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Review

Balancing immunity and tolerance: Deleting and tuning lymphocyte repertoires

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ABSTRACT **Immunological self-tolerance is ensured by eliminating or inhibiting self-reactive lymphocyte clones, creating physical or functional holes in the B- and T-lymphocyte antigen receptor repertoires. The nature and size of these gaps in our immune defenses must be balanced against the necessity of mounting rapid immune responses to an everchanging array of foreign pathogens. To achieve this balance, only a fraction of particularly hazardous self-reactive clones appears to be physically eliminated from the repertoire in a manner that fully prevents their recruitment into an antimicrobial immune response. Many self-reactive cells are retained with a variety of conditional and potentially flexible restraints: (*i*) their ability to be triggered by antigen is diminished by mechanisms that tune down signaling by their antigen receptors, (*ii*) their ability to carry out inflammatory effector functions can be inhibited, and (*iii*) their capacity to migrate and persist is constrained. This balance between tolerance and immunity can be shifted, altering susceptibility to autoimmune disease and to infection by genetic or environmental differences either in the way antigens are presented, in the tuning molecules that adjust triggering set points for lymphocyte responses to antigen, or in the effector molecules that eliminate, retain, or expand particular clones.**

Understanding autoimmune disease depends on achieving a clear picture of how the immune system's primary function, defense against infection and parasitism, is balanced against the secondary goal of minimizing immune-mediated damage to self. Precisely how immunity and tolerance are balanced has yet to be resolved in molecular terms, but many old and new studies are yielding a clear cellular framework (Fig. 1). In essence, immunity and tolerance result from clone survival, activation, and expansion on the one hand, and from clone inhibition and elimination on the other. Whether a clone is triggered toward immunity or tolerance depends on the balance between how antigens are presented to the lymphocyte and how triggering of the cell's antigen receptors has been tuned. This balance is normally set to ensure a robust immune response when antigen is presented from a microbial pathogen but not when the antigen mimics or comes from ones own cells. In individuals with inherited susceptibility to autoimmune disease, one or more elements underpinning this balance must be shifted to increase the likelihood that particular self antigens permit or trigger an immune response. This paper reviews the evidence for this general framework with specific examples of elements that shift the balance between immunity and tolerance for both B and T lymphocytes.

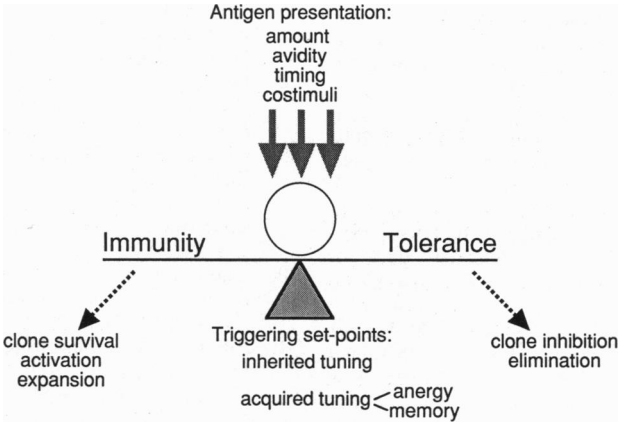

FIG. 1. Elements that balance tolerance and immunity.

Examples of Tolerance by Clone Elimination

Elimination Within Bone Marrow and Thymus. There are now many clear examples of immature self-reactive B and T cells that are physically eliminated within central lymphoid organs. For B cells, this includes self-reactive cells that recognize antigens on cell surfaces (1–3) or that recognize nucleic acids (4). Examples of self-reactive T cells that are eliminated in the thymus include cells that recognize provirus-encoded self superantigens (5–8), major histocompatibility complex (MHC)-encoded alloantigens (9–11), cellular autoantigens (12), and abundant serum components (13, 14). Self-reactive B and T cells that are eliminated while immature appear to do so by apoptosis within the central lymphoid tissues (15, 16), removing essentially any chance that they could participate in an immune response in peripheral lymphoid tissues. In B cells, elimination follows a 1–2 day delay, when the cells are reversibly arrested approximately at the stage when light chain gene rearrangement normally occurs (16). The *RAG* genes remain transcriptionally active in these cells (17), and secondary immunoglobulin gene rearrangements can thus replace self-reactive light chains with other specificities—a process dubbed "receptor editing" (17–19).

