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MECHANISMS OF DISEASE

Regulation of Immune Responses by T Cells

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The T-cell branch of the immune system can respond to a virtually infinite variety of antigens, in part because it includes a very large repertoire of T-cell clones, each with a unique receptor for antigen. It is inevitable that this diverse repertoire contains T cells with receptors that can recognize the body’s own antigens — self-reactive T cells — and instigate harmful autoimmunity. For this reason, a means of restraining such T cells is essential. The controls depend on two mechanisms that not only avert autoimmunity but also maintain protective immunity: shaping of the T-cell repertoire in the thymus and regulation of T cells in the periphery.

The proposal that peripheral regulatory mechanisms have a key role in the immune response, advanced more than three decades ago by Richard Gershon, was based on two concepts — homeostasis and the potential for autoimmunity.¹ The immune system is indeed a homeostatic organization that must regulate itself to avert insufficient immunity and suppress excessive responses. Protective immunity has a considerable potential for error because it entails the production of potent proinflammatory molecules and killer cells that can destroy not only invading microorganisms and cancer cells but also normal cells. To ensure effective immunity to foreign antigens but avert pathogenic autoimmunity in the periphery, the immune system must control the magnitude and class of immune responses but also discriminate self from nonself. The control of magnitude and class is accomplished by intrinsic homeostatic mechanisms, whereas self–nonself discrimination is mediated largely by suppressor T cells, a term originally coined by Gershon¹ (Fig. 1).

These two immunoregulatory mechanisms have direct clinical relevance to autoimmune diseases, allograft rejection, responses to pathogens, and antitumor immunity. It is likely that an understanding of the molecular and cellular mechanisms of immune regulation will generate new ways of preventing and treating immune-mediated diseases. How T cells mediate these mechanisms is the topic of this review.

CENTRAL SELECTION

Immature T cells migrate from the bone marrow to the thymus, where they begin to express receptors for antigen. The majority of these receptors have two chains, α and β, and are called α/β receptors. The receptors recognize an immunogenic peptide held in the cleft of a major-histocompatibility-complex (MHC) molecule — the MHC–antigen-peptide complex. In the thymus, epithelial cells and other antigen-presenting cells display a wide variety of complexes composed of self peptides bound to MHC molecules. T cells with receptors that bind these complexes with sufficient strength (i.e., avidity) survive (positive selection), whereas T cells with very-low-avidity interactions with the complexes die. From the outset of the process of creating a repertoire of mature T cells, therefore, positive selection favors anti-self T cells.²-⁷ But a second process, termed negative selection, causes the death of
T cells with receptors having a high avidity for self peptides. Negative selection is the major mechanism of self-tolerance, and autoimmune disease can be the result if it fails.

Only 3 percent of the T-cell precursors that enter the thymus survive positive and negative selection. This purged population, composed of T cells with receptors of low and intermediate avidity to self (for brevity, we subsequently will omit mentioning the receptor when referring to avidity), leaves the thymus and inhabits lymphoid organs. Curiously, the emigrants from the thymus were selected for survival because of their ability to bind to self peptides with low avidity, yet they constitute the population of T cells that deals with foreign antigens. An explanation for this paradox is that a given T-cell receptor can cross-react with multiple peptides; this cross-reactivity maintains the flexibility required by the immune system to adapt to a changing environment. Moreover, because foreign antigens are not normally present in the thymus, T cells with the potential for high-avidity binding to foreign peptides evade negative selection and escape into the periphery (i.e., other lymphoid tissues and organs).

**PERIPHERAL REGULATION**

As a consequence of selection within the thymus, some T cells with intermediate avidity for self antigens enter the periphery, where they have the potential to become pathogenic effector cells. To avoid pathogenic autoimmunity, various peripheral regulatory mechanisms fine-tune the self-reactive T-cell repertoire. These mechanisms suppress the expansion of self-reactive clones with an avidity that is not sufficiently high to eliminate them intrathymically but is high enough to induce pathogenic autoimmunity in the periphery. Thanks to these peripheral regulatory mechanisms, autoimmune disease does not occur despite the presence of numerous self-reactive clones in the mature T-cell population.
**CONTROL OF THE MAGNITUDE AND CLASS OF IMMUNE RESPONSES**

