

Review

The anatomy of T-cell activation and tolerance

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ABSTRACT The mammalian immune system must specifically recognize and eliminate foreign invaders but refrain from damaging the host. This task is accomplished in part by the production of a large number of T lymphocytes, each bearing a different antigen receptor to match the enormous variety of antigens present in the microbial world. However, because antigen receptor diversity is generated by a random mechanism, the immune system must tolerate the function of T lymphocytes that by chance express a self-reactive antigen receptor. Therefore, during early development, T cells that are specific for antigens expressed in the thymus are physically deleted. The population of T cells that leaves the thymus and seeds the secondary lymphoid organs contains helpful cells that are specific for antigens from microbes but also potentially dangerous T cells that are specific for innocuous extrathymic self antigens. The outcome of an encounter by a peripheral T cell with these two types of antigens is to a great extent determined by the inability of naive T cells to enter nonlymphoid tissues or to be productively activated in the absence of inflammation.

Current evidence indicates that self-reactive lymphocytes can be inactivated at all stages of their development by a variety of mechanisms. Perhaps the best understood is clonal deletion of lymphocytes at an early stage of development in the primary lymphoid organs—e.g., T cells in the thymus—if their antigen receptors have a high affinity for self antigens expressed there. However, this process alone cannot account for tolerance that exists toward antigens that are not well expressed in the primary lymphoid organs. Examples of such antigens are proteins that are expressed exclusively in the parenchymal tissues, proteins that are only expressed at certain developmental stages of the organism, and the infinite variety of otherwise innocuous environmental proteins present in the air and diet. Because peptides derived from these proteins are presented to mature T cells outside of the thymus, peripheral tolerance cannot be explained by a developmentally regulated susceptibility to deletion, as in the case of immature T cells, but rather depends on factors that are extrinsic to the self-reactive T cell.

In recent years, it has become clear that a full understanding of immune tolerance cannot be achieved with reductionist *in vitro* approaches that separate the individual lymphocyte from its *in vivo* environment. The *in vivo* immune response is a well-organized process that involves multiple interactions of lymphocytes with each other, with bone-marrow-derived antigen-presenting cells (APCs), as well as with nonlymphoid cells and their products. The anatomic features that are designed to optimize immune tolerance toward innocuous self antigens and increase the efficiency of recognition and elimination of pathogens are the subjects of this review.

Thymic Tolerance

The thymus is the primary lymphoid organ where immature precursors develop into mature T cells (1). It is also the site where immature T cells that express self-reactive T-cell antigen receptors (TCRs) are eliminated (2). The thymus is organized into three physically distinct areas: the outer subcapsular zone, the cortex, and the inner medulla (1). The subcapsular zone is a thin space that lies directly below the outer capsule that encases the thymus. This area contains the most immature CD4⁻ CD8⁻ T-cell precursors that have recently migrated to the thymus from the bone marrow. From the subcapsular zone, the immature T cells migrate deeper into the thymus and enter the cortex, which is richly populated by a special type of epithelial cell that expresses high levels of class I and II major histocompatibility complex (MHC)-encoded molecules. In the cortex, the T-cell precursors begin to express both CD4 and CD8 and to rearrange their TCR α and β genes such that each cell will express a different rearranged TCR- $\alpha\beta$ heterodimer. The specificity of the TCR expressed by a given CD4⁺ CD8⁺ thymocyte for a peptide-MHC complex expressed on another cell (the APC) will determine the thymocyte's fate. Evidence from fetal thymic organ cultures suggests that a CD4⁺ CD8⁺ thymocyte that expresses a TCR with low but measurable avidity for a self peptide-MHC complex will be "positively selected," that is, it will be allowed to differentiate into a mature CD4⁺ CD8⁻ (if its TCR is specific for a self peptide–class II MHC complex) or a CD4⁻ CD8⁺ (if its

TCR is specific for a self peptide–class I MHC complex) T cell that will exit the thymus and seed the secondary lymphoid tissues (3, 4). In contrast, cortical CD4⁺ CD8⁺ thymocytes that express TCRs that have no avidity for self peptide-MHC complexes do not survive and die by an apoptotic mechanism. Cortical epithelial cells are essential for the process of positive selection because they display the self peptide-MHC complexes that are recognized by CD4⁺ CD8⁺ thymocytes and also provide essential differentiation factors (5). Positively selected T cells continue their journey toward the central region of the thymus by first crossing the cortico-medullary junction and then entering the medulla. The medulla contains the products of positive selection—i.e., mature CD4⁺ CD8⁻ or CD4⁻ CD8⁺ T cells, that exit the thymus into the blood and seed the secondary lymphoid tissues, where they will be available to be activated by a foreign peptide-MHC complex.

The population of positively selected cortical T cells will include cells that have a strong avidity for self peptide-MHC complexes that are expressed on thymic APCs. These T cells are potentially autoreactive and must be eliminated. Work from many laboratories has shown that this occurs via physical deletion involving apoptosis (6). Experiments with TCR transgenic mice that also express the antigen the transgenic T cells are specific for have shown that clonal deletion can occur either early in the CD4⁺ CD8⁺ stage in the cortex (7, 8) or later in the CD4⁺ CD8⁺ stage in the medulla (9, 10). The site where a given self-reactive T cell will be deleted in the thymus is probably determined by the location of the cells that present the relevant self peptide-MHC complex (6). In cases where T cells are deleted early in the CD4⁺ CD8⁺ stage in the cortex, the relevant complex is probably presented by cortical epithelial cells. Because blood-borne macromolecules

Abbreviations: APC, antigen-presenting cell; HEL, hen egg lysozyme; IL, interleukin; MHC, major histocompatibility complex; PALS, periaortic lymphatic sheath; TCR, T-cell antigen receptor; TNF, tumor necrosis factor; TGF- β , transforming growth factor β ; HEV, high endothelial venule.

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have poor access to the cortex (11), cortical clonal deletion is probably most relevant for antigens that are produced by the cortical epithelial cells themselves—e.g., MHC molecules and housekeeping proteins—or are produced in large amounts by other cells in the cortex—e.g., thymic hormones. In several cases where T cells are deleted in the medulla, the relevant antigen is preferentially produced by bone marrow-derived medullary APCs (9) or is preferentially picked up from the serum, processed, and presented by medullary APCs (10). This process is facilitated by the concentration of interdigitating dendritic cells, the most potent inducers of clonal deletion (12), at the corticomedullary junction (1).

The Peripheral Tolerance Paradox

Because clonal deletion of self-reactive T cells in the thymus is such an efficient process, one might speculate that this is the only T-cell tolerance mechanism that is required. However, not all self antigens are expressed in, or gain access to, the thymus. Self antigens that are not expressed in the thymus are by definition expressed in areas of the body that may be patrolled by mature T cells. Tolerance to these types of antigens is particularly problematic because, unlike immature T cells that are programmed to die when stimulated, peripheral T cells are designed to respond in a positive fashion. Therefore, the challenge of the immune system is to retain the capacity to activate peripheral T cells that are specific for microbial antigens but silence the functions of peripheral T cells that are specific for extrathymic self antigens without being able to delete these T cells in the thymus. It is therefore important to define the events that result in effective antimicrobial T-cell immunity if we are to understand the critical steps that must be avoided if full-

blown reactivity to extrathymic self antigens is to be prevented.