Elimination After Arrival in Peripheral Lymphoid Tissues. A considerable proportion of the new B cells produced each day in the bone marrow is exported, and once the peripheral lymphoid tissues have become saturated many of these emigrants die 1–3 days after lodging in the outer T-cell zones of the splenic white pulp cords (20–22). The subset of B cells that survives enters primary follicles, is retained for many cycles of recirculation, and exhibits a skewed repertoire of V_H genes (V_H gene are the coding segments of the variable regions of the immunoglobulin heavy chain) compared with the repertoire of newly formed B cells (20–26). This skewing is likely to reflect negative selection of self-reactive cells (27, 28), although it could also involve positive selection of cells that recognize particular autoantigens, idiotypes, or foreign antigens (20, 24–26).

A clear example of B-cell negative selection at this stage occurs in B cells that

Abbreviations: LPS, lipopolysaccharide; CD40L, CD40 ligand; T_Hn cell, type *n* helper T cell; MHC, major histocompatibility complex; IL-*n*, interleukin *n*; PTP1C, protein tyrosine phosphatase 1C.

A clear example of B-cell negative selection at this stage occurs in B cells that recognize serum lysozyme expressed as a transgene-encoded self antigen (27, 28) or as a tolerizing exogenous antigen (29). The lysozyme-binding B cells are eliminated by apoptosis 1–2 days after migrating to and stopping in the outer T-cell zones adjacent to follicles in spleen and lymph node (27, 29). The process depends on continued binding of autoantigen and on competition with other B cells that are not binding the antigen, suggesting that antigen–receptor signaling in B cells that have bound antigen reduces their attraction to a limited set of follicular niches (27, 28). The stroma of these niches is a network of follicular dendritic cells which may produce chemoattractive and trophic factors for recirculating B cells, much as the bone marrow stroma does for pre-B cells (30) and the thymic epithelium does for T-cell precursors (31). The competitive basis for culling at this stage may explain the retention of a larger fraction of newly formed B cells (20), lack of V-region skewing (25, 32), and greater prevalence of autoantibody-forming cells (33–35) when the peripheral B-cell pool is lymphopenic. A similar process may eliminate B cells that recognize IgG (36), single-stranded DNA (37), T-cell-specific surface antigens (38), or the range of autoantigens bound by the V_H81X element (39–41).

In contrast to B cells, a much smaller fraction of newly formed T cells is exported from the thymus (42–45). Like B cells, however, the peripheral T-cell pool appears to saturate at a tightly regulated size (43), and this may also depend on competition for limiting niches that provide trophic factors. Likely candidates for these niches are the interdigitating dendritic cells, since they are a unique stromal element of the T-cell zones, and genetic defects in dendritic cell maturation prevent proper T-cell zones from forming and reduce recirculating T-cell numbers (46, 47). By analogy with B cells, peripheral T-cell competition might preferentially eliminate self-reactive clones, explaining the increased occurrence of autoimmune diseases in individuals with T-cell lymphopenia (48–51). Several examples of peripheral T-cell deletion could reflect such a process: (*i*) disappearance from a diverse repertoire of $V_\beta6^+$ and $V_\beta14^+$ T cells that recognize superantigens (52, 53); (*ii*) the decline in anergic male-specific T cells that accompanies expansion of other clones following transfer to *nude* male mice (54); and (*iii*) deletion of T cells recognizing a transgene-encoded simian virus 40 T antigen that occurs when competing T cells are present (55).

Elimination After Activation in Peripheral Lymphoid Tissues. B cells that present antigens to $CD4^+$ T cells in peripheral lymphoid tissues can be eliminated at this step by delivery of a death signal from Fas ligand on T cells acting through CD95 (Fas/APO-1) receptors on the B cell (56–60). Naive B cells that suddenly bind antigen become activated, resistant to CD95-induced apoptosis (59), and competent to respond by clonal expansion and antibody production (60, 61). By contrast, self-reactive B cells whose antigen receptors have been desensitized by chronic binding of autoantigen remain sensitive to CD95-induced apoptosis, do not induce cytokine production by T cells, and are not triggered into clonal expansion or antibody production (60–62). CD95–Fas-mediated death can thus abort activation of anergic self-reactive B cells if they present either self antigens or cross-reactive foreign antigens to T cells while trapped in the T-cell zone (28, 30). Strong support for this idea comes from the striking accumulation of autoantibody-producing plasmablasts in the T-cell zones of CD95-deficient *lpr* mice (63).

Peripheral T cells can also be killed following antigen encounter (64–70), at least in part from death signals through CD95–Fas (71–76). T cells that have been exposed to interleukin 2 (IL-2) for a period become sensitive to CD95-induced death (66, 68, 71), while cells that have been acutely costimulated through CD28 or tumor necrosis factor are initially death resistant (77, 78). The relevance of this process may be both to abort activation of anergic self-reactive T cells that are bystanders to antiforeign responses and to terminate excessive clonal expansion (79).