**GENERAL INTRINSIC MECHANISMS**

Intrinsic mechanisms induced when the T-cell receptor engages MHC–antigen-peptide complexes are a major point of control over the magnitude and class of immune responses (Fig. 2). One of these mechanisms entails the avidity and duration of binding of the T-cell receptor with MHC–antigen-peptide complexes — avid binding of relatively long duration favors activation, whereas a weak, brief encounter does not.\(^{19,20}\) Notably, engagement of the receptors can induce not only activation and differentiation of the T cell but also apoptosis.\(^{18}\)

Other receptor–ligand interactions are also pivotal. One of them involves CD40 ligand, a cell-surface molecule that makes an early appearance on activated T cells.\(^{21}\) It is essential for T-cell–induced antibody formation by B cells and for causing antigen-presenting cells (e.g., dendritic cells) to trigger cell-mediated immune responses. The interaction of CD40 ligand with CD40, its receptor on B cells and dendritic cells, causes up-regulation of two surface proteins on these cells, CD80 and CD86.\(^{22,23}\) When CD80 and CD86 interact with CD28 on T cells, the outcome is T-cell activation; by contrast, an interaction with cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) on T cells causes anergy (i.e., a nonspecific state of inactivation) or immune tolerance (i.e., an antigen-specific state of inactivation). Blockade of the CD80–CD28 or CD40 ligand–CD40 pathways induces anergy\(^{24,26}\) or tolerance, whereas blockade of CTLA-4 enhances the immune response.\(^{27}\)

**REGULATORY FUNCTIONS EXERTED BY CONVENTIONAL T CELLS BY MEANS OF INTRINSIC MECHANISMS**

The two main populations of mature T cells are CD4+ (helper) T cells and CD8+ (cytotoxic or killer) T cells. Antigen-induced activation of helper cells causes them to differentiate into subgroups of type 1 helper T (Th1) and type 2 helper T (Th2) cells.\(^{28-30}\) These subgroups produce distinctive cytokines, which constitute another level of control of CD4+ T cells.\(^{31}\) Th1 cells secrete interferon-γ, which induces cellular immune responses and inhibits Th2 cells. Th2 cells secrete interleukin-4 (which participates in activating B cells) and transforming growth factor β (TGF-β) and interleukin-10 (which inhibit Th1 cells).\(^{30,32}\) Other cytokine-secreting subgroups of CD4+ cells, termed “type 1 regulatory T (Tr1)” or “type 3 regulatory T (Tr3) cells,” secrete the immunosuppressive cytokines interleukin-10, TGF-β, or both.\(^{33-35}\) The emergence of Th1, Th2, and other cytokine-secreting T cells after antigen activation has a major role in regulating T-cell functions.\(^{36-38}\) All these cytokine-secreting T cells constitute a set of conventional T cells that exert regulatory functions on immune response to both self and foreign antigens. They are specifically activated by antigen but mediate suppression nonspecifically through their cytokines. Collectively, these cells, by controlling the magnitude and class of immune response, contribute to the nonspecific inhibition of pathogenic autoimmunity.
does not affect high-avidity T cells, which are almost exclusively anti-foreign, and thereby optimizes immune responses to foreign antigens.\(^{17,39}\)

**Subgroups of Suppressor Cells**

There is emerging evidence that distinct subgroups of CD4+, CD8+, and natural killer T cells mediate regulatory mechanisms (Fig. 3). Each of these subgroups uses distinctive receptors and effector mechanisms, and each exerts its influence at different stages of the immune response.\(^{40,41}\) Natural killer T cells and specialized CD4+ regulatory T cells are “natural suppressor cells,” which are present in the immune repertoire before the activation of T cells by antigen, and act primarily during the early phases of innate or primary immune responses or both. These two subgroups probably effect suppression by regulating the magnitude or class of the immune response. In contrast, CD8+ T suppressor cells differentiate into effector cells during the primary immune response and function as suppressor cells during the secondary and memory phases of immunity.\(^{15,17,42,43}\) They are primarily involved in self–nonself discrimination.

