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Primer: mechanisms of immunologic tolerance

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SUMMARY

Successful adaptive immunity against a broad range of pathogens depends on the diversity of randomly generated T-lymphocyte and B-lymphocyte receptors. A subset of these receptors will be self-reactive and must be regulated to prevent autoimmunity. The process of immunologic tolerance addresses this problem by either purging autoreactive receptors from the system or tuning down their reactivity sufficiently to prevent disease. Immature lymphocytes generate a novel receptor during development in the thymus or bone marrow. Engagement of self antigens by these nascent receptors leads to their purging, either by the apoptotic death of the lymphocyte or by the initiation of receptor editing, a process in which the autoreactive receptor is replaced. If the lymphocytes mature further, the activation threshold of autoreactive cells can be tuned by the co-expression of inhibitory receptors or negative signaling molecules, allowing the persistence of the receptor without an increased risk of autoimmunity. T-cell and B-cell receptors that escape these checkpoints can still be regulated in the peripheral immune system by both purging and tuning mechanisms. A separate set of mechanisms, mediated by various regulatory cells, also operates to tune peripheral receptors in a cell-extrinsic fashion. The combined action of these processes ensures that the organism does not suffer autoimmune pathology, even if autoreactive receptors are generated and maintained in the immune system.

KEYWORDS anergy, autoimmunity, self-reactivity, tolerance, tuning

REVIEW CRITERIA

This review is based on our overview of the immunology literature published in the English language. Because the field under review is broad, we did not perform specific keyword searches to include material in the review. Instead, the authors reviewed numerous publications in the field that were published between 1946 and 2005.

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INTRODUCTION

The adaptive immune system aims to mount highly specific responses against unique molecular determinants on pathologic agents that are encountered during the lifetime of a vertebrate. This is made possible by populations of T and B cells that each express a unique receptor generated by random rearrangements or mutations in the genes encoding them. The randomness of this process is necessitated by the fact that the immune system cannot predict the exact set of pathogens that might affect its host; however, this also results in a quandary—a large majority of receptors generated in this way will actually target determinants derived from the host's own genome. The activation and differentiation of lymphocytes bearing such receptors can result in autoimmune disease and, therefore, must be avoided wherever possible. This constitutes the problem of immunologic tolerance. The immune system's solution to this problem involves two simple strategies: eliminating the autoreactive receptor from the mature cellular repertoire (purging), or compromising the ability of such receptors to mediate disease (tuning). As we discuss below, multiple and redundant mechanisms that aim to purge or tune autoreactive receptor-bearing cells have been described at different stages in the lymphocyte's life cycle (Figure 1).

AUTOREACTIVE RECEPTORS ARE PURGED DURING DEVELOPMENT

The cellular precursor that gives rise to T or B cells is found in the bone marrow and does not express either T-cell receptors (TCRs) or B-cell receptors (BCRs). Differentiation of this precursor into the T-cell lineage follows its migration to the thymus, while development along the B-cell lineage occurs in the bone marrow. The unique specificity of each immature lymphocyte is then generated by the action of recombination-activating genes that randomly rearrange the subunits of a functional BCR or TCR from genomic cassettes in the differentiating lymphocyte. The repertoire

of receptors generated in this way can contain 20–30% autoreactive TCRs[1] or 55–75% self-reactive BCRs.[2] The first phase of immunologic (central) tolerance acts on these immature lymphocytes while they are still in the central lymphoid organs, to purge any newly generated receptor that is capable of strongly engaging antigen in its environment. This purging process has been called 'negative selection' and operates on the assumption that most of the epitopes that the lymphocyte will encounter in the immature state derive from abundant self antigens in the bone marrow or thymus.

