that are not specific for the microbe. Based on such results, the doubling time of antigen-stimulated CD8 T cells is estimated to be $\sim$6 hours. The clonal expansion of antigen-specific CD4 helper T cells might be less, in the order of 100- to 1,000-fold, and the magnitude of B cell expansion during immune responses in vivo has not yet been defined. A fraction of this expanded pool of lymphocytes differentiates into effector cells—antibody secreting cells (from B lymphocytes), cytokine-producing helper T cells, and cytotoxic T lymphocytes, some of which can be long-lived. After immunization or infection, plasma cells producing specific antibodies can be found in the bone marrow years later, and the antibodies they produce presumably continue to provide rapid protection against a repeated infection with the same pathogen. As the antigen is eliminated and the adaptive immune response subsides, the antigen-specific lymphocytes are deprived of survival stimuli and the majority die by apoptosis. At the end of the active response, the only survivors are some long-lived effector cells and memory lymphocytes.

Given the complexities of cell–cell interactions in immune responses and the easy mobility of lymphocytes and other cells of the immune system, it has been a mystery how these interactions are controlled with precision. A clue that intercellular communication must be regulated in some way was suggested by the old morphological observation that in lymphoid tissues, T and B lymphocytes are segregated in distinct anatomic compartments. We now know that this segregation is maintained in large part by the family of cytokines called chemokines, mentioned above as the signals for dendritic cell migration. Although chemokines were discovered as mediators of leukocyte recruitment in inflammation, it is now known that they control the normal traffic of lymphocytes, and other leukocytes, through tissues. T and B lymphocytes express receptors for different chemokines, which are produced in different regions of lymphoid tissues and serve to keep the lymphocytes in place. Activation and differentiation of the lymphocytes changes the expression of chemokine receptors, allowing the cells to leave their normal residence and migrate to regions where they can interact with other cells (Sallusto and Lanzavecchia, 1999). The elucidation of these roles of chemokines, and of the regulation of chemokine receptors on lymphocytes that are associated with their differentiation, are some of the most exciting recent developments in immunology. The fact that chemokine receptors also play an essential role in the process of infection with HIV, the causative agent of the AIDS, only serves to add fuel to the interest in these molecules and their receptors (Huang et al., 1996; Berger et al., 1999). The function of chemokines in the immune system has led to studies revealing equally important roles of these proteins in the development of other organs and the controlled migration of cells even in nonlymphoid tissues.

Because immune responses have the potential to cause injury to host tissues, it is not surprising that they are tightly controlled. It is now clear that lymphocytes express receptors capable of delivering both positive and negative signals. We have mentioned previously that CD28 on T cells recognizes B7 molecules on APCs and is involved in T cell activation. Activated T cells express another receptor called CTLA-4 (CD152), which also recognizes the same B7 molecules but shuts off responses by inhibiting TCR and CD28-mediated signals (Lee et al., 1998). B lymphocytes respond to antigen recognition by their antigen receptors and express receptors that recognize the Fc portions of antigen complexed with antibody to shut off B cell activation once enough antibody is produced. Natural killer cells are activated to kill virus-infected cells using a number of receptors, but are prevented from killing uninfected host cells by inhibitory receptors that recognize class I MHC molecules of the host. One of the most interesting questions in immunology is how the balance between these opposing signals is adjusted to keep the system at rest normally, to enable it to respond rapidly to encounters with pathogens, and to return it to its basal resting state as the pathogen is eliminated.