Lymphocyte Migration Through Secondary Lymphoid Tissues

After T cells leave the thymus, they recirculate between the blood and lymph with intermediate stops in secondary lymphoid tissues (13) (lymph nodes, Peyer's patches, and the spleen) (1). The secondary lymphoid tissues are designed to co-localize antigen, APCs, and naive T and B cells. Peripheral lymph nodes are strategically located throughout the body to collect and process antigens arriving via the lymphatic vasculature. Lymphatic vessels originate as closed-end capillaries that collect tissue-derived lymph fluid and lymphocytes. Not surprisingly, the richest networks of lymphatic vessels are found in sites of greatest foreign antigenic load—e.g., skin and mucosal surfaces (1). Lymph and lymphocytes are carried into lymph nodes by afferent lymphatic vessels, pass through the lymph node cortex and medulla and exit via efferent lymphatic vessels (Fig. 1A). This flow pattern ensures that lymph-borne antigens will percolate through the densely clustered lymphocytes and APCs that make up the inner part of the lymph node, optimizing the chances that effective antigen presentation will occur. Macrophages line the subcapsular space in a position where they can phagocytose particles and produce proinflammatory cytokines that, as detailed below, play a key role in adaptive immunity. Interdigitating dendritic cells, the most potent APCs at activating naive T cells (14), are interspersed throughout the central paracortex that also contains mainly T cells plus a few B cells. Most B cells are located in follicles in close association with follicular dendritic cells, although a few T cells are also present. A key feature of this arrangement is the juxtaposition of T and B cells with the dendritic cell types that are critical for

their activation. The spleen has a similar organization but is unique among secondary lymphoid organs because it lacks afferent lymphatic drainage and is thus dedicated to process blood-borne antigens exclusively. The spleen is divided into the red pulp, which contains red blood cells and macrophages, and the white pulp, which has T- and B-cell-rich areas like the lymph nodes (Fig. 1B). A central artery enters the spleen and branches into arterioles that end in splenic sinuses present in the red pulp. The arterioles dump their contents of blood cells and fluids into the sinuses, providing the major means by which blood-borne material enters the spleen. T cells and interdigitating dendritic cells are concentrated along the length of the arterioles to form the periarterial lymphatic sheath (PALS) that constitutes the T-cell-rich zone of the spleen and is analogous to the lymph node paracortex. Follicles rich in B cells and follicular dendritic cells are attached to the PALS at various points along its length, and both the follicles and PALS are surrounded by the marginal zone. The marginal zone is a unique structure of the spleen and consists of a mixture of T and B cells, including memory B cells.

Secondary lymphoid tissues are not static structures; rather, lymphocytes are constantly coming in and going out. This is an essential feature of the immune system, which must move its lymphocyte repertoire through the secondary lymphoid tissues where lymph- or blood-borne antigenic material will be captured and presented. Naive—i.e., cells that have never been stimulated—T and B cells use molecules like L-selectin to exit the blood and enter lymph nodes by binding to specialized blood vessels called high endothelial venules (HEVs) that are located in the paracortex (15). In contrast, naive lymphocytes enter the splenic white pulp via an HEV- and L-selectin-independent mechanism, probably by leaving the red

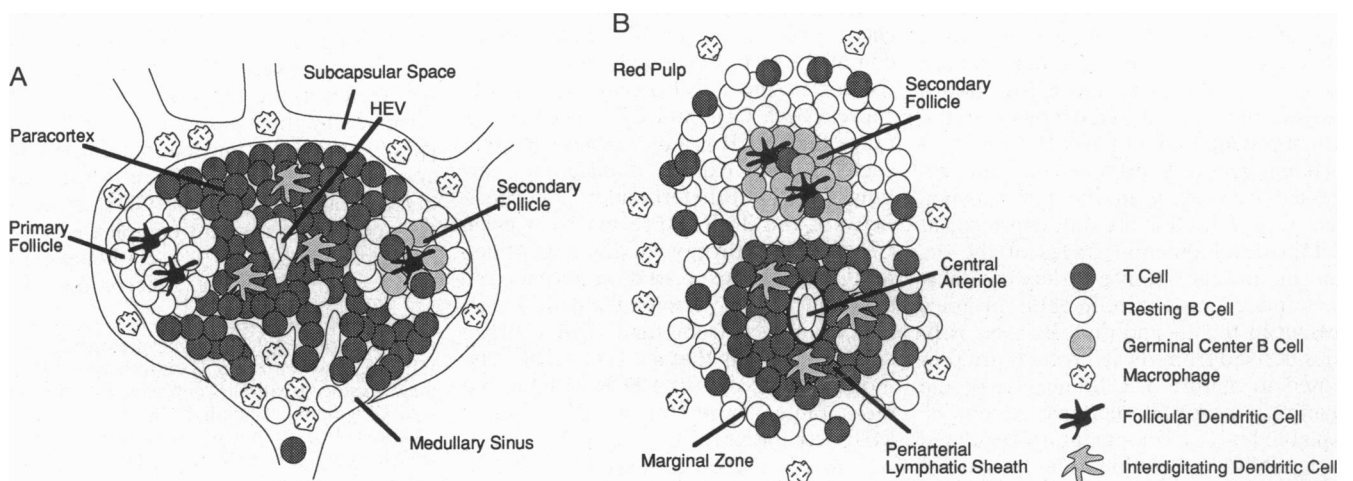


FIG. 1. Schematic depiction of the structure of a lymph node (A) and an area of splenic white pulp (B). The white pulp is shown as a transverse cross-section through one branch of the central arteriole.

pulp sinus and directly entering the marginal zone (1, 15, 16). Naive T cells are deposited into the T-cell zones, whereas naive B cells migrate to primary follicles. Both cell types remain in a given secondary lymphoid tissue site for 10–20 h, and, if they do not encounter their cognate antigen, they leave and recirculate to another secondary lymphoid tissue (17). As described below, the tropism of lymphocytes for secondary lymphoid tissues is lost following activation and replaced with a tropism for nonlymphoid tissues.

Peripheral T-Cell Activation by Microbial Antigens

As diagrammed in Fig. 2A, introduction of foreign antigen dramatically alters the behavior of those lymphocytes within secondary lymphoid tissue that are specific for the antigen in question. Foreign antigen that is deposited in tissue may travel passively to the nearest lymph node by way of the draining afferent lymphatic vessels (1). Alternatively, immature dendritic cells present in the tissue—e.g., Langerhans cells of the skin—may take up the antigen and, in response to inflammatory cytokines, migrate via the afferent lymph to the node (18–20). Antigen that is present in the blood will be taken up by red pulp macrophages or diffuse from the red pulp into the white pulp. Although B cells and macrophages probably take up

and process free antigen that enters the lymph node and spleen, many studies indicate that antigen-bearing, interdigitating dendritic cells are essential for the initial activation of naive T cells (14). In addition, the immature dendritic cells that migrate from the tissue may be critical because these types of cells have been shown to take up and process large amounts of antigen, a property that is lost in mature dendritic cells (14, 21).

The co-localization of dendritic cells and naive T cells in the paracortex and PALS increases the chances that naive T cells with the appropriate TCRs will find an antigen-bearing APC. The interaction of CD28 (22) expressed by the T cell and B7-1 or B7-2 (22) expressed by the dendritic cells plays an important role in the clonal expansion of naive T cells in the paracortex. Paracortical dendritic cells express B7-2 molecules *in situ* (23), and immature tissue dendritic cells increase expression of B7 molecules during their migration from tissue to the lymph node (24). Once activated by antigen-bearing dendritic cells, the specific T cells proliferate in the paracortex and become competent to receive further activation signals from antigen-bearing macrophages and B cells (14). The latter population includes antigen-specific B cells that use their surface immunoglobulin molecules to efficiently internalize and process the antigen and present peptide–MHC complexes.