**Natural Killer T Cells**

Natural killer T cells are a distinctive population of T cells. They have properties of natural killer cells but express \(\alpha/\beta\) T-cell receptors that consist
of an invariant alpha chain (Va24–JaQ) paired preferentially to various Vβ chains.\textsuperscript{44} These cells specifically recognize glycolipids related to the glycolipid α-galactosylceramide that often occurs in pathogenic microorganisms and tumor cells. The α-galactosylceramide binds to CD1d, an MHC molecule on antigen-presenting cells. The CD1d–glycolipid complex triggers natural killer T cells to lyse targets and secrete cytokines.\textsuperscript{45-47} Mammalian antigens such as isoglobotrihexosylceramide and bacterial glycosphingolipid antigens are structurally related to α-galactosylceramide and can stimulate natural killer T cells.\textsuperscript{40,46,49} Natural killer T cells were originally thought to mediate the innate immune responses that lyse tumor cells and pathogens,\textsuperscript{50} but they also are involved in autoimmune diseases.\textsuperscript{51,52} When stimulated by contact with antigen, natural killer T cells develop heightened killer-cell activity and secrete large amounts of interleukin-4, interferon-γ, TGF-β, and interleukin-10, all known to be involved in the activation of cells that mediate inflammation, innate immunity, and Th2-type immunity.\textsuperscript{45,46,53,54}

Natural killer T cells influence a variety of autoimmune diseases in animal models. Prominent among these are murine models of insulin-
dependent diabetes and multiple sclerosis, which involve primarily Th1 cells. In these diseases the secretion by natural killer T cells of the Th2-favoring cytokines interleukin-4 and interleukin-10 is probably an important inhibitory mechanism. In the nonobese diabetic mouse, injection of cell populations enriched for natural killer T cells prevents type 1 diabetes, whereas depletion of natural killer T cells early in the evolution of diabetes accelerates the onset of diabetes. In murine models of inflammatory bowel disease and multiple sclerosis, depletion of natural killer T cells accelerates the onset of disease, while activation of natural killer T cells by treatment with α-galactosylceramide ameliorates or prevents disease. These effects are abrogated in mice that are deficient in CD1d.

Natural killer T cells have also been implicated in human autoimmune diseases. In monozygotic twins that are discordant for type 1 diabetes, the twin with diabetes has fewer Vα24–JαQ natural killer T cells than the twin without diabetes, suggesting protection against the disease by natural killer T cells. However, studies that compared patients who had diabetes with healthy controls, including discordant twins, found that the numbers of natural killer T cells and the production of interleukin-4 are unaltered during the course of type 1 diabetes. These results do not necessarily refute the hypothesis that natural killer T cells defend against type 1 diabetes — they may indicate that development of the autoimmune disease is controlled by several subgroups of immunoregulatory cells acting in concert.

**SPECIALIZED CD4+ REGULATORY T CELLS**

Specialized CD4+ regulatory T cells, initially characterized by the expression of a CD25 molecule on their surface, were identified in studies of the multiple autoimmune diseases that develop in lymphopenic neonatally thymectomized or genetically athymic mice. In these animals, insulinitis, thyroiditis, gastritis, and a wasting disease develop spontaneously. Adoptive transfer of the mice with CD4+CD25+ T cells inhibits the development of autoimmunity. These experiments indicate that CD4+CD25+ regulatory cells are directly involved in the suppression of autoimmunity in immunodeficient mice.

In young normal animals, CD4+ regulatory T cells emerge from the thymus and populate peripheral lymphoid organs. There they probably persist into adulthood and are responsible, in part, for preventing autoimmunity in immunocompetent adults. However, the idea that these cells are a distinct lineage is complicated by the fact that the CD25 molecule that was originally used to identify them is the α chain of the interleukin-2 receptor, which is expressed on virtually all T cells during antigen-induced activation.

The role of CD4+CD25+ cells in autoimmunity was pursued in transgenic mice that carried a gene for a pathogenic myelin-specific T-cell receptor that is involved in the induction of experimental autoimmune encephalomyelitis, a model of multiple sclerosis. Despite the fact that more than 95 percent of the T cells in these mice expressed the pathogenic receptor, experimental autoimmune encephalomyelitis did not develop. If, however, the transgenic mice were mated with RAG−/− mice (which lack the recombinase enzymes required to form all T-cell receptors except the transgenic receptor), florid experimental autoimmune encephalomyelitis developed in the recombinase-deficient offspring. It seems from this experiment that the very few T lymphocytes with nontransgenic receptors in the transgenic mice could block the induction of experimental autoimmune encephalomyelitis. Subsequently, both CD4+CD25+ and CD4+CD25− cells were found to suppress the initiation of experimental autoimmune encephalomyelitis. All this suggests that CD25 is not the essential feature of CD4+ regulatory cells.