T-cell development

In this developmental window T cells are located between the cortex and medulla of the thymus. The dendritic cells at this site, together with stromal cells in the medulla, are probably the major antigen-presenting cells (APCs) that induce negative selection of autoreactive T cells. These APCs are, however, limited in their capability to purge all of the autoreactive TCRs because they only have access to a subset of all self antigens. The failure of the thymus to delete T cells specific for antigens in distant peripheral organs, for example the pancreas, could lead to the costly development of tissue-specific autoimmune disorders later in life. A partial solution to this dilemma is the recent discovery of the 'ectopic' expression of tissue-specific gene products within the thymic medulla.[3] Such an unusual transcriptional profile in the medulla is made possible by the product of genes, such as autoimmune regulator (*AIRE*), which possess domains that can mediate the activity of UBIQUITIN LIGASES and transcription factors. Patients with mutations in this gene develop autoimmune disorders in a variety of peripheral organs, collectively called APECED (autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy) syndrome,[4] presumably because of the inability of the thymus to delete organ-specific autoreactive T cells. Similarly, a type 1 (insulin-dependent) diabetes mellitus susceptibility allele might increase the incidence of the disease by lowering the level of ectopic expression of insulin in thymic medullary epithelial cells.[5]

B-cell development

The purging of autoreactive BCRs in the bone marrow follows similar rules, but does not require a dedicated APC. There is also no evidence yet of genes, such as *AIRE*, that might help turn on tissue-specific genes ectopically in the bone marrow. Furthermore, deletion of immature B cells requires high concentrations of multimeric antigen complexes. Engagement of moderate levels of antigen results in a developmental arrest that does not necessarily lead to cell death. Instead, migration of the arrested cell out of the bone marrow is delayed and the recombination-activating genes are re-expressed in an attempt to generate a different receptor that might not be autoreactive.[6] This form of receptor purging, known as 'receptor editing', can be distinguished from apoptotic purging by the presence of recombination intermediates or excision circles in the surviving B cells (Figure 2). In mouse model systems, up to 50% of the peripheral polyclonal population of receptors can be generated by this process.[7] Editing is also likely to have a role in correcting BCR autoreactivity generated in mature B lymphocytes following somatic hypermutation. On rare occasions, an autoreactive TCR can also be downregulated and replaced with a newly rearranged one.[8]

Limitations of central purging

The processes of negative selection and receptor editing are unlikely to purge all possible autoreactive receptors that are generated during lymphocyte development. Apart from the problems associated with tissue-specific proteins and anticipating genes that will be expressed only later in life, it is also difficult for the immune system to decide on the affinity of a receptor towards self antigens that might allow it to be functionally autoreactive in the mature state (and, therefore, purged in the immature state). This is further confounded by the fact that lymphocytes change their sensitivity to an antigen at various stages during their mature life,[9] raising the possibility that a receptor with too low an affinity to be negatively selected on an immature lymphocyte might still trigger autoimmunity at certain stages of maturity in the periphery. Additional mechanisms are, therefore, necessary for the continued policing of the random repertoire of lymphocyte receptors.

CONTROL OF PERIPHERAL ACTIVATION

During development in the thymus or bone marrow, the antigen receptors are coupled to intracellular signaling cascades that trigger cell death, adaptation, or receptor editing in response to antigen engagement (Figure 2). As the lymphocyte matures, signaling cascades

GLOSSARY

UBIQUITIN LIGASES
Enzymes that link proteins to ubiquitin and thereby target them for degradation; have a role in the development of lymphocyte anergy