Elimination After Activation and Somatic Hypermutation. Remodeling of B-cell antigen receptors by immunoglobulin gene hypermutation in germinal centers can create strongly self-reactive receptors from innocuous or beneficial precursors (80–84). While germinal center centrocytes selectively survive if they bind foreign antigens immobilized on follicular dendritic cells (85), they are also triggered into apoptosis if they bind free soluble antigen (29, 86, 87). Centrocyte survival vs. apoptosis might result from different intracellular signals elicited by antigen linked to complement C3d components on the surface of follicular dendritic cells compared with antigen-receptor cross-linking by free antigen. Support for this hypothesis comes from the following: (*i*) the failure of germinal center/memory responses when complement is depleted (88, 89), deficient (90), cannot be fixed (91), or when C3d binding to CD21 is blocked (92, 93); (*ii*) the costimulatory effect of co-clustering CD21, the C3d receptor on B cells, with antigen receptors (94, 95); and (*iii*) the failure of germinal center development in the absence of CD19 (96), which mediates synergistic signaling between CD21 and antigen receptors (97).

Examples of Tolerance by Clone Retuning

Anergy in B Cells. Self-tolerance can also be achieved by functionally altering self-reactive cells instead of physically eliminating them. In B cells, a period of exposure to a relatively weak, costimulator-deficient antigenic stimulus, such as soluble anti-immunoglobulin antibody (98), serum lysozyme (99, 100), or single-stranded DNA (37, 101), renders the B cell much more difficult to activate into proliferation and antibody secretion by a subsequent immunogenic antigen challenge. Anergy in B cells reflects retuning of the cells' antigen receptors. Receptors of the IgM class are decreased on the surface of anergic cells by a factor of 20–50 due to a selective block in transport from the endoplasmic reticulum (102). In anergic cells that have managed to enter follicles, IgD receptors are expressed normally (99, 102), but their capacity to trigger tyrosine phosphorylation of the associated CD79 ($Ig\alpha$,$Ig\beta$) chains or $pp72^{syk}$ kinase or elevation of intracellular calcium is greatly diminished (61). As a consequence, antigens or anti-immunoglobulin antibodies are unable to trigger anergic B cells into proliferation by T-cell independent routes (61). Cognate interaction with specific T cells leads to CD95–Fas-mediated death of the anergic cells rather than clonal expansion (28, 60, 61), unless the antigen receptors on anergic cells are suddenly crosslinked much more strongly, for example, by presenting lysozyme antigen as a membrane-bound array (61). Reactivation of anergic B cells by highly aggregated viral antigen has been proposed in mice expressing a transgene-encoded lymphocytic choriomeningitis virus (LCMV) antigen (103). Anergic B cells are thus not inert, but the "gain" in their antigen-receptor signaling apparatus has been tuned down such that a greater immunogenic stimulus is needed to activate them compared with naive B cells. The relevance of tuning down self-reactive B cells' activatibility and allowing them to reach the outer T-cell zones in the periphery, rather than simply eliminating the cells from the repertoire in the bone marrow, most likely stems from the need to balance tolerance and immunity (Fig. 2).

Anergy in T Cells. The strategy of balancing immunity and tolerance by tuning down the activatibility of self-reactive clones also appears common in the T-cell repertoire. Thus, T cells become much more difficult to activate into clonal expansion after they have been presented with superantigens (64, 104–107), alloantigens (10, 108, 109), or peptide antigens (54, 65, 69, 110–114) in a weak, chronic, or costimulator-deficient manner. Like B cells, tuning down T-cell activatibility in many cases involves downregulation of antigen-receptor and co-receptor numbers on the cell surface to varying degrees (10, 65, 109, 115–117). Signals that promote proliferation appear profoundly diminished even in anergic T cells that display normal T-cell receptor (TCR) densi-