The Immune System Uses Diverse Effector Mechanisms to Eliminate Different Types of Microbes

The immune system has to protect the host from an almost unlimited diversity of microbes. This is achieved by building into the system a large potential for specialization, which ensures that the system responds appropriately to each type of microbe in a way that optimizes elimination of that particular microbe. B lymphocytes recognize and respond to extracellular microbes and their toxins, produce antibodies that neutralize these microbes and toxins, and promote their elimination by phagocytes. They do so mainly under the direction of

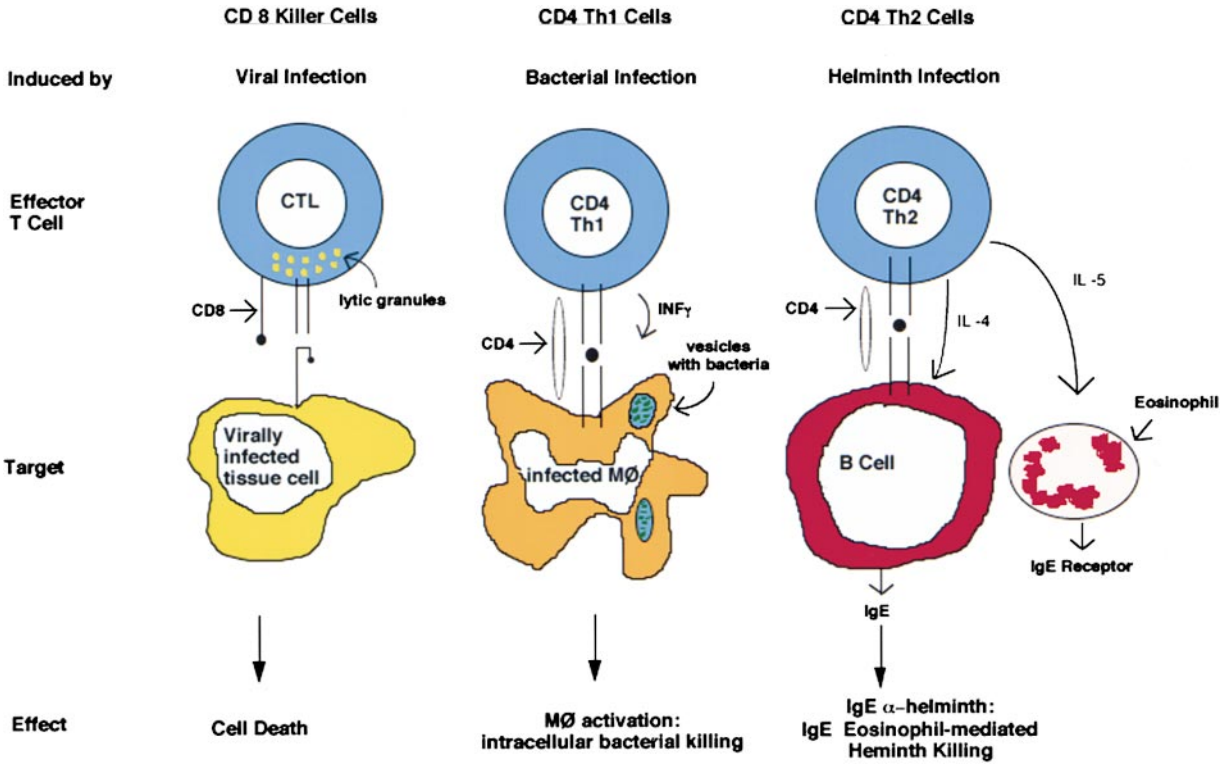

Figure 4. The Three Well-Defined Effector Functions of Lymphocytes

There are three types of well-characterized effector T cells. The CD8 effector T cells are primarily reactive against intracellular pathogens such as viruses and bacteria that live in the cytosol. CD4 Th1 cells are reactive to intracellular pathogens, or to pathogens that have been ingested by a phagocyte, whereupon they release interferon γ, which activates strong antimicrobial activity in the macrophage; Th1 cells also activate B cells making antibody that promotes phagocytosis of the pathogen. $CD4^+$ Th2 cells activate B cells to produce nonphagocytic antibodies, including IgE, and also activate nonphagocytic leukocytes, mainly eosinophils; Th2 cells are especially strongly activated by parasitic worm infection, which induces high levels of IgE and eosinophilia. The activated eosinophils express a surface receptor for IgE and can attack the parasitic worms coated with IgE.

helper T lymphocytes, which activate B lymphocytes to make antibodies and activate phagocytes to eliminate microbes that grow within intracellular vesicles or that are ingested from the extracellular environment.