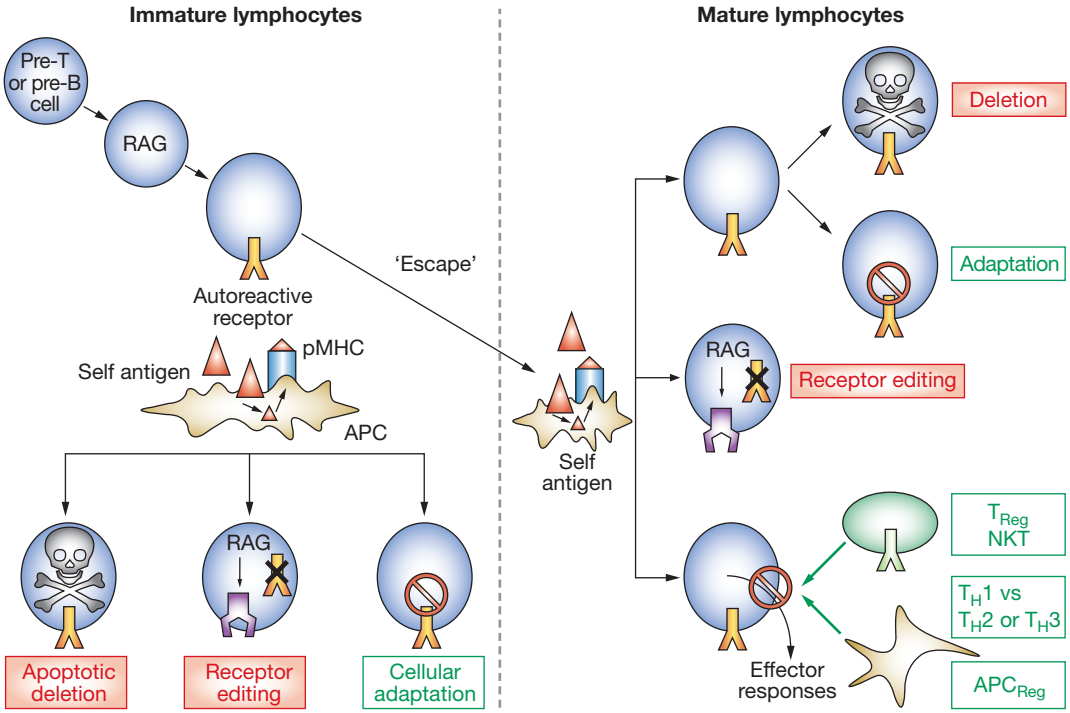

Figure 1 Mechanisms that purge or tune autoreactive receptors in the immune system. Some of the T-cell and B-cell receptors generated by random recombination in developing lymphocytes possess the ability to react to a self antigen. To control the ability of these receptors to cause damage, the immune system purges them (red labels) or tunes them (green labels). Purging in the immature lymphocyte is achieved by inducing cell death (apoptotic deletion) or changing the receptor (receptor editing) as soon as the receptor engages antigen. In the case of T cells, the antigen has to be processed and presented on appropriate MHC molecules, while B cells can recognize intact soluble or membrane-bound antigens. Autoreactive receptors can be alternately tuned by the induction of negative intracellular signaling (adaptation), to prevent the self antigen from transducing positive signals to the lymphocyte. Autoreactive receptors that escape these processes and end up on mature lymphocytes can be further purged or tuned as a result of antigen engagement on resting antigen-presenting cells (for T-cell receptors) or in the absence of T-cell help (for B-cell receptors) or merely as a result of the chronicity of self-antigen stimulation. Finally, the ability of these receptors to induce pathology can be tuned down by the action of specialized cells (regulatory T cells, natural killer cells, regulatory antigen-presenting cells) that can change the kind of effector responses (T helper cell type 1 to type 2 or type 3) delivered by the activated autoreactive-receptor-bearing lymphocytes.
APC, antigen-presenting cell; APC_{Reg}, regulatory antigen-presenting cell; NKT, natural killer T cell; pMHC, peptide–MHC complex; RAG, recombination-activating genes; T_H1, helper T cell type 1; T_H2, helper T cell type 2; T_H3, helper T cell type 3; T_{Reg}, regulatory T cell.

proximal to the receptor are rewired to enable positive signals to be rapidly transduced, which initiate proliferation and differentiation in response to antigen recognition. This change also means that purging of autoreactive receptors in the mature T-cell and B-cell population can no longer be regulated by simply testing for receptor occupancy (as in the bone marrow and thymus). One solution to this problem is to consider the environmental context of antigen recognition while deciding the fate of the lymphocyte following antigen engagement in the periphery.

B-cell activation

The idea that antigen engagement alone is insufficient to fully activate a lymphocyte was first promulgated by Bretscher and Cohn in their two-signal model.[10] Subsequent experiments have demonstrated that the simultaneous recognition of antigen by a B cell and a helper T cell allows the T cell to deliver secondary signals (help) required for the complete realization of the B cell's activation program. Except for a minor subpopulation of T-cell-independent B1 cells, the vast majority of B cells that engage