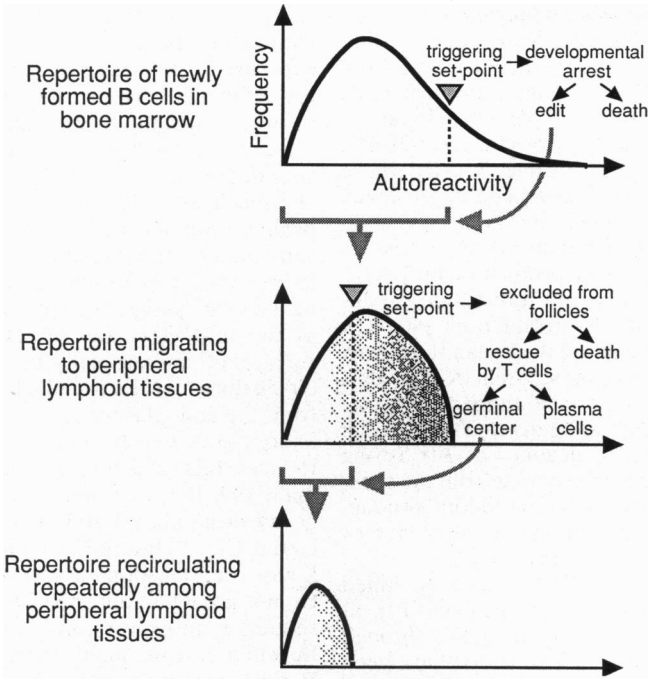

FIG. 2. Self-reactive B cells are eliminated from the repertoire in a series of steps that balances the need for self-tolerance against the need to preserve clones to fight infection. (*Top*) Hypothetical distribution of clones plotted by degree of autoreactivity (on the *x* axis) against clone frequency (*y* axis). Degree of autoreactivity is a function of the amount of autoantigen presented and the avidity with which it is bound by the clone. Most newly formed B-cell clones have low but appreciable self-reactivity, but only a subset appear to exceed an inherited set point and trigger either elimination in the bone marrow or editing to lower self-reactivity. Clones with less autoreactivity are exported to the periphery, illustrated by the hypothetical distribution of clones in *Middle*. By this time, repeated binding of autoantigens has tuned down surface immunoglobulin signaling in the more self-reactive end of the spectrum, illustrated by shading. Binding of autoantigen and competition for follicular niches also trigger exclusion and death of the more self-reactive clones in the T-cell zones, although these cells can potentially be rescued by T cells if they bind foreign antigens with much higher avidity than they bind to self. As a result of follicular competition, together with hypermutation and further selection in germinal centers, the B-cell repertoire that recirculates for weeks or months among lymphoid tissues is skewed toward a small subset of B cells with the least autoreactivity (*Bottom*). A much larger range of newly produced clones is nevertheless available in the T-cell zones of the spleen (*Middle*) to be tested for its fit against microbial antigens and potentially recruited for transient antibody responses or remodeling in germinal centers.

ties, and this tuning down occurs either proximal to the receptor (116, 118–123) or at some more distal step closer to the nucleus (124). Like the situation with B cells, anergic T cells are also not inert but may participate in immune responses under certain circumstances; their activation threshold appears raised (125), but they retain the capacity to produce some cytokines or effector functions (126–130). The chief effect appears to be on clonal expansion after export from the thymus (53, 54, 65, 69), either because the T cells make little IL-2 or because they lose the capacity to proliferate in responses to IL-4 (130, 131).

Examples of Tolerance by Inhibiting Clone Effector Functions

Inhibition of Plasma Cell Differentiation. The reduced potential for triggering clonal expansion by antigen in anergic self-reactive B cells can be circumvented by nonspecific mitogens, such as bacterial lipopolysaccharide (LPS) (132). Under these circumstances differentiation into antibody-secreting plasma cells is inhibited by the continued binding of autoantigen to surface immunoglobulin receptors (132). The same phenomenon has been described in naive B cells exposed to anti-immunoglobulin antibodies and appears to reflect an inhibitory effect of chronic antigen-receptor signaling on the plasma cell differentiation program (133–135).

Inhibition of Inflammatory Cytokine Production. Interferon γ (IFN-γ) produced by type 1 helper T cells (T_H1 cells) plays a dominant role in promoting inflammation by activating macrophages and by promoting B-cell isotype switching to IgG2a (in the mouse), which in turn fosters inflammation by virtue of that isotype's propensity to fix complement and trigger macrophage Fc receptors. By contrast, T_H2 cells producing IL-4 and -10 can inhibit these proinflammatory effectors by inhibiting development of IFN-γ-producing T_H1 cells and by instructing B cells to switch to the noncomplement fixing isotype, IgG1 (in the mouse) (136). Clone inhibition along these lines may be important in curtailing inflammation by self-reactive T cells (50, 137–139).

Effects of Antigen Presentation on the Balance Between Tolerance and Immunity

Triggering of clone elimination and inhibition vs. survival and expansion depends on the way self antigens are presented (Fig. 1). Antigen presentation encompasses, first, the amount and avidity with which antigen is bound, primarily affecting the magnitude of triggering in either direction. Timing of antigen presentation and association with costimuli, by contrast, appear to be the two main cues preferentially guiding responses toward either tolerance or immunity.