Surprisingly, the initial interaction between splenic antigen-specific B cells and helper T cells occurs in the T-cell-rich PALS (25–27), a site where very few B cells reside.

Some of the activated T (28, 29) and B cells (30) then migrate from the T-cell zone into follicles to initiate the germinal-center reaction. In the follicles, activated CD4⁺ T cells produce critical lymphokines that promote B-cell proliferation and isotype switching and express CD40 ligand that binds to CD40 on the B cells. Immunohistochemical analyses have localized CD40 ligand-expressing, lymphokine-producing T cells in close proximity to antibody-forming B cells in the PALS and follicles (31). CD40 signaling in B cells has been shown to be important for stimulating B-cell proliferation (32, 33) and antibody production (32) and for enhancing the T-cell costimulatory activity of B cells (34). CD40 ligand has been recently shown to deliver an activation signal to T cells that is important for their clonal expansion (35, 36). The importance of the CD40–CD40 ligand interaction is demonstrated by the findings that primary and secondary antibody responses and germinal-center formation are blocked by anti-CD40 ligand antibody treatment *in vivo* (37, 38) and that CD40 ligand-deficient mice (39) and humans (40) fail to produce IgG or form germinal centers.

The proliferating B-cell blasts push nonactivated follicular B cells out of the

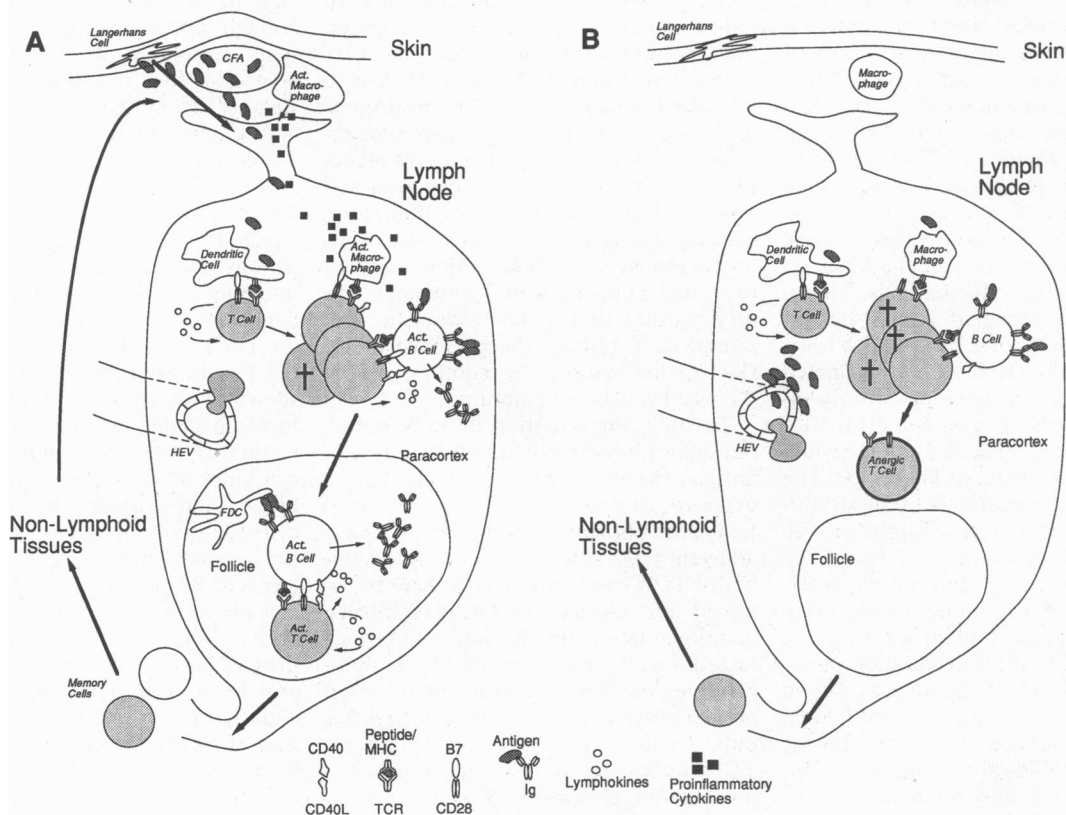


FIG. 2. Diagrammatic representation of the events associated with the induction of T-cell immunity by injection of antigen in complete Freund's adjuvant (CFA) (A) or peripheral tolerance by soluble antigen alone (B). FDC, follicular dendritic cell.

way and begin to form the characteristic germinal center, composed of a dark zone containing actively proliferating B-cell blasts that are undergoing somatic hypermutation of immunoglobulin heavy and light chain genes and a light zone containing nonmitotic B cells (41, 42). Antigen-antibody complexes, produced in the T-cell zone by B cells during their initial interaction with T cells, are bound by follicular dendritic cells (43). These complexes serve as a source of antigen for selection of the highest affinity B cells, presumably those cells that have acquired the most beneficial immunoglobulin somatic mutations. It is thought that these B cells capture antigen very efficiently and thus outcompete other antigen-binding B cells for immunoglobulin- or CD40-mediated survival signals provided by follicular dendritic cells and helper T cells. The germinal-center reaction peaks about 2 weeks after initial antigen exposure and wanes thereafter (26), due in part to the dying off of many of the antigen-specific T and B cells. However, by way of a poorly understood process, some of the antigen-specific T and B cells survive to become memory cells that are capable of mounting rapid and efficient secondary responses (44).

During the course of the primary immune response, activated T cells lose L-selectin that is involved in lymph node entry and increase expression of adhesion molecules on their surface—e.g., LFA-1 and VLA-4—that permit extravasation into nonlymphoid tissues (44). The trafficking of activated lymphocytes into tissues is governed by interactions with vascular endothelium in inflamed tissues (45). Inflammatory mediators and cytokines—e.g., interleukin 1 (IL-1) and tumor necrosis factor α (TNF- α)—produced by macrophages and parenchymal cells in response to microbial molecules, induce the expression of selectins, integrin ligands, and immobilized chemokines on nearby vascular endothelial cells. Therefore, previously activated T cells that express higher levels of LFA-1 and VLA-4 are preferentially recruited into inflamed tissues, although in cases of chronic inflammation, naive T cells can also enter because the local vascular endothelium takes on the properties of HEV (15). The net effect of this process is to allow antigen-specific lymphocytes to migrate to the actual sites of inflammation where the invading pathogen has entered the body.

In summary, T-cell immunity to microbial antigens depends on inflammation at the site of entry; co-localization of microbial antigen, APCs, and naive T cells in secondary lymphoid tissues; and clonal expansion and survival of antigen-specific T cells, followed by their migration into follicles and then into nonlymphoid tissues. As outlined below, peripheral tolerance can operate at any of these steps depending on the nature of the self antigen.