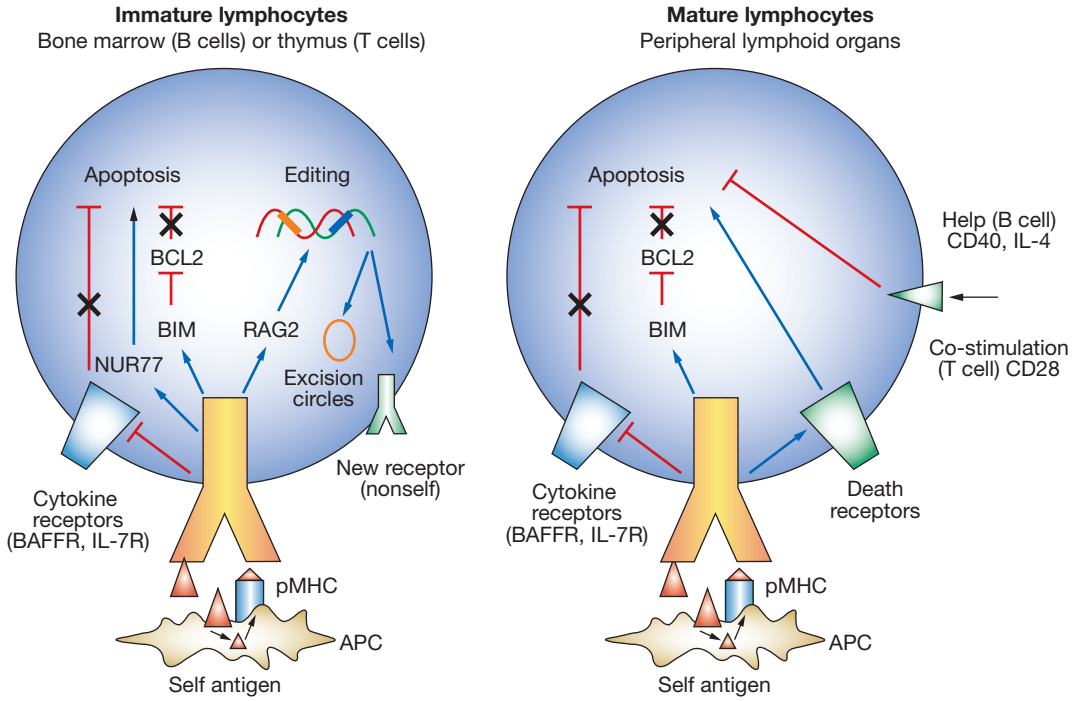

Figure 2 Intracellular cascades involved in purging autoreactive receptors. Although specific molecules might differ between B and T lymphocytes, similar pathways are involved in maintaining tolerance by purging autoreactive receptors in both cell types. Immature lymphocytes that engage self antigens upregulate molecules, such as BCL2-like protein 11, that inhibit the antiapoptotic activity of BCL2 family proteins. In T cells the orphan nuclear receptor 77 is thought to directly initiate cell death. Tolerogenic signaling in B cells also fails to upregulate critical growth-mediating cytokine receptors (e.g. the BAFFR death receptor), and the resulting loss of trophic signaling leads to cell death. Receptor editing involves the upregulation of recombination activating gene proteins, followed by renewed recombination in the genomic locus, generating excision circles. This allows the cell to try and generate a new, nonautoreactive receptor. Similar mechanisms might also operate on B cells in the periphery (not shown). In addition, peripheral lymphocytes could be signaled by the engagement of death receptors, such as FAS (also known as CD95) or the tumor necrosis factor receptor, which directly trigger cell death. The second signal (co-stimulation for T cells and T-cell help for B cells) often provides an antiapoptotic advantage to the lymphocyte either by actively preventing cell death or by increasing metabolism.
APC, antigen-presenting cell; BAFFR, tumor necrosis factor receptor superfamily member 13c; BCL2, B cell leukemia/lymphoma 2; BIM, BCL2-like protein 11; IL, interleukin; IL-7R, interleukin-7 receptor; NUR77, orphan nuclear receptor 77; pMHC, peptide–MHC complex; RAG, recombinase-activating protein.

antigen in the absence of T-cell help display features of abortive activation and undergo apoptosis within 2–3 days, without differentiating to secrete high-affinity antibodies. This allows for productive B-cell activation to be regulated at the level of helper-T-cell activation. Furthermore, this also reduces the probability of self-reactive antibody production, because it would require association in the periphery of two different cell types that have individually escaped negative selection. Aberrations in this checkpoint can result in antibody-mediated autoimmune disease. A case in point is the mouse mutant strain, sanroque, in which deregulated expression of molecules involved in T-cell help results in a lupus-like syndrome.[11]