An important discovery, with many physiological and pathological implications, has been the finding that activated helper T cells exist in specialized subsets that perform distinct functions by producing distinct sets of cytokines (Kim et al., 1985; Mosmann et al., 1986). In response to phagocytosed and intracellular microbes, helper T cells differentiate into the Th1 subset, which produces cytokines (principally IFN-γ) that activate phagocytes to destroy the infectious agent. In striking contrast, helminths induce the differentiation of helper T cells into Th2 cells, whose cytokines (IL-4 and IL-5) induce IgE and eosinophil-mediated destruction of the pathogens (Figure 4). These subsets also play distinct roles in diseases, with Th1 cells being involved in most inflammatory autoimmune diseases and Th2 cells in allergic disorders mediated in part by IgE. The existence of T cell subsets has become one of the dominant themes of immunology, and an enormous effort is being devoted to elucidating the biological and molecular basis of CD4 T cell differentiation into these subsets. Cytotoxic T lymphocytes function mainly to kill cells harboring microbes whose antigens enter the cytosol, such as viruses and some intracellular bacteria, and thus eliminate the reservoirs of infection (Figure 4).

As adaptive immunity has become increasingly specialized and sophisticated, microbes have evolved new ways to fight back. The ability of viruses, bacteria, and other microbes to evade host immunity by altering their major antigens is well recognized. Many viruses inhibit pathways of antigen processing and presentation in infected cells and are thus able to hide from T cells. For instance, some viruses interfere with the biosynthesis or assembly of MHC class I molecules, so that infected cells do not express the MHC class I:peptide complexes that are needed to stimulate CD8 T lymphocytes (Ploegh, 1998). The specificity of natural killer cells might be an adaptation against these viral evasion mechanisms because natural killer cells are activated by infected cells that specifically lack one or more MHC class I molecules (Ljunggren and Karre, 1990; Yokoyama and Seaman, 1993). This is an elegant example of the constant evolutionary struggle between microbes and hosts, with each adapting to the other. Other viruses, such as HIV, evade immunity by infecting and destroying the T lymphocytes themselves.

The Selection of Immune Receptor Repertoires and Their Maintenance in the Periphery

The generation of highly diverse repertoires of antigen receptors by somatic recombination of receptor gene segments has an intrinsic flaw, which is that the same

process produces receptors that are either of no use to the individual or, worse, actually harmful to the individual because they recognize self-antigens and may cause autoimmunity. This potential problem is largely corrected by selection processes that eliminate both useless and harmful lymphocytes before the cells mature to a stage of functional competence. The processes of repertoire selection are best defined in T lymphocytes. During their maturation in the thymus, immature T lymphocytes that recognize self-peptides complexed to self-MHC molecules are positively selected to survive (von Boehmer, 1994). This process of positive selection preserves only T cells that can see self-MHC-bound antigens in the periphery because these antigens will be displayed by the self-MHC molecules in each individual. Also in the thymus, T cells that recognize self-MHC:self-peptide complexes with high affinity are deleted from the repertoire by apoptosis (Nossal, 1994). This process of negative selection is also called central tolerance because it is the form of tolerance that is induced in the central or generative lymphoid organ. The factors that determine whether a particular clone of T cells with receptors of one specificity will be selected positively or negatively, or simply be neglected to die, are not yet well understood. Similar selection processes in the bone marrow appear to shape the repertoire of mature B lymphocytes. Once T and B cells leave the central lymphoid organs and enter the periphery, there is evidence that further contact with the positively selecting ligand maintains their numbers and also their sensitivity to activation. In T cells, this has been shown using mice that lack the selecting ligand as a result of genetic deletion. Mice that lack MHC class I molecules do not allow transferred CD8 T cells to survive (Tanchot et al., 1997; Goldrath and Bevan, 1999), while mice that lack MHC class II molecules do not allow CD4 T cells to survive (Ernst et al., 1999; Viret et al., 1999). Mature B cells that have their B cell antigen receptors removed also die rapidly (Lam et al., 1997). All these findings are consistent with the hypothesis that ongoing interactions with self-ligands in the peripheral lymphoid tissues are required to ensure the survival of the cells of the adaptive immune system. These findings again highlight how the consequences of antigen recognition by lymphocytes are tightly controlled. Thus, positively selected mature but naïve lymphocytes normally recognize some endogenous self-antigens well enough to stay alive but not so well that they are activated. When a microbial antigen is introduced, some of the same lymphocytes receive signals that are strong enough to initiate the activation process. This, of course, might be partly attributable to the fact that normally, the absence of infection and innate immunity prevents lymphocyte activation. However, this is unlikely to be the full answer because an active response to one microbe accompanied by innate immunity does not result in bystander activation of other lymphocytes that do not recognize their cognate antigens—even though these lymphocytes are being kept alive all the time by recognition of endogenous self-antigens.