Developmentally Regulated Antigens

If autoimmunity is to be prevented, the aforementioned sequence of events must be avoided when peripheral self antigens are presented. This situation could occur when developmentally regulated self antigens, for example proteins associated with pregnancy, lactation, puberty, and aging, appear in the blood for the first time after mature T cells have seeded the secondary lymphoid organs. In these cases, it is likely that new proteins are presented to a mature T cell repertoire that has not had the chance to purge itself of T cells specific for peptide-MHC complexes derived from these proteins. Thus, these peptide-MHC complexes would be expected to be just as “foreign” to the immune system as those derived from microbes. Clues about a possible explanation for this paradox come from experiments performed by Dresser (46, 47), which showed that the immunogenicity of purified antigens was determined by factors in addition to foreignness. He showed that even foreign antigens failed to induce immunity unless they were injected with an adjuvant (46). Adjuvants are components of microbes, for example lipopolysaccharide or muramyl dipeptide, that induce inflammation (48). Dresser (47) and others (49) also showed that injection of foreign proteins without an adjuvant induced a state of long-lasting immunological tolerance such that subsequent injection of the same protein together with an adjuvant failed to induce immunity. These results suggested that: (i) adjuvants create an inflammatory *in vivo* environment that is conducive to adaptive immunity; and (ii) in the absence of this environment, antigen exposure results in tolerance. According to this model (50, 51), developmentally regulated self proteins would not cause autoimmunity because they would be presented to T cells in the absence of inflammation, whereas microbial antigens would induce immunity because they would always be presented to T cells in the presence of inflammation caused by components of their cell walls or membranes.

To study this situation in more detail, our laboratory directly tracked the fate of antigen-specific T cells following *in vivo* exposure to a soluble antigen alone or to that same antigen emulsified in Freund’s adjuvant (29). A low but detectable number of TCR transgenic T cells were transferred into normal syngeneic recipients, and the number and location of the transferred T cells was followed by immunohistology or flow cytometry by using an anti-clonotypic antibody that recognizes only T cells that express the transgenic TCR. Following antigen injection in adjuvant, antigen-specific T cells proliferated extensively in the paracortical region of the draining lymph nodes and after several days appeared in draining lymph

node follicles. After day 5, the antigen-specific T cells gradually disappeared from the lymph nodes. Adoptively transferred mice primed in this way produced high levels of antigen-specific IgG and developed a local delayed-type hypersensitivity reaction when challenged with antigen, suggesting that humoral and cellular immunity had been induced (M.K.J., unpublished observation). In contrast, when adoptively transferred mice were injected intravenously with soluble antigen alone, the antigen-specific T cells proliferated transiently in the paracortical regions of peripheral lymph nodes but never went into follicles, and most of the T cells rapidly disappeared. These animals did not produce antibody (M.K.J., unpublished observation). Given the dependence of germinal-center formation on CD4⁺ T cells (39, 40, 52), it is likely that this failure is related to the inability of antigen-specific T cells to enter the follicles. When adoptively transferred mice that were first injected with antigen alone were rechallenged with antigen in adjuvant, the few remaining TCR transgenic T cells proliferated poorly in the draining lymph nodes, suggesting that their activation was impaired. A very similar set of observations was reported by Kyburz *et al.* (53) using CD8⁺ TCR transgenic T cells specific for a viral peptide-class I MHC complex. Therefore, in these situations, even though the antigen (chicken ovalbumin or viral protein) was completely foreign to the host, administration of the antigen under noninflammatory conditions caused an abortive type of T-cell activation that resulted in tolerance instead of immunity.

A scenario by which adjuvants shift the T-cell response toward immunity (Fig. 2A) instead of peripheral tolerance (Fig. 2B) is shown in Fig. 2. As noted above, the CD28-B7 and CD40 ligand-CD40 interactions play key roles in the productive immune response, and, thus, adjuvants may work by causing the induction of B7 on APCs or CD40 ligand on the responding T cells. Microbial products have been shown to induce B7 and other costimulatory molecules on normally B7-negative resting B cells (54), and inflammatory cytokines produced in response to microbial products cause immature dendritic cells to migrate from the inflamed site to the regional lymph nodes and express high levels of B7 (18–20, 24). Therefore, in the absence of inflammation, fewer costimulatory APCs would be predicted to be present in the draining lymph nodes. Several lines of evidence indicate that this situation favors T-cell tolerance. Prevention of CD28-B7 interactions *in vivo* has been shown to inhibit T-cell immunity (55–59) and result in antigen-specific T-cell tolerance in several (56–58), but not all (55), instances. Injection of B7-negative resting B cells induces T-cell tol-

erance *in vivo* (60, 61), although it has been argued (61) that this is related to a tolerogenic program that occurs in naive T cells whenever they are stimulated by an APC other than a dendritic cell and not by the poor costimulatory function of resting B cells. However, the ability of B cells to induce T-cell tolerance is greatly enhanced if recipient mice are also treated with anti-CD40 ligand antibody (62) or if the B cells are CD40-deficient (63). One explanation for this is that inhibition of CD40 signaling prevents the induction of B7-dependent T-cell costimulatory activity in the B cells (34). An alternative explanation, however, is that CD40 ligand delivers a critical activation signal to the T cell that prevents death or induction of functional unresponsiveness (35, 36). Independent of the mechanism, the ability of B-cell antigen presentation in the absence of dendritic-cell antigen presentation to induce T-cell unresponsiveness may be an important tolerance mechanism for self antigens expressed only by B cells—e.g., peptide–MHC complexes derived from variable components of immunoglobulins (61).

Adjuvants may also facilitate T-cell immunity by stimulating cells of the innate immune system to produce inflammatory cytokines that promote T-cell survival. Injection of superantigens that directly crosslink the TCR- β chain on T cells and class II MHC molecules on APCs induces transient T-cell proliferation followed by unresponsiveness and death (64), as in the case of peptide antigen injection in the absence of adjuvant (29, 53). Marrack and coworkers (65) have shown that the T cells that express the appropriate TCR- β chains survive much longer if lipopolysaccharide is injected along with the superantigen. TNF- α appears to be partially responsible for this effect. In addition, the disappearance of T cells that is induced by the injection of antigen in the absence of adjuvant does not occur if the T cells lack Fas (66), implicating Fas–Fas ligand interactions in this peripheral deletion pathway.

Tolerance also results when CD8⁺ T cells are suddenly exposed to their cognate antigen in the periphery. Rocha and von Boehmer (67) showed that when CD8⁺ T cells from female donors expressing a transgenic TCR specific for the male H-Y antigen were transferred into male recipients, the T cells proliferated transiently, and then most of the cells died leaving behind a population that was functionally unresponsive due to loss of surface TCR and CD8 molecule expression (66). These surface molecule changes were not noted in the aforementioned cases where peripheral tolerance was induced by the injection of soluble antigen in the absence of an adjuvant (29, 53). Loss of TCR and accessory molecules may be a consequence of chronic stimulation because in the H-Y system the antigen in

question (H-Y-expressing male cells) cannot be readily cleared. Functional unresponsiveness related to TCR and CD8 modulation may also be involved in immunological tolerance to the fetus. Pregnancy-associated loss of TCR and CD8 has been described for CD8⁺ TCR transgenic T cells in pregnant mice that carry a fetus expressing the class I MHC molecule for which the transgenic TCR is specific (68). Tolerance does not persist in the mother postpartum, suggesting that the tolerogens are fetal cells that migrate into the mother. Although it is possible that tolerance in these cases is caused by antigen presentation in a non-inflammatory environment as diagrammed in Fig. 2B, it is equally possible that chronic stimulation by antigens that cannot be easily cleared results in “exhaustion” of the T cells (69).