T-cell activation

The activation of a helper T cell thus becomes a key event in initiating a peripheral adaptive immune response and must, therefore, be tightly regulated. Applying the two-signal philosophy to T-cell activation, Lafferty and Cunningham[12] proposed that naive helper T cells require an antigen-independent co-stimulatory signal to fully differentiate into effector cells. Such a signal

GLOSSARY

B7 FAMILY OF LIGANDS
Molecules expressed by activated antigen-presenting cells that are a primary source of delivering co-stimulation for T cells

can be provided by APCs, which upregulate the B7 FAMILY OF LIGANDS to engage receptors (such as CD28) on the T cell, as they sample the MHC–peptide complexes on the same cell surface. The co-stimulatory molecules themselves are only upregulated if a non-antigen-specific signal from pathogens or tissue damage is detected by the APC[13]. This process was first appreciated by Janeway, who described the ability of microbial 'patterns' to engage the Toll-like receptor family and trigger APC activation.[14] In the absence of such patterns, most self antigens are probably presented on APCs with low levels of co-stimulation, and the T-cell responses elicited are probably not productive. A combination of the two models has been developed to explain the activation of $CD8^+$ T cells, which additionally require interactions with helper T cells specific for the same antigen. These interactions can be orchestrated sequentially between the helper T cell, APC, and $CD8^+$ T cell. Such a triple cellular entente ensures that rare autoreactive $CD8^+$ T cells that have escaped developmental purging are not easily converted to cytolytic cells.

TUNING IS AN ALTERNATIVE TO PURGING

The induction of apoptotic cell death or the initiation of receptor editing in cells that engage a self-reactive receptor effectively purges the receptor from the immune repertoire. Alternatively, the consequences of bearing an autoreactive receptor can be nullified by functionally compromising the ability of the cell to respond, at least to the amounts of the cognate self antigen that are likely to be expressed.[15] We will broadly catalog such mechanisms of self-tolerance as 'tuning'. Tuning can operate by altering the properties of the cell bearing the receptor (cell-intrinsic tuning or adaptation, illustrated in Figure 3) or by exploiting interactions with dedicated cells not necessarily bearing the same receptor (extrinsic tuning).

B-cell anergy

Mature peripheral B cells were first shown to undergo functional inactivation, by an adaptation process called 'clonal anergy', in response to high doses of nonparticulate antigen that was administered intravenously.[16] Subsequently, studies that modeled tolerance in transgenic mice, by mating mice expressing soluble lysozyme to those generating lysozyme-specific BCRs, demonstrated that clonally anergic B cells can survive for extended periods after chronic self-antigen engagement. Such cells, however, displayed a variety of negative adaptations aimed at dampening the signals emanating from the chronic stimulus (Figure 3), including the upregulation of phosphatases, such as src homology 2 domain-containing protein tyrosine phosphatase 1 (SHP1) and src homology 2 domain-containing inositol phosphatase (SHIP), to counteract the positive action of receptor-linked kinases, such as spleen tyrosine kinase (SYK).[17,18] Interestingly, these lymphocytes continued to display basal oscillations of calcium and translocation of the nuclear factor of activated T cells (NFAT) family of transcription factors to the nucleus, which is indicative of an active biochemical process that maintained the cellular adaptation.[19] The anergic B cells were, however, poorer at migrating to the follicular niches required for optimal interaction with helper T cells, as well as differentiating into plasma cells, even in response to a strong mitogenic signal like bacterial endotoxin.

T-cell adaptive tolerance

Similar experiments using TCR-transgenic mice have demonstrated an adaptation process in peripheral T cells, known as 'adaptive tolerance', that follows chronic stimulation by self antigens.[20] The biochemical gridlocks downstream of the TCR in adaptively tolerant T cells include a block in the function of zeta-chain associated protein kinase, ultimately resulting in a poor ability to mobilize intracellular calcium and activate transcription factors, such as NFAT, despite antigen recognition (Figure 3). In addition, higher basal expression of ubiquitin ligases, such as Cbl-b, GRAIL (gene related to anergy in lymphocytes), or itchy homolog E3 ubiquitin protein ligase (itch), might allow for the faster degradation of components of the positive signaling machinery in such cells.[21] The adapted T cells are thus less capable of transcribing most cytokine genes or initiating clonal expansion despite continued engagement of the antigen. Interestingly, removal of the T cell from the chronic stimulus results in the recovery of its responsiveness.[22] This is consistent with a form of cellular adaptation that allows the T cell to dynamically alter its signaling properties to match the levels of ambient antigenic stimulation.[23] It is probable that such mechanisms would still allow an autoreactive receptor to be useful in fighting an infectious agent bearing a similar epitope, albeit only at a higher antigen concentration.