**FOXP3**

Recently, the transcription factor FOXP3, a member of the forkhead family of DNA-binding transcription factors, was found to be highly expressed in CD4+ regulatory T cells. Mutations in the FOXP3 gene cause the fatal autoimmune and inflammatory disorder of scurvy in mice and the clinical and molecular features of the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome in humans. Both mice with scurvy and patients with the IPEX syndrome have defects in T-cell activation and low numbers and impaired suppressor functions of CD4+CD25+ T cells. In mice that overexpress FOXP3, CD4+CD25− and CD4−CD8+ T cells have suppressor activity, indicating that expression of FOXP3 is linked to this function. Moreover, expression of the FOXP3 gene specifies CD4+ function of regulatory T cells, irrespective of CD25 expression.
strong evidence that the presence of FOXP3, and not CD25, defines CD4+ regulatory T cells. That activated CD4+CD25− and CD8+ T cells express FOXP3 suggests that this transcription factor is, indeed, linked specifically to the suppressor function.79-82

Definition of Specialized CD4+ Regulatory T Cells
In light of these findings, earlier studies of CD4+ regulatory T cells that relied on CD25 as the defining marker have to be reexamined or reinterpreted. Other cell-surface activation molecules, including CTLA-4 and GITR (the glucocorticoid-induced tumor necrosis factor receptor family–related gene) may also distinguish CD4+ regulatory T cells.93,70,83-84 Analysis of these markers in relation to FOXP3 expression will refine the identification of the CD4+ regulatory T cells. A precise definition of these cells is important because clinical interventions employing infusion of CD4+ regulatory T cells into patients is now being pursued as immunosuppressive therapy for autoimmune diseases.85-87

Mechanism of Suppression by Specialized CD4+ Regulatory T Cells
Although antigens can specifically activate CD4+ regulatory T cells, it is unclear whether the suppressor function of the cells is antigen-specific. The effector phase of CD4+ regulatory T cell–mediated suppression does not seem to involve the T-cell receptor; antigen presentation by MHC molecules (i.e., MHC restriction), which is necessary for the helper function of CD4+ helper cells, is not essential for the suppressor function of CD4+ regulatory T cells. The suppression depends, at least in part, on cell contact, but the surface molecules involved in the contact are unknown.83 TGF-β and interleukin-10, which participate in the intrinsic mechanisms of suppression, also have been implicated in suppression by CD4+ regulatory T cells. Whether the specialized CD4+ regulatory T cells also function by means of self–nonself discrimination is unclear.

CD8+ SUPPRESSOR T CELLS
Qa1 and Autoimmune Disease
Evidence indicates that CD8+ suppressor T cells effect specific immunosuppression by deleting or suppressing potentially pathogenic self-reactive T-cell clones in the periphery. The existence of this pathway was revealed by the following two findings: CD8+ T cells participate in resistance to the reinduction of experimental autoimmune encephalomyelitis,88,89 and they suppress relapses of the disease.43 The mechanism entails preferential down-regulation of potentially pathogenic T-cell clones that have intermediate avidity for peptides derived from myelin basic protein. Figure 4 shows the regulatory pathways that come into play when T cells become activated during their first encounter with conventional self or foreign antigens. An early step is the differential expression of a unique set of self peptides presented by an MHC class Ib molecule — Qa1 in mice and HLA-E in humans — on some but not all activated T cells.39,90-92 The expression of the Qa1–self-peptide complex on antigen-activated T cells is a function of the intermediate-avidity interaction between T-cell receptors on T cells and MHC–antigen-peptide complexes presented by conventional antigen-presenting cells during the T-cell activation.37 The Qa1–self-peptide complexes, as surrogate structures expressed on target T cells, are recognized by the αβ T-cell receptors on regulatory CD8+ T cells and trigger the CD8+ T cells to differentiate into effector cells. These effector cells in turn down-regulate any activated intermediate-avidity T cells expressing the same Qa1–self-peptide complexes during the secondary immune response.93 Moreover, CD4+ T cells with specificity for myelin basic protein peptides can be used as a vaccine to induce CD8+ T cells that protect against experimental autoimmune encephalomyelitis.94,95 The protection is blocked by antibodies against the Qa1 molecule, and severe symptoms of experimental autoimmune encephalomyelitis develop in molecularly engineered CD8-deficient or Qa1-deficient mice exposed to myelin-associated peptides.42 All these studies in mice suggest the possibility that CD8+ suppressor T cells participate in the remissions of multiple sclerosis.15