As noted above, antigen-specific CD4⁺ T cells did not enter follicles following a tolerogenic injection of antigen in the absence of an adjuvant (29). A similar type of follicular exclusion has been shown for tolerized antigen-specific B cells. Goodnow *et al.* (70) have produced double transgenic mice that express hen egg lysozyme (HEL)-specific immunoglobulin on all of their B cells and also express a soluble form of HEL. HEL-specific T cells are tolerant in these mice and, thus, the transgenic B cells are constantly exposed to HEL in the absence of T-cell help. The transgenic B cells are not deleted but become functionally unresponsive because of a proximal block in secreted immunoglobulin signaling (71). When transferred into HEL, single transgenic mice that possess a diverse B-cell repertoire, the unresponsive B cells from the double transgenic mice accumulated in the T-cell zones, did not enter follicles, and died shortly thereafter (72). Because this phenomenon was not observed in the intact, double transgenic mice, the authors concluded that the follicular exclusion of the transferred unresponsive B cells was due to failed competition with normal B cells. These investigators (73) and others (74, 75) also demonstrated that activated germinal-center B cells that are suddenly exposed to a cross-reactive antigen for which there is no T-cell help leave the germinal center and enter the T-cell zone but are unable to reenter the germinal center and die. This is probably an important tolerance mechanism that eliminates B cells that acquire immunoglobulin somatic mutations that confer reactivity for a self antigen present in the lymphoid tissue. Therefore, in three different tolerogenic situations—activation of naive T cells in the absence of inflammation, stimulation of naive T-cell zone B cells in the absence of T-cell help, or confrontation of activated germinal center B cells with a cross-reactive antigen in the absence of T cell help—the specific lymphocyte population is excluded from the follicle (or

germinal center) and dies or becomes unresponsive. The lengths to which the immune system has gone to exclude potentially self-reactive lymphocytes from follicles suggests that the follicle provides an essential microenvironment for the generation of memory T and B cells. The actual mechanism by which T or B cells are excluded from follicles is unclear, however, although ineffective competition for follicular niches and failure of the production of a chemoattractant by follicular cells are possibilities.

In summary, the bulk of the evidence to date suggests that T-cell tolerance specific for developmentally regulated antigens (antigens that appear after a mature T-cell repertoire has formed) is induced in secondary lymphoid tissues as a consequence of an abortive form of T-cell activation that occurs when an inflammatory environment is absent.

Nonlymphoid Antigens

A second class of antigens that peripheral T cell tolerance must deal with are proteins expressed exclusively in nonlymphoid tissues, for example proteins expressed only in the pancreas, liver, or kidney. Because these proteins are not expressed in the thymus, there is no opportunity to clonally delete T cells specific for peptide–MHC complexes derived from them.

The first line of protection for these types of self antigens is related to the fact that naive T cells are relatively confined to blood and secondary lymphoid organs and cannot efficiently enter uninflamed nonlymphoid tissues (13). Therefore, nonlymphoid antigens are tolerated, in part because naive T cells do not get access to the tissues that express them. This could explain the results of Ohashi *et al.* (76), who studied double transgenic mice expressing lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) in the β islet cells of the pancreas and also a TCR specific for LCMV-GP/H-2D^b on all CD8⁺ T cells. Despite the fact that most of the T cells in these animals were specific for LCMV-GP peptide–H-2D^b, they did not develop diabetes. Tolerance was not due to deletion or T-cell anergy since the functional response of transgenic T cells, when measured *in vitro*, was the same as that of T cells from TCR transgenic mice that did not express LCMV-GP. When the doubly transgenic animals were infected with LCMV, the pancreas was infiltrated with TCR transgenic T cells and the β cells were destroyed. These results are consistent with the idea that the naive TCR transgenic T cells did not enter the pancreas and thus ignored the antigen-bearing β cells. However, activation of the T cells in secondary lymphoid tissue by the virus infection would be expected to cause the expression of adhesion receptors re-

quired for extravasation, thus allowing the T cells to enter the pancreas and destroy the β cells.

Although the results mentioned above suggest that it is limited, some degree of surveillance of most nonlymphoid tissues by naive T cells probably does occur. A peripheral tolerance mechanism would be required to deal with this, especially for CD8⁺ T cells that recognize class I MHC molecules that are expressed on most nonlymphoid tissues. Evidence for peripheral tolerance in this situation comes from studies by Arnold and coworkers (77–79) who have constructed doubly transgenic mice expressing TCR specific for class I MHC K^b molecules on all CD8⁺ T cells and also K^b in the liver but not the thymus. The absence of K^b expression in the thymus was shown by lack of its detection by immunohistology and reverse transcriptase-PCR. The K^b-reactive TCR transgenic cells had reduced levels of TCR and CD8 expression and failed to respond to K^b *in vivo* or *in vitro*. Therefore, tolerance was probably induced as naive TCR transgenic T cells recognized K^b in the liver. Although it is possible that tolerance could have been induced in regional lymph nodes, this seems unlikely since it would have required that the T cells recognized passively acquired K^b on the surface of a lymph node APC. Naive T-cell surveillance may be higher in tissues like the liver that have a rich blood supply and where blood flow is reduced in sinusoids providing a better opportunity for naive T cells to leave the circulation.

One explanation for why self antigen-MHC presentation by nonlymphoid tissues induces tolerance instead of autoimmunity is that most nonlymphoid tissues do not express costimulatory molecules like B7 that are required to activate naive T cells and, perhaps, prevent tolerance. This idea has been validated by the results of Flavell and coworkers (80, 81) using transgenic mice that express I-E class II MHC molecules alone, B7-1 alone, or I-E plus B7-1 on pancreatic β cells. Coexpression of both I-E and B7-1, but not I-E or B7-1 alone, on pancreatic β cells resulted in T-cell-mediated destruction of the pancreas instead of tolerance. Furthermore, transplanted I-E⁺, B7-1⁺ islets were rejected by normal mice but not by the transgenic mice that express only I-E in the pancreas, demonstrating that the T cells in the latter mice were truly tolerant to I-E and not simply ignorant of it. This important set of results shows that nonlymphoid tissues that express class II MHC molecules are capable of inducing CD4⁺ T-cell tolerance, whereas nonlymphoid tissues that express both class II MHC and costimulatory molecules induce T-cell immunity. It should be noted, however, that constitutive expression of class II MHC molecules is not the normal situation. In a normal individual, even if a

CD4⁺ naive T-cell specific for a self peptide-class II MHC complex were to wander into a nonlymphoid tissue, the T cell would not be activated because these tissues normally do not express class II MHC molecules. Evidence for a lack of constitutive self antigen-class II MHC presentation is provided by the finding that transgenic B7 expression alone in pancreatic β cells (80–82) or keratinocytes (83, 84) does not induce CD4⁺ T-cell-mediated autoimmunity. It is therefore likely that ignorance is the major mechanism of tolerance for class II MHC-restricted, tissue-specific peptides in normal individuals. However, class II MHC molecules are upregulated on many nonlymphoid costimulation-deficient tissues during inflammation, and this could serve as a means of terminating chronic T-cell responses. The finding that constitutive expression of B7-1 by keratinocytes greatly increases and prolongs cutaneous delayed-type hypersensitivity reactions to exogenously applied contact sensitizers is consistent with this possibility (83, 84).

Antigens Expressed in Privileged Sites

Some organs—e.g., brain, gonads, and eye—are considered immune-privileged sites because foreign antigens that are placed within them do not provoke an immune response and often tolerance is induced instead. These organs maintain even stronger barriers to routine entry of lymphocytes than other nonlymphoid tissues. Such restriction may be required to avoid disruption of normal physiologic function of these sites by the destructive force of the immune response. One example is the blood-brain barrier, where tight junctions between endothelial cells of the brain vasculature prevent the access of lymphocytes to the central nervous system (15, 85). The importance of the blood-brain barrier as a self-tolerance mechanism is illustrated by experimental models of autoimmunity involving the central nervous system. One example, experimental autoimmune encephalomyelitis, can be induced by CD4⁺ T cells specific for certain components of myelin (86, 87). However, this can only be achieved in certain strains of mice that are notable for increased vascular sensitivity within the central nervous system (87, 88). Even in this context disease often requires the use of pertussis toxin that is known to further disrupt the blood-brain barrier (89–91).