Lymphocyte co-inhibitory receptors

Cell-intrinsic tuning is not achieved solely by the calibration of kinases and phosphatases downstream of the self-reactive receptor. Lymphocytes can also upregulate an ever increasing family of co-inhibitory molecules that serve to raise the threshold for their receptor activation.[24] Many of these receptors, including cytotoxic T-lymphocyte-associated protein 4 (CTLA4), programmed cell death 1 (PD1), the T-cell immunoglobulin mucin (TIM) family in T cells, and CD22 and Fc receptor II in B cells, are upregulated, even by naive lymphocytes after activation. Continued expression of such molecules in response to persisting autoantigens, however, can modulate cellular responsiveness. An exception to this is the membrane phosphatase CD5, which is upregulated during the development of immature T and B cells and might continue to modulate the sensitivity of the antigen receptor in the mature peripheral cell.[25]

The significance of such tuning mechanisms to the regulation of autoimmunity is evident in induced or natural mutation of several key molecules involved in tuning. Mice deficient for the ubiquitin ligase itch develop a high titre of autoantibodies, while rats lacking Cbl-b develop type I diabetes mellitus. Polymorphisms in the *CTLA4* locus have recently been associated with Graves' disease, autoimmune hypothyroidism, and type 1 diabetes mellitus.[26]

SPECIALIZED CELL LINEAGES TUNE THE AUTOREACTIVE REPERTOIRE

The regulation of lymphocyte responses at the level of APC activation allows the immune system to curtail the probability of self-reactive receptor-bearing cells triggering autoimmunity. The nonspecific nature of APC activation itself, however, limits the scope of this mechanism, especially in the context of an ongoing response to an infection. For example, the presence of inflammatory stimuli from the pathogen could result in the upregulation of co-stimulatory molecules on the APC, which could then also present self antigens to any autoreactive lymphocytes that are available to respond. A potential solution to this problem is the existence of lineages of lymphocytes capable of regulating the activation and differentiation of other lymphocytes. The most prominent of these are $CD25^+$ regulatory T cells (T_{Regs}), which develop in the thymus and express the transcription factor forkhead box P3 (Foxp3).[27] Although the mechanisms leading to the generation of Foxp3 are not yet clear, immature T cells are committed to a Foxp3-expressing lineage during thymic development in mouse models.[28] This commitment requires the expression of high-affinity self-reactive receptors and represents an alternative fate to negative selection.[29] T_{Regs} represent 5–7% of the normal repertoire of peripheral $CD4^+$ T cells and have been shown to reduce the frequency of a variety of autoimmune diseases, including diabetes mellitus and rheumatoid arthritis in mouse models. The ability of T_{Regs} to dominantly inhibit the activation of naive T cells in an antigen-specific fashion might allow them to individually regulate the autoreactive receptors that escaped purging in the thymus.[30] This arm of the immune system, therefore, acts like a proofreading process for potential errors during negative selection in the thymus.[31] There are reports that at least some characteristics of T_{Regs} can be acquired by a very small number of naive T cells that are stimulated in specialized cytokine milieu, including transforming growth factor-β. These T cells have been called 'induced T_{Regs}', but their relevance to self-tolerance is not clear. Mutations affecting the human X-chromosome-linked *FOXP3* locus result in the multiorgan autoimmune disorder IMMUNE DYSREGULATION, POLYENDOCRINOPATHY, ENTEROPATHY, AND X-LINKED INHERITANCE.[32]