Amount of Antigen Presented. The amount of antigen presented to B or T cells has long been recognized as having an important bearing on both tolerance and immunity (140–144). Self or foreign antigens produced or administered in too small amounts either fail to induce tolerance (140–144), promote thymic T-cell survival instead of tolerance (145–148), induce tolerance in T cells but not B cells (149), or tolerize only the higher affinity cells (27, 142, 149). By the same token, too little antigen can fail to trigger an immune response or selectively provide T_H1 responses (150).

Antigens that are made in large amounts are not necessarily presented to B or T cells in large amounts. For B cells, abundant self antigens that are sequestered inside cells, hidden within native protein folds, or limited to extralymphoid tissues may not trigger tolerance. As a result, autoantibodies to these antigens may be more easily triggered by sudden display on dying cells (151) or exposure to immunogenic foreign antigens that happen to crossreact (144, 152). For T cells, abundant antigens within extralymphoid tissues, such as myelin sheaths, thyroid gland, or pancreatic islets, may be presented in only trace amounts to thymic or circulating T cells and thus fail to trigger tolerance or induce only limited retuning (ref. 55 and 153–161; S. Akkaraju, W. Ho, M. M. Davis, K. Canaan, and C.C.G., unpublished data). Similarly, tolerance can fail to be triggered by cryptic self peptides that are processed inefficiently from intact antigens (162, 163) or by self peptides that are inefficiently bound by products of particular MHC alleles (164, 165). Self-reactive clones that escape tolerance in these ways can potentially be triggered to mount an immune response if they are stimulated by a crossreactive foreign antigen (155, 156, 166) or if they migrate into organs where they encounter large amounts of self antigen that is combined with an immunogenic costimulus (167, 168). Inefficient tolerance induction due to this facet of the *Ir* gene effect may explain the impor-

tant role of particular MHC alleles in autoimmune disease susceptibility (165).

Avidity of Antigen Binding. The avidity with which antigen is recognized by lymphocytes, including both the K_{on}/K_{off} at single ligand–receptor sites and the effect of multivalent binding, also plays a decisive role in triggering tolerance and immunity. Self-reactive B cells with high single-site affinity are preferentially tolerized, while clones with low affinity for abundant autoantigens can escape (27, 149), except when those autoantigens are displayed to the B cell in a multivalent array, such as anchored on blood cell surfaces (169). Extensive receptor crosslinking by high avidity multivalent self antigens, such as cell surface molecules, appears necessary to trigger clone elimination in the bone marrow (2, 170). Similarly, developing thymic T cells that recognize peptide–MHC complexes with a low affinity and rapid K_{off} (M. Davis, personal communication) escape tolerance and are stimulated to mature and survive, whereas cells with more stable binding are triggered to die (145–147).

In immunogenic contexts, by contrast, high affinity B cells are preferentially retained and stimulated during T-cell-dependent antibody responses, and binding of high avidity multivalent antigen arrays is necessary to provide T-cell-independent antibody responses (142, 171). Similarly, weak binding antagonizes or anergizes peripheral T cells, whereas stable higher affinity binding triggers activation and clone expansion (172). A key consequence of these effects of avidity is that tolerance is conditional among lower avidity, self-reactive clones, so that they can potentially be stimulated into an immune response if their receptors bind with much higher avidity to antigens that come from infectious microorganisms.

Timing of Antigen Presentation. Timing affects the triggering of tolerance or immunity both in terms of when an antigen is presented to a clone and whether it is presented as an acute burst or as a chronic stimulus. Antigen presented to lymphocytes when they are still immature (typically a self antigen) tends to trigger tolerance by anergy or elimination, whereas antigen presented only after the cells have matured and migrated to the periphery (the usual pattern for antigens of infectious origin) typically provokes activation (6, 8, 173–177). Among mature T and B cells, proliferation is promoted by an acute burst of antigenic stimulation (the typical pattern for antigens of infectious origin), whereas chronic stimulation, as occurs for most self antigens, leads to tuning down of receptors and cell death (28, 53, 64, 65, 67–69, 100, 178).

Costimuli Associated with Presented Antigens. Spatial and temporal association between presented antigens and immunogenic costimuli is an important cue for triggering immunity or tolerance, as conceived for B cells by Bretscher and Cohn (179) and for T cells by Lafferty and Cunningham (180). For both cell types, immunogenic costimuli can come from activation of other nearby lymphocytes, from activation of the innate immune system, or from molecular signatures of microorganisms themselves.