Immune ignorance may also be an important mechanism of tolerance for other immune-privileged sites. Thus, extensive tight junctions also exist among Sertoli cells lining the seminiferous tubules in the testis, as well as among the retinal pigment epithelial cells in the eye (92, 93). However, it is now clear that active immunoregulatory mechanisms employed by resident tissue cells are also critical for main-

taining immune privilege (94). For example, Sertoli cells (95) and ocular tissues (96) constitutively express Fas ligand and can kill previously activated T cells that normally express Fas. Thus, Bellgrau *et al.* (95) showed that, while allogeneic testis tissue transplanted under the kidney capsule survived for weeks without any evidence of rejection or lymphocyte infiltration, allogeneic testis tissue derived from Fas ligand-deficient mutant *gld* mice was promptly rejected. Similarly, injection of herpes simplex virus type 1 into the anterior chamber of the eye resulted in initial inflammatory infiltration of the eye in normal and *gld* mice (96). However, while extensive apoptosis in infiltrating cells and little tissue damage were subsequently seen in normal mice, the course in *gld* mice was characterized by expansion of the inflammatory infiltrate and significant tissue damage. Soluble mediators may also contribute to the immunosuppressive environment found within immune-privileged sites. For instance, ocular fluids contain high concentrations of transforming growth factor β (TGF- β), as well as certain immunosuppressive neuropeptides (97). Similar immunosuppressive microenvironments are also present in the brain, testis, and placenta (97). Preferential skewing of the immune response toward humoral rather than cell-mediated immunity to antigens placed into immune-privileged sites, perhaps by favoring T-cell differentiation toward the less destructive Th2 phenotype, may be another possible mechanism of avoiding tissue damage (97, 98).

Mucosal Antigens

The mucosal surfaces are exposed to the greatest number of foreign antigens. However, while effective immunity usually develops against microbial pathogens invading through the mucosa, immune tolerance is maintained against innocuous antigens that are components of diet and air. Some discrimination between potentially pathogenic intestinal contents and food is provided by the physical barriers created by the intestinal epithelium. Integrity of adhesion among intestinal epithelial cells and the layer of mucus coating the epithelium are critical in protecting the intestine from bacterial penetration (99–101). However, a barrier that is even more specific against immunogenic luminal contents is created by secreted IgA molecules. Production of IgA is dependent on absorption of antigens into Peyer's patches, lymph node-like structures that are scattered throughout the intestine. Specialized intestinal epithelial cells, called M cells, efficiently translocate bacteria and macromolecules into Peyer's patches, where particles with adjuvant properties are likely to lead to a productive immune response, including IgA production (102). In contrast, soluble food

proteins can directly pass from the gut lumen into the bloodstream (103–105) and thus would be likely to induce T-cell tolerance as a result of noninflammatory antigen presentation, as shown in Fig. 2B. Support for this comes from the finding that oral administration of soluble foreign protein antigens in the absence of an adjuvant results in systemic T-cell tolerance, whereas feeding foreign antigen plus a mucosal adjuvant, such as cholera toxin, results in T-cell immunity and IgA production (106–108). Another reason for normal tolerance of food antigens may be due to the local suppressive microenvironment in the gut. Immunosuppressive molecules such as TGF- β and IL-10 are made abundantly in the intestine. In addition to resident parenchymal cells, another source of these molecules may be regulatory T cells that are preferentially activated by fed antigens (109, 110). By whatever mechanism, TGF- β and IL-10 clearly play a role in tolerance to mucosal antigens because TGF- β 1- and IL-10-deficient animals develop inflammatory bowel disease (111–113). Interestingly, mice lacking TGF- β 1 also develop systemic autoimmunity, suggesting an important systemic role of this cytokine in regulating the immune response.

Summary

Clonal deletion via apoptotic cell death appears to be the intrinsic response of immature thymocytes that receive high levels of TCR signaling. The structure of the thymus is designed to maximize exposure to thymic self peptide–MHC complexes by passing immature thymocytes through a gauntlet of cortical epithelial cells and corticomedullary dendritic cells. In contrast, peripheral tolerance, which by definition must act on mature T cells that are specific for extrathymic self antigens, depends on factors other than the avidity of the TCR for self peptide–MHC complexes. Many of these factors consist of anatomic barriers that limit the migration and activation of self-reactive T cells. The homing receptors expressed by naive cells restrict T cells to secondary lymphoid tissues, limiting the detection of tissue-specific self antigens expressed in nonlymphoid tissues. In addition, class II MHC and costimulatory molecules are not expressed by non-lymphoid tissues, preventing productive activation of self-reactive T cells that do gain access. T cells that recognize developmentally regulated self antigens in secondary lymphoid organs do so in a noninflammatory environment that does not allow the T cells to enter follicles or become memory cells. Finally, immune-privileged tissues that are highly susceptible to immune-mediated destruction protect themselves from T-cell attack with immunosuppressive cytokines and surface molecules. Together, these mech-

anisms limit the activation of naive T cells to secondary lymphoid tissues that drain sites of inflammation, and control the effector functions of activated T cells that enter nonlymphoid tissues. The fact that these limitations are only overcome during microbial infections prevents the T-cell system from mounting full-blown autoimmune responses to peripheral self antigens.