Central tolerance cannot by itself prevent responses to self-antigens that are not present in the generative lymphoid organs. Tolerance to these peripheral tissue antigens is induced and maintained by several mechanisms. In T cells, these mechanisms include functional anergy, which results from recognition of self-antigens in the absence of adequate costimulation (Schwartz, 1992), and apoptotic cell death, which results from repeated stimulation by persistent self-antigens (Zuniga-Pflucker et al., 1995). Less is known about mechanisms of peripheral B cell tolerance. Failure of self-tolerance can eventually result in the development of autoimmune diseases, which are among the most common and enigmatic of immunological diseases (Rose and Mackay, 1998). There is great hope that animal models and genomic approaches will provide valuable clues about the genetic basis and pathogenesis of autoimmune diseases. Many gene knockout or mutant mouse strains develop what looks like autoimmunity. The best studied of these genes are involved in anergy or activation-induced apoptosis, which suggests that defects in these genes cause autoimmunity by disrupting one or more of the mechanisms of peripheral self-tolerance (Van Parijs and Abbas, 1998). It remains to be seen how much these animal models will teach us about human autoimmune diseases. One example of this is the so-called autoimmune disease accompanied by lymphoproliferation seen in mice and humans with mutations in CD95 (Fas). In such people, the phenotype is often seen with just one chromosome mutated, perhaps because CD95 is expressed as a trimer on the cell surface, such that a single mutant protein subunit needs to be defective in order to prevent normal CD95 function.

Summary: Where We Have Been and Where We Are Heading

The impressive advances in immunology are largely a triumph of reductionist approaches. Our ability to isolate and analyze pure and homogenous populations of T and B lymphocytes is one reason we know more about the proteins and genes of lymphocytes than about any other cell type in mammals. Lymphocytes have also served as the models for identifying many genes, receptors, signal transduction mechanisms, and other processes that are relevant to virtually all biological systems. It has been only forty years since the function of lymphocytes as the cellular mediators of adaptive immunity was established by Gowans. In this brief period, the signals and controls operative in lymphocyte function have been described in reasonable detail using these reductionist approaches. The greatest gaps in our knowledge are in the areas where reductionist approaches fail. For instance, we know relatively little about the development of adaptive immune responses in vivo, and even less about the largely neglected area of innate immunity, particularly its role in inducing the interplay between innate and adaptive immunity. We also do not understand how different signals are integrated in lymphocytes, especially during physiological immune responses, again because such responses usually have to be studied in vivo, where the multiple interactions involved are difficult to dissect. The next wave of basic research in immunology is likely to focus on immune responses in intact organisms, both experimental animals and humans. Several technical advances are making such studies feasible, such as accurate quantitation of rare antigen-specific lymphocytes in vivo, their detection in tissue sections, and real-time observation of cell–cell interactions using fluorescent probes. Recent studies also suggest that certain immune responses may generate protective or regulatory T cells that specifically shut off

other responses. Understanding how to control and manipulate effector and regulatory lymphocytes is a central goal of immunology research.