T_{Regs} are by no means the only subset of T cells capable of tuning the peripheral repertoire. T cells using TCRs of restricted diversity, such as NATURAL KILLER T CELLS and γδ T CELLS have been reported to ameliorate the extent of disease progression in, for example, diabetes mellitus, rheumatoid arthritis, and systemic lupus erythematosus.[33] It is not clear, however, if these cells are part of a process that regulates autoreactivity or only help to control damage during and after an inflammatory response. Subsets of dendritic cells and B cells have also been ascribed regulatory roles in certain models of immune activation.[34,35]

IMMUNOLOGIC TOLERANCE AIMS TO PREVENT AUTOIMMUNE PATHOLOGY

Although the initiation of autoimmunity is mainly caused by activation of cells bearing self-reactive receptors, the resultant pathology is mediated by the subsequent actions of activated lymphocytes. Tolerance processes operate to constrain such activity to levels below pathologic thresholds, but might not always eliminate activity completely.

GLOSSARY

IMMUNE DYSREGULATION, POLYENDOCRINOPATHY, ENTEROPATHY, AND X-LINKED INHERITANCE
A genetic disease caused by mutations in *FOXP3*, resulting in a lack of regulatory T cells and a broad spectrum of autoimmune diseases

NATURAL KILLER T CELLS
A unique lineage of T cells that also have properties of innate natural killer cells

γδ T CELLS
T cells that express γ and δ chains as their T-cell receptor

GLOSSARY

TYPE 1 T HELPER (T_H1) CELL
T-cell phenotype associated with interferon-γ and associated proinflammatory cytokines

TYPE 2 T HELPER (T_H2) CELL
T-cell phenotype associated with interleukin-4, interleukin-5 and interleukin-13 production

TYPE 3 T HELPER (T_H3) CELL
T-cell phenotype eliciting IgA and associated with the production of transforming growth factor-β and interleukin-10