Costimuli that guide antigen-binding B cells towards immunity rather than tolerance include signals from activated T_H cells, such as CD40 ligand (CD40L) and IL-2 and -4. Both CD40L and IL-4 synergize with antigen-receptor crosslinking on B cells to promote clonal expansion and block cell death (181, 182). The importance of CD40L as a proimmunity signal is underscored by the profound humoral immunodeficiency in children and mice with inherited CD40L deficiency (182). Activation of the innate immune system, particularly the complement cascade, also provides important costimulatory signals to B cells (183). Foreign microorganisms often trigger complement via the alternate pathway and become covalently decorated with the complement cleavage product, C3d. This renders these foreign antigens potent immunogens, at least in part through coclustering the C3d receptor on B cells, CD21, with antigen receptors (94, 95). Consistent with an important proimmunity role, antibody responses are diminished when complement is depleted (88, 89), deficient (90), cannot be fixed (91), or when C3d binding to CD21 is blocked (92, 93). Finally, molecules that represent unique signatures of microorganisms, such as the LPS moiety of Gram negative bacteria or double-stranded RNA, represent potent signals for B-cell clonal expansion that act synergistically with antigen-receptor signaling (184, 185).

T-cell clonal expansion is also strongly favored by costimuli from a variety of sources. IL-2 is a potent costimulus from other activated T cells that can substitute for costimuli from the innate immune system in a variety of settings (112, 186). IL-2 provides a possibly important costimulatory signal from particular $\alpha\beta$ or $\gamma\delta$ T-cell subsets that specialize in recognizing molecular signatures of pathogens such as N-formylated peptides (187) or particular metabolites (188, 189). CD80 (B7.1) and CD86 (B7.2/B70) are key costimulators for triggering IL-2 production and T-cell clonal expansion that act by engaging the receptor CD28 on T cells (190). CD86/B7.2 molecules are displayed on the surface of antigen-presenting lymphocytes, such as B cells, when they have been acutely activated by antigen or bacterial LPS (61, 191–193). Cells of the innate immune system, such as macrophages and dendritic cells, display the B7 family of molecules when activated either by a variety of early warning signs of infection, such as tumor necrosis factor, interferons, complement, or by molecular signatures of microorganisms themselves, such as LPS (191, 194, 195).

Coordination of Timing and Costimuli. The display and reception of costimuli are coordinated with the timing of antigen presentation in ways that reinforce their effects in guiding clones toward either immunity or tolerance. In the B-cell lineage, immature B cells do not express the costimulatory CD21 receptor for C3d (16, 196), and antigen stimulation fails to trigger CD86/B7.2 (J. G. Cyster and C.C.G., unpublished observations). Moreover, immature B cells that have bound antigen lose responsiveness to LPS (16, 98, 197, 198). In mature B cells, sudden antigen stimulation provokes display of CD86/B7.2, but after two days CD86/B7.2 expression returns to baseline despite sustained antigenic stimulation, and it is not elevated on B cells that have been chronically stimulated by antigen (28, 61, 192, 193).

Effects of Triggering Set Points on the Balance Between Tolerance and Immunity

The intracellular signaling threshold required to trigger an antigen-binding clone toward immunity or tolerance is as important as the amount, avidity, time, or costimuli of the antigens bound. Different triggering set points can either be inherited as genetic polymorphisms in signal-tuning molecules (Table 1) or be acquired by retuning within individual clones due to previous antigenic encounters that lead to anergy or memory.

Inherited Changes in B-Cell Tuning. Regulating the amount of intracellular second messengers elicited by antigen plays a central role in balancing the fraction of the B-cell repertoire that is eliminated or tuned down against the need to preserve responsive clones for fighting infections. This is most clearly illustrated by inherited deficiencies in two protein tyrosine phosphatases, PTP1C (protein tyrosine phosphatase 1C; also call SHP or HCP) and CD45.

PTP1C is a cytosolic enzyme that can be recruited and activated via two Src homology 2 (SH2) domains (199). In B cells that are exposed to antigen, PTP1C is recruited to at least two cell surface targets: CD22, a lectin that is rapidly tyrosine