Immunology, more than any of the other modern biological sciences, is ripe for taking basic research to clinical applications. The same principles of vaccination will be applied to tumors, for which dendritic cell-based vaccines and adjunct cytokine treatments are in clinical trials. Many of the current attempts to treat autoimmune and allergic diseases and prevent graft rejection based on immunological principles have focused on inhibiting the effector mechanisms used by the adaptive immune response. Clearly the future lies in controlling specific adaptive immune responses that are causing damage, thus striking at the causes of these disorders. Perhaps the most exciting prospects are for inducing tolerance to autoantigens, allergens, and grafts. As we understand the nature of the antigens against which such reactions are directed and the signals involved in lymphocyte activation and inhibition, we should be able to exploit this knowledge to develop rational strategies for inducing tolerance. We have mentioned the possibility of blocking costimulators to shut off immune responses, and clinical trials of this approach in many diseases are under way. Equally interesting is the possibility of targeting specific lymphocytes. For instance, it might be possible to control specific alloreactive lymphocytes by attaching powerful toxins to the MHC antigens of the graft, perhaps presented as multimeric complexes that will be recognized and bound by specific lymphocytes. The same principle could be used to target pathogenic lymphocytes that recognize complexes of self-antigens and self-MHC molecules and cause autoimmune reactions. As we learn more about lymphocytes that shut off immune responses—by secreting, for instance, immunosuppressive cytokines such as TGFβ—it might be possible to selectively activate such lymphocytes and induce them to home to the tissues that are the targets of pathologic immune reactions.

There is also a growing appreciation that the immune system may contribute to diseases that do not, on face value, appear to result from primary immunological abnormalities. For instance, T cell–mediated immune responses against the proteins of vascular endothelium and smooth muscle may exacerbate the progression of atherosclerosis (Libby and Ridker, 1999). Immunotherapies may also benefit diseases that are not immunological in origin by neutralizing or eliminating potentially harmful proteins, as has been shown recently in a mouse model of Alzheimer's disease (Schenk et al., 1999).

We are hopeful that the improved understanding of immune mechanisms, especially those that involve the adaptive immune system, will lead to antigen-specific regulation of many diseases. We expect tremendous gains in our knowledge of adaptive immunity and an ability to alter such responses from aggressive to benign in the case of overly active immune responses to allergens, autoantigens, or engrafted tissues. We can also look forward to increasing the potency of similar adaptive responses to viruses such as HIV, malaria, tuberculosis, and metastatic tumors. This, we believe, will follow both basic science and advanced clinical research, which is so necessary a component of translation from experimental animal models to success in treating a wide range of immune-mediated diseases. It is this ability to overcome nature in the twenty-first century that we look forward to.

References

Abbas, A.K., Lichtman, A.H., and Pober, J.S. (1997). Cellular and Molecular Immunology, Third Edition (Philadelphia: W.B. Saunders).

Agrawal, A., Eastman, Q.M., and Schatz, D.G. (1998). Transposition mediated by RAG1 and RAG2 and its implications for the evolution of the immune system. Nature *394*, 744–751.

Alam, S.M., Davies, G.M., Lin, C.M., Zal, T., Nasholds, W., Jameson, S.C., Hogquist, K.A., Gascoigne, N.R., and Travers, P.J. (1999). Qualitative and quantitative differences in T cell receptor binding of agonist and antagonist ligands. Immunity *10*, 227–237.