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- Picker, L. J. & Siegelman, M. H. (1993) in *Fundamental Immunology*, ed. Paul, W. E. (Raven, New York), 3rd Ed., pp. 145–197.
- Kappler, J. W., Roehm, N. & Marrack, P. (1987) *Cell* **49**, 273–280.
- Hogquist, K. A., Gavin, M. A. & Bevan, M. J. (1993) *J. Exp. Med.* **177**, 1469–1473.
- Ashton-Rickardt, P. G., Van Kaer, L., Schumacher, T. N. M., Ploegh, H. L. & Tonegawa, S. (1993) *Cell* **73**, 1041–1049.
- Anderson, G., Jenkinson, E. J., Moore, N. C. & Owen, J. J. T. (1993) *Nature (London)* **362**, 70–73.
- Sprent, J. & Webb, S. R. (1995) *Curr. Opin. Immunol.* **7**, 196–205.
- Kisielow, P., Bluthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. (1988) *Nature (London)* **333**, 742–746.
- Bogen, B., Dembic, Z. & Weiss, S. (1993) *EMBO J.* **12**, 357–363.
- Surh, C. D. & Sprent, J. (1994) *Nature (London)* **372**, 100–103.
- Zal, T., Volkman, A. & Stockinger, B. (1994) *J. Exp. Med.* **180**, 2089–2099.
- Nieuwenhuis, P., Stet, R. J. M., Wegenaar, J. P. A., Wubbena, A. S., Kampinga, J. & Karrenbeld, A. (1988) *Immunol. Today* **9**, 372–375.
- Matzinger, P. & Guelder, S. (1989) *Nature (London)* **338**, 74–76.
- Mackay, C. R., Marston, W. L. & Duder, L. (1990) *J. Exp. Med.* **171**, 801–817.
- Steinman, R. M. (1991) *Annu. Rev. Immunol.* **9**, 271–296.
- Girard, J. P. & Springer, T. A. (1995) *Immunol. Today* **16**, 449–457.
- Arbores, M. L., Ord, D. C., Ley, K., Rattech, H., Maynard-Curry, C., Otten, G., Capon, D. J. & Tedder, T. F. (1994) *Immunity* **1**, 247–260.
- Smith, M. E. & Ford, W. L. (1983) *Immunology* **49**, 83–94.
- Macatonia, S. E., Knight, S. C., Edwards, A. J., Griffiths, S. & Fryer, P. (1987) *J. Exp. Med.* **166**, 1654–1667.
- Larsen, C. P., Steinman, R. M., Witmer-Pack, M. D., Hankins, D. F., Morris, P. J. & Austyn, J. M. (1990) *J. Exp. Med.* **172**, 1483–1494.
- Cumberbatch, M. & Kimber, I. (1995) *Immunology* **84**, 31–55.
- Sallusto, F., Cella, M., Danieli, C. & Lanzavecchia, A. (1995) *J. Exp. Med.* **182**, 389–400.
- June, C. H., Bluestone, J. A., Nadler, L. M. & Thompson, C. B. (1994) *Immunol. Today* **15**, 321–331.
- Inaba, K., Witmer-Pack, M., Inaba, M., Hathcock, K. S., Sakuta, H., Azuma, M., Yagita, H., Okumura, K., Linsley, P. S., Ikehara, S., Muramatsu, S., Hodes, R. J. & Steinman, R. M. (1994) *J. Exp. Med.* **180**, 1849–1860.
- Larsen, C. P., Ritchie, S. C., Pearson, T. C., Linsley, P. S. & Lowry, R. P. (1992) *J. Exp. Med.* **176**, 1215–1220.
- Gray, D. (1988) *Immunology* **65**, 73–79.
- Jacob, J., Kassir, R. & Kelsoe, G. (1991) *J. Exp. Med.* **173**, 1165–1175.
- Liu, Y. J., Zhang, J., Lane, P. J. L., Chan, E. Y. T. & MacLennan, I. C. M. (1991) *Eur. J. Immunol.* **21**, 2951–2962.
- Fuller, K. A., Kanagawa, O. & Nahm, M. H. (1993) *J. Immunol.* **151**, 4505–4512.
- Kearney, E. R., Pape, K. A., Loh, D. Y. & Jenkins, M. K. (1994) *Immunity* **1**, 327–339.
- Jacob, J. & Kelsoe, G. (1992) *J. Exp. Med.* **176**, 679–687.
- Van den Eertwegt, A. J. M., Noelle, R. J., Roy, M., Shepherd, D. M., Aruffo, A., Ledbetter, J. A., Boersma, W. J. A. & Claassen, E. (1993) *J. Exp. Med.* **178**, 1555–1565.
- Noelle, R. J., Roy, M., Shepherd, D. M., Stamenkovic, I., Ledbetter, J. A. & Aruffo, A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 6550–6554.
- Liu, Y. J., Joshua, D. E., Williams, G. T., Smith, C. A., Gordon, J. A. & MacLennan, I. C. M. (1989) *Nature (London)* **342**, 929–931.
- Ranheim, E. A. & Kipps, T. J. (1993) *J. Exp. Med.* **177**, 925–935.
- Grewal, I. S., Xu, J. & Flavell, R. A. (1995) *Nature (London)* **378**, 617–620.
- van Essen, D., Kikutani, H. & Gray, D. (1995) *Nature (London)* **378**, 620–623.
- Foy, T. M., Shepherd, D. M., Durie, F. H., Aruffo, A., Ledbetter, J. A. & Noelle, R. J. (1993) *J. Exp. Med.* **178**, 1567–1575.
- Foy, T. M., Laman, J. D., Ledbetter, J. A., Aruffo, A., Claassen, E. & Noelle, R. J. (1994) *J. Exp. Med.* **180**, 157–163.
- Xu, J., Foy, T. M., Laman, J., Elliott, E. A., Dunn, J. J., Waldschmidt, T. J., Elsemore, J., Noelle, R. J. & Flavell, R. A. (1994) *Immunity* **1**, 423–431.
- Korthauer, U., Graf, D., Mages, H. W., Briere, F., Munoredevi, P., Malcolm, S., Ugazio, A. G., Notarangelo, L. D., Levinski, R. J. & Krocak, R. A. (1993) *Nature (London)* **361**, 539–543.
- Berek, C., Berger, A. & Apel, M. (1991) *Cell* **67**, 1121–1129.
- MacLennan, I. C. M. (1994) *Annu. Rev. Immunol.* **12**, 117–139.
- Szakai, A. K., Kosco, M. H. & Tew, J. G. (1989) *Annu. Rev. Immunol.* **7**, 91–111.
- Sprent, J. (1994) *Cell* **76**, 315–322.
- Imhof, B. A. & Dunon, D. (1995) *Adv. Immunol.* **58**, 345–416.
- Dresser, D. W. (1961) *Nature (London)* **191**, 1169–1171.
- Dresser, D. W. (1962) *Immunology* **5**, 378–388.
- Warren, H. S., Vogel, F. R. & Chedid, L. A. (1986) *Annu. Rev. Immunol.* **4**, 369–388.
- Chiller, J. M., Habicht, G. S. & Weigle, W. O. (1971) *Science* **171**, 813–815.
- Janeway, C. A., Jr. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **54**, 1–13.
- Matzinger, P. (1994) *Annu. Rev. Immunol.* **12**, 991–1045.