Table 1. Receptor tuning molecules in B and T cells

	B cells	T cells
Molecules that tune up	CD21/CD19, CD45	CD4/CD8, CD28, CD45
Molecules that tune down	CD22, FcR2B, PTP1C	CTLA4, CD5, p58/Ly49, PTP1C

phosphorylated after antigen receptor engagement (200); and FcR2B1, a receptor for aggregated IgG that is tyrosine phosphorylated only when coclustered with antigen receptors by the binding of immune complexes (201). B cells that carry a loss-of-function PTP1C-mutation, viable motheaten (202, 203), have an exaggerated intracellular calcium response to free antigen indicating that recruitment of PTP1C to a target such as CD22 plays an essential role in tuning down antigen-receptor signaling (204). Exaggeration of the intracellular response to antigen by PTP1C deficiency causes both high and low avidity self-reactive B cells to be eliminated in the bone marrow (204). The balance between immunity and tolerance is altered by PTP1C deficiency in a complex way, however, because, in addition to immunodeficiency and B lymphopenia, viable motheaten mice also have exaggerated activity of the B1 B-cell subset which secretes IgM autoantibodies (205). These autoantibody-producing B cells may escape elimination and have exaggerated activation because they develop in sites where they are sheltered from the self antigens they recognize (206) or because PTP1C has different effects on signaling in B1 cells.

CD45, by contrast, is a transmembrane protein with two cytosolic tyrosine phosphatase domains that augments antigen–receptor signaling, possibly by removing inhibitory phosphate groups at the C terminus of Src family protein tyrosine kinases (199, 207). B cells that carry a targeted deficiency in CD45 have a depressed intracellular calcium and ERK/MAP kinase response to antigen (Cyster, J. G., Healy, J. I., Kishihara, K., Mak, T. W., Thomas, M. L., and C.C.G., unpublished results). This depressed response is still sufficient to allow high-avidity, self-reactive B cells to be eliminated in the bone marrow, but lower avidity, self-reactive B cells are now positively selected into the recirculating repertoire in preference to B cells that have little or no self-reactivity (Cyster, J. G., Healy, J. I., Kishihara, K., Mak, T. W., Thomas, M. L., and C.C.G., unpublished results). A similar effect may explain the production of autoantibodies in mice lacking the *lyn* tyrosine kinase (209, 210).

The B-cell receptors for C3d (CD21) and the Fc portion of IgG (FcRIIb1) are also key tuning molecules. CD21 enhances the intracellular calcium response to antigen, shifting the balance in favor of clonal expansion and immunity when foreign antigens are presented in a complement-decorated form (94, 95, 211). Coclustering of surface immunoglobulin with CD21 recruits the CD21-associated cell surface molecule CD19 that becomes tyrosine phosphorylated and in turn recruits cytoplasmic signaling molecules, such as vav (97). Consistent with a role in shifting the balance towards immunity, antibody responses to foreign antigens are markedly reduced by genetic deficiency in complement (90), CD19 (96, 212), or vav (213, 214). By contrast, FcRIIb1 diminishes the intracellular calcium response to antigen, shifting the balance against further clonal expansion and immunity when antibody accumulates and causes antigen to be presented in an IgG-decorated form (215–217).When coclustered with antigen receptors on B cells in this way, FcRIIb1 becomes tyrosine phosphorylated and dampens intracellular signaling by recruiting PTP1C (201).

Tuning in T Cells. Similar receptor-tuning strategies appear to operate in T cells. CD45 positively regulates the intracellular calcium response in T cells (199, 207). Relatively few T cells mature in CD45-deficient mice (218), possibly because most low-avidity, MHC-reactive T cells make little signaling response to promote survival and positive selection. The MHC coreceptors CD4 and CD8 also enhance the response to antigen–MHC by recruiting intracellular tyrosine kinases to the antigen–receptor complex (207). Overexpression of CD8 or CD4 skews T-cell selection toward less self-reactive cells (219, 220). Conversely, genetic deficiency of CD4 or CD8 or inability of CD8 to bind to MHC results in fewer T cells maturing and exiting the thymus, possibly skewing the circulating repertoire toward more avid self-reactivity (221–223).

CD28 and CTLA4, two cell surface molecules that bind the B7-family of ligands on antigen-presenting cells, appear to tune up and tune down T-cell activation respectively. CD28 engagement acts synergistically with T-cell receptor (TCR) crosslinking to enhance JNK activation (224) and phosphorylation of IκB (225), augment IL-2 secretion, and promote clonal expansion of mature T cells (190). By contrast, CTLA4 binds B7 ligands with higher affinity (190) and may interfere with CD28 both by outcompeting for binding and by transmitting an active inhibitory signal (226–228). Consistent with these opposing roles, immune responses are diminished as a result of CD28 deficiency (229), whereas CTLA4 deficiency results in exaggerated peripheral T-cell activation and clonal expansion (230, 231).