Altman, J.D., Moss, P.A.H., Goulder, P.J.R., Barouch, D.H., McHeyzer-Williams, M.G., Bell, J.I., McMichael, A.J., and Davis, M.M. (1996). Phenotypic analysis of antigen-specific T lymphocytes. Science *274*, 94–96.

Babbitt, B.P., Allen, P.M., Matsueda, G., Haber, E., and Unanue, E.R. (1985). Binding of immunogenic peptides to Ia histocompatibility molecules. Nature *317*, 359–361.

Banchereau, J., and Steinman, R.M. (1998). Dendritic cells and the control of immunity. Nature *392*, 245–252.

Benacerraf, B., and McDevitt, H.O. (1972). Histocompatibility-linked immune response genes. Science *175*, 273–279.

Berger, E.A., Murphy, P.M., and Farber, J.M. (1999). Chemokine receptors as HIV-1 coreceptors: roles in viral entry, tropism, and disease. Annu. Rev. Immunol. *17*, 657–700.

Bjorkman, P.J. (1997). MHC restriction in three dimensions: a view of T cell receptor/ligand interactions. Cell *89*, 167–170.

Bjorkman, P.J., Saper, M.A., Samraoui, B., Bennett, W.S., Strominger, J.L., and Wiley, D.C. (1987). Structure of the human class I histocompatibility antigen, HLA-A2. Nature *329*, 506–512.

Burnet, F.M. (1959). The Clonal Selection Theory (London: Cambridge University Press).

Cresswell, P. (1994). Assembly, transport, and function of MHC class II molecules. Annu. Rev. Immunol. *12*, 259–293.

Dustin, M.L., Olszowy, M.W., Holdorf, A.D., Li, J., Bromley, S., Desai, N., Widder, P., Rosenberger, F., van der Merwe, P.A., Allen, P.M., and Shaw, A.S. (1998). A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. Cell *94*, 667–677.

Ernst, B., Lee, D.S., Chang, J.M., Sprent, J., and Surh, C.D. (1999). The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. Immunity *11*, 173–181.

Foy, T.M., Aruffo, A., Bajorath, J., Buhlmann, J.E., and Noelle, R.J. (1996). Immune regulation by CD40 and its ligand GP39. Annu. Rev. Immunol. *14*, 591–617.

Garboczi, D.N., Ghosh, P., Utz, U., Fan, Q.R., Biddison, W.E., and Wiley, D.C. (1996). Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature *384*, 134–141.

Garcia, K.C., Degano, M., Stanfield, R.L., Brunmark, A., Jackson, M.R., Peterson, P.A., Teyton, L., and Wilson, I.A. (1996). An alphabeta T cell receptor structure at 2.5 A and its orientation in the TCR-MHC complex. Science *274*, 209–219.

Germain, R.N. (1994). MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. Cell *76*, 287–299.

Goldrath, A.W., and Bevan, M.J. (1999). Low-affinity ligands for the TCR drive proliferation of mature CD8+ T cells in lymphopenic hosts. Immunity *11*, 183–190.

Gowans, J.L., McGregor, D.D., Cowen, D.M., and Ford, C.E. (1962). Initiation of immune responses by small lymphocytes. Nature *196*, 651–655.

Harding, F.A., McArthur, J.G., Gross, J.A., Raulet, D.H., and Allison, J.P. (1992). CD28-mediated signaling co-stimulates murine T cells

and prevents induction of anergy in T-cell clones. Nature *356*, 607–609.

Hendrick, S.M., Cohen, D.I., Neilsen, E.A., and Davis, M.M. (1984). Isolation of cDNA clones encoding T cell–specific membrane-associated proteins. Nature *308*, 149–153.

Hiom, K., Melek, M., and Gellert, M. (1998). DNA transposition by the RAG1 and RAG2 proteins: a possible source of oncogenic translocations. Cell *94*, 463–470.

Huang, Y., Paxton, W.A., Wolinsky, S.M., Neumann, A.U., Zhang, L., He, T., Kang, S., Ceradini, D., Jin, Z., Yazdanbakhsh, K., et al. (1996). The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat. Med. *2*, 1240–1243.