Inactivation of T Cells with Intermediate Avidity for Both Self and Foreign Antigens
Qa1-dependent CD8+ T cells were found to inhibit the immune response to a conventional protein antigen, hen-egg lysozyme (HEL), when it behaves as a self antigen in HEL-transgenic mice (i.e., mice that express HEL during thymic development). However, these cells enhance the immune response to HEL when it behaves as a foreign antigen in wild-type mice.17 Thus, these Qa1-dependent CD8+ T cells participate both in
the development of tolerance to self antigen and in the responses of T cells to foreign antigens. The use of a panel of HEL-specific CD4+ T-cell clones, each with a different avidity for HEL, showed that the susceptibility of the activated clones to the down-regulation by CD8+ T cells is determined by the avidity of the interactions, which activates the T-cell clones. In a wide range of doses of antigen used to activate the T-cell clones, Qa1-dependent CD8+ T cells selectively down-regulate the HEL-specific clones of intermediate — but not high or low — avidity, regardless of whether these clones are derived from wild-type or from HEL transgenic mice.

**AN AVIDITY MODEL OF PERIPHERAL T-CELL REGULATION**

**EVADEING AUTOIMMUNITY AND OPTIMIZING THE IMMUNE RESPONSE TO FOREIGN ANTIGENS**

CD8+ T cells selectively suppress T cells that have intermediate affinity for self or foreign antigens;
This has important clinical and biologic implications. Because the repertoires of anti-self and anti-foreign T-cell receptors that naive T cells display differ owing to negative selection in the thymus, the consequences of selective down-regulation of T cells with intermediate avidity must also differ. On the one hand, autoimmunity is averted because potentially pathogenic self-reactive T cells reside in the pool of intermediate-avidity self-reactive T cells. On the other hand, the selective down-regulation of T cells with intermediate avidity for foreign antigens indirectly augments the outgrowth of T cells with high avidity for foreign antigens (i.e., T cells that are essential for protective immunity). Thus, a unified mechanism of suppression ensures self-tolerance and optimizes protective immunity.

The HLA-E System and Evasion of Autoimmunity in Humans

Some investigations have begun to uncover a parallel in humans to the Qa1-dependent regulatory CD8+ pathway. In vitro, human CD8+ T cells can be induced to differentiate into regulatory cells whose function depends on HLA-E, the human homologue of Qa1. Pathogenic self-reactive T cells can serve as a vaccine that induces CD8+ regulatory T cells that have the capacity to protect mice against autoimmune disease. This observation supports the search for ways of inducing or enhancing peripheral immunoregulatory pathways in humans.

It is likely that multiple sclerosis, type 1 diabetes, and rheumatoid arthritis will arise if control of self-reactive T cells is lost. Means of activating the HLA-E–dependent CD8+ T-cell pathway could lead to new treatments of autoimmune diseases. And because the specificity of the regulation by Qa1-dependent and HLA-E–dependent CD8+ T cells is not at the level of antigens that activate the target T cells, this approach permits treatment and prevention of autoimmune disease without knowledge of the provocative antigen.

In addition to diseases that arise as a consequence of a failure of immune suppression, there are clinical situations in which excessive suppression of T cells is a dominant feature. The spread of certain types of cancer may be due to excessive suppression of antitumor immunity. In such instances, blockade of the CD8+ suppressor-mediated pathway, perhaps with antibodies specific for HLA-E–self-peptide complexes, could be useful. Such antibodies might be of value during attempts to induce antitumor T cells by means of vaccination. Because T cells that are reactive with tumors often express intermediate-avidity T-cell receptors, the CD8+ suppressor T-cell pathway may target these antitumor T cells and interfere with successful induction of antitumor immunity. Blockade of this suppressor pathway could resurrect protective antitumor immunity.

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