Other cell surface molecules that function to tune down the response to antigen in T cells are CD5, which is present on most T cells, and the p58 and Ly49 molecules that are present on NK cells and a small subset of T cells. In thymocytes, deficiency of CD5 exaggerates signaling and proliferative responses in TCR crosslinking and causes elimination of self MHC-reactive clones that normally would be efficiently triggered to survive and mature (214). Like CD22 in B cells, the cytoplasmic tail of CD5 becomes tyrosine phosphorylated after the TCR is crosslinked, raising the possibility that it may also recruit PTP1C. A similar mechanism may be employed by p58 and Ly49, which are tyrosine phosphorylated and transmit inhibitory signals to NK cells following binding of self-MHC proteins (232).

Acquired Changes in Tuning. Clones of B and T cells also acquire changes in their set points as a result of previous antigen encounters. Thus, anergy in B and T cells that have been exposed to self antigen in a weak, chronic, and costimulator-deficient form in many cases results from reduced intracellular signaling compared with naive cells (61, 116, 118–123). How this retuning is achieved is not known, although both decreased surface receptor expression and increased activity of intracellular tuning molecules such as PTP1C may contribute. As opposed to anergic cells, memory B and T cells that have previously been activated and expanded by foreign antigen respond more vigorously and with a lower threshold when antigen is presented again (142). Switching membrane immunoglobulin isotype to IgG in memory B cells may enhance signaling, and memory B cells also express higher levels of CD45 and CD21. In T cells, antigen-receptor signaling may be tuned up by changing the splicing of CD45 mRNA to CD45RO or CD45RB$^-$ forms that associate with the TCR or coreceptors more efficiently (233).

Effector Molecules Affecting the Balance Between Immunity and Tolerance

While initiation of clone expansion or elimination depends on the balance between antigen presentation and set-point tuning, distinct molecular pathways are subsequently required to execute these processes. These effector molecules (Table 2), when defective, also shift the balance between tolerance and immunity.

Autoantibody production due to lack of CD95–Fas or Fas ligand (234–236) are clear examples of inherited defects in effector molecules for eliminating self-reactive clones. As discussed above, Fas is required to eliminate self-reactive B cells and possibly self-reactive T cells after activation in peripheral lymphoid tissues

Table 2. Effector molecules for eliminating, retaining, and expanding lymphocyte clones

Molecules that primarily delete	CD95 (Fas/APO1), ICE
Molecules that primarily retain or expand	BTK, ITK, Bcl-2, Bcl-X
Molecules that promote expansion or deletion depending on context	TNF-R, CD40, CD30, IL-2

TNF-R, tumor necrosis factor receptor.

(60, 79). Interestingly, autoantibody production also occurs in children with CD40L deficiency (237), perhaps because CD40L is required to induce CD95/Fas expression on anergic self-reactive B cells and abort their activation (J. C. Rathmell and C.C.G., unpublished data). IL-2 plays a comparable role in rendering T cells sensitive to elimination through CD95–Fas, and defects in this step may explain the paradoxical shift toward excessive T-cell numbers and autoantibody production in IL-2-deficient or IL2-receptor deficient mice (238–240), or in nonobese diabetic (NOD) mice that make an altered form of IL-2 (241).

Bcl-2 and Bcl-x represent a class of effector molecules that shift the balance toward immunity by promoting clone survival. Bcl-2 protein is elevated in the subset of mature B cells selected to recirculate among primary follicles (242, 243), suggesting that bcl-2 may be induced by trophic signals in limiting follicular niches and account for competitive B-cell selection (30). Consistent with this idea, genetic deficiency in bcl-2 results in peripheral B-cell lymphopenia (244, 245). By contrast, constitutive overexpression of bcl-2 increases the number of recirculating mature B cells (246, 247), inhibits elimination of self-reactive B cells at several stages (16, 27, 29, 248), and predisposes to autoantibody production (247). Mutations in the tyrosine kinase btk also cause peripheral B-cell lymphopenia and humoral immunodeficiency (249–253), as well as an inability to survive competitive selection in the periphery (254). Less bcl-2 protein is present in btk-deficient B cells from *xid* mice suggesting that btk may be required for bcl-2 induction when B cells enter follicular niches (30, 255). Inherited btk deficiency shifts the balance away from immunity for both foreign and self antigens, resulting in varying degrees of humoral immunodeficiency (249–253) and suppressing autoantibody production in autoimmune-prone strains, such as NZB/W (208, 256).

Concluding Remarks

I have tried to illustrate how immunity and tolerance are balanced and how that balance can vary from one individual to another. Glimpses into the molecular circuitry underpinning this balance have come from a small but illuminating set of inherited mutations. More extensive genetic analysis in mice and humans will yield a thorough understanding of this molecular circuitry and provide pharmaceutical targets and rational strategies to prevent and cure autoimmune diseases.

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