Janeway, C.A., Jr. (1989). Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harb. Symp. Quant. Biol. *54*, 1–13.

Janeway, C.A., Travers, P., Walport, M., and Capra, D. (1999). Immunobiology: The Immune System in Health and Disease, Fourth Edition (London: Garland Publishing).

Jenner, E. (1798). An inquiry into the causes and effects of the variola vacciniae, a disease discovered in some of the western counties of England, particulary Gloucestershire, and known by the name of Cowpox (London: for the author by Sompson Lew).

Kappler, J.W., Skidmore, B., White, J., and Marrack, P. (1981). Antigen-inducible, H-2-restricted, interleukin-2-producing T cell hybridomas. Lack of independent antigen and H-2 recognition. J. Exp. Med. *153*, 1198–1214.

Katz, D.H., Hamaoka, T., Dorf, M.E., Maurer, P.H., and Benacerraf, B. (1973). Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (Ir) gene in the control of lymphocyte interactions in responses controlled by the gene. J. Exp. Med. *138*, 734–739.

Kenyon, N.S., Chatzipetrou, M., Masetti, M., Ranuncoli, A., Oliveira, M., Wagner, J.L., Kirk, A.D., Harlan, D.M., Burkly, L.C., and Ricordi, C. (1999). Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. Proc. Natl. Acad. Sci. USA *96*, 8132–8137.

Kim, J., Woods, A., Becker-Dunn, E., and Bottomly, K. (1985). Distinct functional phenotypes of cloned Ia-restricted helper T cells. J. Exp. Med. *162*, 188–201.

Kirk, A.D., Burkly, L.C., Batty, D.S., Baumgartner, R.E., Berning, J.D., Buchanan, K., Fechner, J.H., Jr., Germond, R.L., Kampen, R.L., Patterson, N.B., et al. (1999). Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. Nat. Med. *5*, 686–693.

Köhler, G., and Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined structure. Nature *256*, 495–497.

Lam, K.P., Kuhn, R., and Rajewsky, K. (1997). In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. Cell *90*, 1073–1083.

Lee, K.M., Chuang, E., Griffin, M., Khattri, R., Hong, D.K., Zhang, W., Straus, D., Samelson, L.E., Thompson, C.B., and Bluestone, J.A. (1998). Molecular basis of T cell inactivation by CTLA-4. Science *282*, 2263–2266.

Libby, P., and Ridker, P.M. (1999). Novel inflammatory markers of coronary risk: theory versus practice. Circulation *100*, 1148–1150.

Linsley, P.S., and Ledbetter, J.A. (1993). The role of the CD28 receptor during T cell responses to antigen. Annu. Rev. Immunol. *11*, 191–212.

Ljunggren, H.G., and Karre, K. (1990). In search of the 'missing self': MHC molecules and NK cell recognition. Immunol. Today *11*, 237–244.

Markees, T.G., Phillips, N.E., Gordon, E.J., Noelle, R.J., Shultz, L.D., Mordes, J.P., Greiner, D.L., and Rossini, A.A. (1998). Long-term survival of skin allografts induced by donor splenocytes and anti-CD154 antibody in thymectomized mice requires CD4(+) T cells, interferon-gamma, and CTLA4. J. Clin. Invest. *101*, 2446–2455.

Medzhitov, R., Preston-Hurlburt, P., and Janeway, C.A., Jr. (1997). A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature *388*, 394–397.

Melchers, F., Rolink, A.G., and Schaniel, C. (1999). The role of chemokines in regulating cell migration during humoral immune responses. Cell *99*, 351–354.

Metchnikoff, E. (1893). Lectures on the comparative pathology of inflammation (London: Kegan, Paul, Trench, Trubner).

Monks, C.R., Freiberg, B.A., Kupfer, H., Sciaky, N., and Kupfer, A. (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. Nature *395*, 82–86.