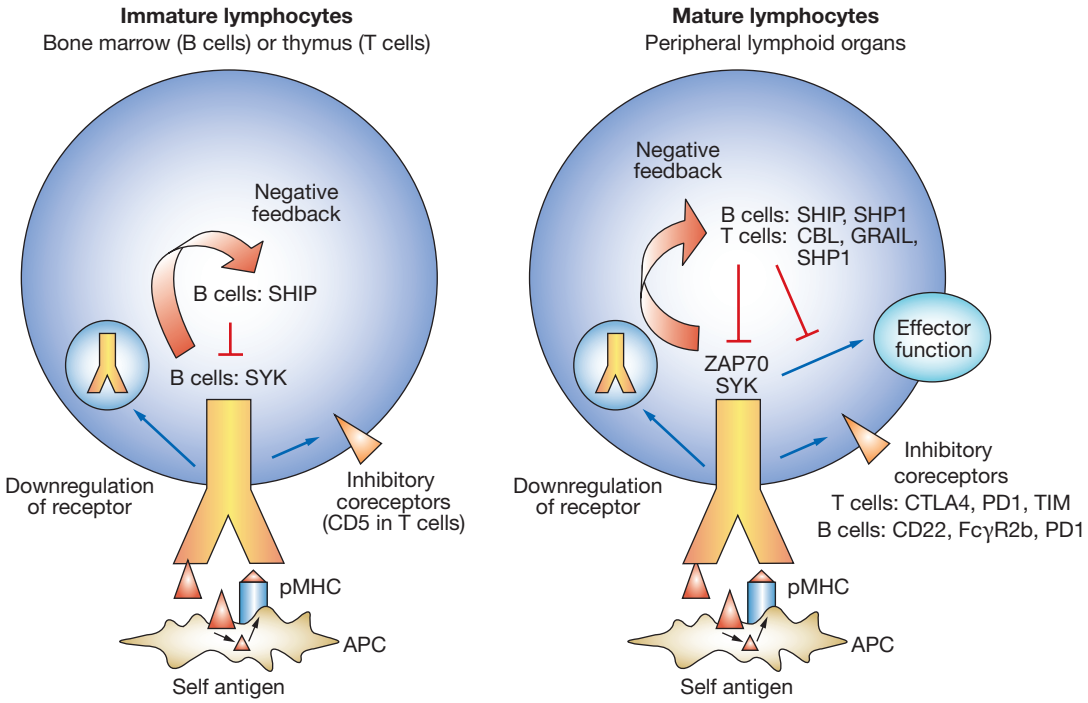

Figure 3 Cell-intrinsic tuning of autoreactive receptors. The ability of a self-reactive receptor to initiate autoimmune pathology can be dampened by cell-intrinsic tuning mechanisms. These include the downregulation of the receptor itself (thereby requiring higher levels of antigen to activate the cell) or recruiting signaling molecules that raise the threshold for the transduction of positive signals downstream of the receptor. Phosphatases, such as SHP1 or SHIP, can dephosphorylate the products of positive enzymes, such as ZAP70 or SYK, while molecules like CBL or GRAIL can ubiquitinylate some components essential for positive signaling and target them for degradation. Alternatively, the sustained upregulation of inhibitory receptors, such as CTLA4, PD1, CD22, which can also be coupled to intracellular negative-signaling molecules, increase the threshold for activation of the lymphocyte.
APC, antigen-presenting cell; CTLA4, cytotoxic T-lymphocyte-associated protein 4; FcγR2b, Fc receptor γ2b; GRAIL; gene related to anergy in lymphocytes; PD1, programmed cell death 1; pMHC, peptide–MHC complex; SHIP, Src homology 2 domain-containing inositol phosphatase; SHP1, src homology 2 domain-containing protein tyrosine phosphatase 1; SYK, spleen tyrosine kinase; TIM, T-cell immunoglobulin mucin; ZAP70, zeta-chain (T-cell receptor)-associated protein kinase.

Pathology can also result from aberrant amplification of such autoreactivity. The enhancement of anti-DNA antibodies by the engagement of Toll-like receptor 9 on autoreactive B cells illustrates this phenomenon.[36]

Similarly, inflammatory pathology associated with diseases, such as multiple sclerosis, diabetes mellitus, and rheumatoid arthritis, seem to require the differentiation of T cells towards a TYPE 1 T HELPER (T_H1) CELL phenotype. Some forms of arthritis, systemic lupus erythematosus, or nephritis are, in turn, exacerbated by antibodies elaborated by the TYPE 2 T HELPER (T_H2) CELL phenotype. In each context, the immune system could potentially ameliorate the concurrent pathology merely by changing the effector class of the lymphocyte response. The control of T-cell class can, therefore, also be included as a peripheral tolerance mechanism (of a tunable nature) when considering the systemic outcome (less pathology) but not the cellular outcome (activation, proliferation, and persistence of the lymphocyte) after self-antigen encounter. This has, indeed, been the rationale behind therapeutic oral or nasal administration of autoantigens to deviate a damaging helper T cell type 1 response towards a type 2 or TYPE 3 T HELPER (T_H3) CELL response.[37]

CONCLUSION

The critical requirement for balancing immunologic tolerance towards self antigens with a lymphocyte repertoire that is as diverse as possible forces the immune system to exist on the

perpetual knife edge of autoimmunity.[38] Rather than merely invest in any one mechanism that can prevent this balance from tipping towards disease, the immune system seems to have evolved multiple, overlapping, and potentially redundant approaches to maintain tolerance. It is, therefore, not surprising that depleting any one mechanism does not automatically result in autoimmunity.[39] It follows from such reasoning that most theoretical models that have been postulated to explain the induction and maintenance of tolerance might overlap with the reality of the phenomenon without exclusively defining it.[40]

KEY POINTS

- Immunologic tolerance operates to prevent randomly generated self-reactive T-cell and B-cell receptors from triggering autoimmunity
- Purging and tuning of the autoreactive receptors are the two strategies used to maintain immunologic tolerance
- Purging is accomplished by the induction of apoptosis in the lymphocyte expressing the autoreactive receptor or by the process of receptor editing, in which the autoreactive receptor is replaced with a newly rearranged one
- Tuning can be cell-intrinsic (adaptation) or cell-extrinsic (regulatory) and allows the self-reactive receptor to persist in the periphery without initiating autoimmunity
- Adaptation is achieved by expression of inhibitory receptors or negative-regulatory signaling molecules in the lymphocyte, which increases its threshold for activation by the autoreactive receptor
- Cell-extrinsic tuning is mediated by regulatory cells, including $CD4^+$ regulatory T cells, natural killer T cells, and antigen-presenting cells, that might prevent activation of the self-reactive lymphocyte or modulate its differentiation process

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Competing interests
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