52. Cosgrove, D., Gray, D., Dierich, A., Kaufman, J., Lemeur, M., Benoist, C. & Mathis, D. (1991) *Cell* **66**, 1051-1066.
53. Kyburz, D., Aichele, P., Speiser, D. E., Hengartner, H., Zinkernagel, R. M. & Pircher, H. (1993) *Eur. J. Immunol.* **23**, 1956-1962.
54. Liu, Y. & Janeway, C. A., Jr. (1991) *Int. Immunol.* **3**, 323-332.
55. Linsley, P. S., Wallace, P. M., Johnson, J., Gibson, M. G., Greene, J. L., Ledbetter, J. A., Singh, C. & Tepper, M. A. (1992) *Science* **257**, 792-795.
56. Lenschow, D. J., Zeng, Y., Thistlewaite, J. R., Montag, A., Brady, V., Gibson, M. I., Linsley, P. S. & Bluestone, J. A. (1992) *Science* **257**, 789-791.
57. Lin, H., Bolling, S. F., Linsley, P. S., Wei, R.-Q., Gordon, D., Thompson, C. B. & Turka, L. A. (1993) *J. Exp. Med.* **178**, 1801-1806.
58. Wallace, P. M., Rodgers, J. N., Leytze, G. M., Johnson, J. S. & Linsley, P. S. (1995) *J. Immunol.* **154**, 5885-5985.
59. Kearney, E. R., Walunas, T. L., Karr, R. W., Morton, P. A., Loh, D. Y., Bluestone, J. A. & Jenkins, M. K. (1995) *J. Immunol.* **155**, 1032-1036.
60. Enyon, E. E. & Parker, D. C. (1992) *J. Exp. Med.* **175**, 131-138.
61. Fuchs, E. J. & Matzinger, P. (1992) *Science* **258**, 1156-1158.
62. Parker, D. C., Greiner, D. L., Phillips, N. E., Appel, M. C., Steele, A. W., Durie, F. H., Noelle, R. J., Mordes, J. P. & Rossini, A. A. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 9560-9564.
63. Buhlmann, J., Foy, T. M., Aruffo, A., Crassal, K. M., Ledbetter, J. A., Green, W. R., Xu, J. C., Shultz, L. D., Roope-sian, D., Flavell, R. A., Last, L., Noelle, R. J. & Durie, F. H. (1995) *Immunity* **2**, 645-653.
64. Webb, S., Morris, C. & Sprent, J. (1990) *Cell* **63**, 1249-1256.
65. Vella, A. T., McCormack, J. E., Linsley, P. S., Kappler, J. W. & Marrack, P. (1995) *Immunity* **2**, 261-270.
66. Singer, G. C. & Abbas, A. (1994) *Immunity* **1**, 365-371.
67. Rocha, B. & von Boehmer, H. (1991) *Science* **251**, 1225-1228.
68. Tafuri, A., Alferink, J., Moller, P., Hammerling, G. J. & Arnold, B. (1995) *Science* **270**, 630-633.
69. Zinkernagel, R. M., Moskophidis, D., Kundig, T., Oehen, S., Pircher, H. P. & Hengartner, H. (1993) *Immunol. Rev.* **131**, 199-223.
70. Goodnow, C. C., Crosbie, J., Jorgensen, H., Brink, R. A. & Basten, A. (1989) *Nature (London)* **342**, 385-391.
71. Cooke, M. P., Heat, A. W., Shokat, K. M., Zeng, Y., Finkelman, F. D., Linsley, P. S., Howard, M. & Goodnow, C. C. (1994) *J. Exp. Med.* **179**, 425-438.
72. Cyster, J., Hartley, S. & Goodnow, C. C. (1994) *Nature (London)* **371**, 389-395.
73. Shokat, K. M. & Goodnow, C. C. (1995) *Nature (London)* **375**, 334-338.
74. Pulendran, B., Kannourakis, G., Nouri, S., Smith, K. G. C. & Nossal, G. J. V. (1995) *Nature (London)* **375**, 331-334.
75. Han, S., Zheng, B., Dal Porto, J. & Kelsoe, G. (1995) *J. Exp. Med.* **182**, 1635-1644.
76. Ohashi, P. S., Oehen, S., Buerki, K., Pircher, H., Ohashi, C. T., Odermatt, B., Mallissen, B., Zinkernagel, R. M. & Hengartner, H. (1991) *Cell* **65**, 305-317.
77. Shonrich, G., Momburg, F., Malissen, M., Schmitt-Verhulst, A.-M., Malissen, B., Hammerling, G. J. & Arnold, B. (1992) *Int. Immunol.* **4**, 581-590.
78. Hammerling, G. J., Schonrich, G., Ferber, I. & Arnold, B. (1991) *Immunol. Rev.* **122**, 47-67.
79. Arnold, B., Schonrich, G. & Hammerling, G. J. (1993) *Immunol. Today* **14**, 12-14.
80. Guerder, S., Picarella, D. E., Linsley, P. S. & Flavell, R. A. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 5138-5142.
81. Guerder, S., Meyerhoff, J. & Flavell, R. (1994) *Immunity* **1**, 155-166.
82. Harlan, D. M., Hengartner, H., Huang, M. L., Kang, Y.-H., Abe, R., Moreadith, R. W., Pircher, H., Gray, G. S., Ohashi, P. S., Freeman, G. J., Nadler, L. M., June, C. H. & Aichele, P. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 3137-3141.
83. Williams, I. R., Ort, R. J. & Kupper, T. S. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 12780-12784.
84. Nasir, A., Ferbel, B., Salminen, W., Barth, R. K. & Gaspari, A. (1994) *J. Clin. Invest.* **94**, 892-898.
85. Schlosshauer, B. (1993) *Bioessays* **15**, 341-346.
86. Zamvil, S. S. & Steinman, L. (1990) *Annu. Rev. Immunol.* **8**, 579-621.
87. Tuohy, V. K., Sobel, R. A. & Lees, M. B. (1988) *J. Immunol.* **140**, 1868-1873.
88. Yong, T., Bebo, Jr., B. F., Sapatino, B. V., Welsch, C. J., Orr, E. L. & Linthicum, D. S. (1994) *J. Neurotrauma* **11**, 161-171.
89. Bernard, C. C. A. & Carnegie, P. R. (1975) *J. Immunol.* **114**, 1537-1540.
90. Linthicum, D. S. & Frelinger, J. (1982) *J. Exp. Med.* **155**, 31-40.
91. Linthicum, D. S., Munoz, J. J. & Blaskett, A. (1982) *Cell. Immunol.* **73**, 299-310.
92. Greenwood, J., Howes, R. & Lightman, S. (1994) *Lab. Invest.* **70**, 39-52.
93. Dynn, M. (1988) in *Cell and Tissue Biology: A Textbook of Histology*, ed. Weiss, L. (Urban & Schwarzenberg, Baltimore), pp. 931-972.
94. Streilein, J. W. (1995) *Science* **270**, 1158-1159.
95. Bellgrau, D., Gold, D., Selawry, H., Moore, J., Franzusoff, A. & Duke, R. C. (1995) *Nature (London)* **377**, 630-632.
96. Griffith, T. S., Brunner, T., Fletcher, S. M., Green, D. R. & Ferguson, T. A. (1995) *Science* **270**, 1189-1192.
97. Ksander, B. R. & Streilein, J. W. (1994) *Chem. Immunol.* **58**, 117-145.
98. Wilbands, G. A. & Streilein, J. W. (1990) *Immunology* **71**, 383-389.
99. Beachey, E. H. (1981) *J. Infect. Dis.* **143**, 325-345.
100. Mantle, M., Basaraba, L., Peacock, S. C. & Gall, D. G. (1989) *Infect. Immun.* **57**, 3292-3299.
101. Hermiston, M. L. & Gordon, J. I. (1995) *Science* **270**, 1203-1207.
102. Keren, D. F. (1992) *Sem. Immunol.* **4**, 217-226.
103. Cornell, R., Walker, W. A. & Isselbacher, K. J. (1971) *Lab. Invest.* **25**, 42-48.
104. Warshaw, A. L., Walker, W. A., Cornell, R. & Isselbacher, K. J. (1971) *Lab. Invest.* **25**, 675-684.
105. Bruce, M. G. & Ferguson, A. (1986) *Immunology* **59**, 295-300.
106. Wilson, A. D., Bailey, M., Williams, N. A. & Stokes, C. R. (1991) *Eur. J. Immunol.* **21**, 2333-2339.
107. Hornquist, E. & Lycke, N. (1993) *Eur. J. Immunol.* **23**, 2136-2143.
108. Xu-Amano, J., Kiyono, H., Jackson, R. J., Staats, H. F., Fujihashi, K., Burrows, P. D., Elson, C. O., Pillai, S. & McGhee, J. R. (1993) *J. Exp. Med.* **178**, 1309-1320.
109. Chen, Y., Kuchroo, V. K., Inobe, J., Hafler, D. A. & Weiner, H. (1994) *Science* **265**, 1237-1240.
110. Powrie, F. (1995) *Immunity* **3**, 171-174.
111. Shull, M. M., Ormsby, I., Kier, A. B., Pawlowski, S., Diebold, R. J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., Annunziata, N. & Doetschman, T. (1992) *Nature (London)* **359**, 693-699.
112. Kulkarni, A. B., Huh, C.-G., Becher, D., Geiser, A., Lyght, M., Flanders, K. C., Roberts, A. B., Sporn, M. B., Ward, J. M. & Karlsson, S. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 770-774.
113. Kuhn, R., Lohler, J., Rennick, D., Rajewski, K. & Muller, W. (1993) *Cell* **75**, 263-274.