Mosmann, T.R., Cherwinski, H., Bond, M.W., Giedlin, M.A., and Coffman, R.L. (1986). Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J. Immunol. *136*, 2348–2357.

Murali-Krishna, K., Altman, J.D., Suresh, M., Sourdive, D.J., Zajac, A.J., Miller, J.D., Slansky, J., and Ahmed, R. (1998). Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity *8*, 177–187.

Nakagawa, T.Y., Brissette, W.H., Lira, P.D., Griffiths, R.J., Petrushova, N., Stock, J., McNeish, J.D., Eastman, S.E., Howard, E.D., Clarke, S.R., et al. (1999). Impaired invariant chain degradation and antigen presentation and diminished collagen-induced arthritis in cathepsin S null mice. Immunity *10*, 207–217.

Nossal, G.J. (1994). Negative selection of lymphocytes. Cell *76*, 229–239.

Ogg, G.S., Jin, X., Bonhoeffer, S., Dunbar, P.R., Nowak, M.A., Monard, S., Segal, J.P., Cao, Y., Rowland-Jones, S.L., Cerundolo, V., et al. (1998). Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science *279*, 2103–2106.

Paul, W.E. (1999). Fundamental Immunology (Philadelphia: Lippincott-Raven), p. 1589.

Ploegh, H.L. (1998). Viral strategies of immune evasion. Science *280*, 248–253.

Rose, N.R., and Mackay, I.R. (1998). The Autoimmune Diseases, Third Edition (San Diego: Academic Press).

Rosenthal, A.S., and Shevach, E.M. (1973). Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J. Exp. Med. *138*, 1194–1212.

Sallusto, F., and Lanzavecchia, A. (1999). Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. J. Exp. Med. *189*, 611–614.

Sant'Angelo, D.B., Waterbury, G., Preston-Hurlburt, P., Yoon, S.T., Medzhitov, R., Hong, S.C., and Janeway, C.A., Jr. (1996). The specificity and orientation of a TCR to its peptide-MHC class II ligands. Immunity *4*, 367–376.

Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., et al. (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature *400*, 173–177.

Schwartz, R.H. (1992). Costimulation of T lymphocytes: the role of CD28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell *71*, 1065–1068.

Tanchot, C., Lemonnier, F.A., Perarnau, B., Freitas, A.A., and Rocha, B. (1997). Differential requirements for survival and proliferation of CD8 naive or memory T cells. Science *276*, 2057–2062.

Tonegawa, S., Hozumi, N., Matthyssens, G., and Schuller, R. (1977). Somatic changes in the content and context of immunoglobulin genes. Cold Spring Harb. Symp. Quant. Biol. *41*, 877–889.

Van Parijs, L., and Abbas, A.K. (1998). Homeostasis and self-tolerance in the immune system: turning lymphocytes off. Science *280*, 243–248.

Viret, C., Wong, F.S., and Janeway, C.A., Jr. (1999). Designing and maintaining the mature TCR repertoire: the continuum of self-peptide:self-MHC complex recognition. Immunity *10*, 559–568.

von Boehmer, H. (1994). Positive selection of lymphocytes. Cell *76*, 219–228.

Weiss, A., and Littman, D.R. (1994). Signal transduction by lymphocyte antigen receptors. Cell *76*, 263–274.

Yokoyama, W.M., and Seaman, W.E. (1993). The Ly-49 and NKR-P1 gene families encoding lectin-like receptors on natural killer cells: the NK gene complex. Annu. Rev. Immunol. *11*, 613–635.

Zinkernagel, R.M., and Doherty, P.C. (1974). Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. Nature *251*, 547–548.

Zuniga-Pflucker, J.C., Di, J., and Lenardo, M.J. (1995). Requirement for TNF-alpha and IL-1 alpha in fetal thymocyte commitment and differentiation. Science *268*, 1906–1909.