T-Cell Tolerance: Central and Peripheral

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Somatic recombination of TCR genes in immature thymocytes results in some cells with useful TCR specificities, but also many with useless or potentially self-reactive specificities. Thus thymic selection mechanisms operate to shape the T-cell repertoire. Thymocytes that have a TCR with low affinity for self-peptide–MHC complexes are positively selected to further differentiate and function in adaptive immunity, whereas useless ones die by neglect. Clonal deletion and clonal diversion (Treg differentiation) are the major processes in the thymus that eliminate or control self-reactive T cells. Although these processes are thought to be efficient, they fail to control self-reactivity in all circumstances. Thus, peripheral tolerance processes exist wherein self-reactive T cells become functionally unresponsive (anergy) or are deleted after encountering self-antigens outside of the thymus. Recent advances in mechanistic studies of central and peripheral T-cell tolerance are promoting the development of therapeutic strategies to treat autoimmune disease and cancer and improve transplantation outcome.

T cells recognize pathogen fragments in the context of surface MHC molecules on host cells. As such, they have the potential to do enormous damage to healthy tissue when they are not appropriately directed, that is, when they respond to self-antigens as opposed to foreign antigens. T lymphocyte tolerance is particularly important, because it impacts B-cell tolerance as well, through the requirement of T cell help in antibody responses. Thus, failure of T-cell tolerance can lead to many different autoimmune diseases. The tolerance of T cells begins as soon as a T-cell receptor is formed and expressed on the cell surface of a T-cell progenitor in the thymus. Tolerance mechanisms that operate in the thymus before the maturation and circulation of T cells are referred to as “central tolerance.”

However, not all antigens that T cells need to be tolerant of are expressed in the thymus, and thus central tolerance mechanisms alone are insufficient. Fortunately, additional tolerance mechanisms exist that restrain the numbers and or function of T cells that are reactive to developmental or food antigens, which are not thymically expressed. These mechanisms act on mature circulating T cells and are referred to as “peripheral tolerance.”

CENTRAL TOLERANCE

T lymphocytes arise from circulating bone-marrow-derived progenitors that home to the thymus. After T lineage commitment and expansion, T-cell receptor (TCR) gene rearrangement

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ensues and gives rise to either $\gamma\delta$ or $\alpha\beta$ progenitors at the CD4 and CD8 double-negative (DN) stage. A small number of $\alpha\beta$ committed DN cells give rise to a large number of CD4 and CD8 double-positive (DP) thymocytes, and somatic recombination of TCR genes results in a remarkably broad repertoire of distinct $\alpha\beta$ TCRs with random specificity. The TCR affinity for self-peptide–major histocompatibility complex (MHC) determines a thymocyte's fate from this point forward (Fig. 1). DP thymocytes expressing TCRs that do not bind self-peptide–MHC complexes die by neglect. Those with a low affinity for self-peptide–major histocompatibility complex MHC complexes differentiate to CD4 or CD8 single-positive (SP) thymocytes—so-called positive selection. However, those with high-affinity TCR for self-peptide–MHC complexes represent a potential threat to the health of the animal, and various mechanisms operate to ensure tolerance to self, including clonal deletion, clonal diversion, receptor editing, and anergy.

In the thymus, one of the main mechanisms of T-cell tolerance is “clonal deletion,” although the selection of regulatory T cells (“clonal diversion”) is also important and is of enormous interest (see Benoist 2012). Thymocytes expressing high-affinity TCR for self-peptide–MHC can avoid the deletion or diversion fates via un-

dergoing secondary gene rearrangement at the TCR α loci, thereby changing the specificity of the TCR. This process is known as “receptor editing.” Although examples of receptor editing exist for T cells (Wang et al. 1998; McGargill et al. 2000, 2002; Buch et al. 2002; Santori et al. 2002), it is unclear how prominent this mechanism is (Holman et al. 2003), and it will not be discussed further here. Finally, a state of unresponsiveness can be induced in self-reactive thymocytes, called “anergy.” Anergy is likely a more prominent tolerance mechanism that operates in the periphery and is discussed further in that section. These four processes—clonal deletion, clonal diversion, receptor editing, and anergy—are the major mechanisms that limit the self-reactivity of the T-cell repertoire and are crucial for immune health.

CLONAL DELETION

In this section, we discuss several fundamental questions of clonal deletion, such as: At which stages of development do thymocytes undergo deletion? Where does it occur anatomically? What cell types induce apoptosis? What is the molecular mechanism?

The thymus is composed of two major anatomical areas—an outer region known as the cortex, which contains DN and DP thymocytes,

Figure 1. A model for the relationship between developmental outcome and TCR affinity for self-peptide–MHC. Cells with TCR that have a low affinity for self die by neglect. Those with an intermediate affinity are positively selected. High-affinity self-reactive clones can die via clonal deletion, and the threshold between positive and negative selection is hypothesized to be steep. Regulatory T cells (Treg) (yellow) have more highly self-reactive TCRs than most conventional T cells (purple). Some Tregs may have very highly self-reactive TCRs and are rescued from deletion via cytokine signaling or by virtue of having a second TCR (dotted line).

and an inner region known as the medulla, which contains SP thymocytes (Fig. 2). Positive selection of thymocytes occurs in the cortex. However, whether or not clonal deletion occurs in the cortex has been controversial. For example, superantigen studies have suggested that deletion occurs at the SP stage (Kappler et al. 1987) (in the thymic medulla), whereas examination of TCR transgenics and endogenous self-antigens had suggested that deletion occurs at the transition from DN to DP (Kisielow et al. 1988; Sha et al. 1988) (in the thymic cortex). This apparent discrepancy can be partially rec-

tified by the observation that superantigens are primarily expressed in the medulla, which is the site where SP thymocytes reside. Likewise, tissue-specific antigens are often expressed exclusively in the medulla (see below for further discussion). In contrast, in TCR transgenic models where the TCR is specific for ubiquitous self-antigens, deletion occurs at the DN-to-DP transition. However, in transgenic models, thymocytes express both TCR β and TCR α chains early at the DN stage and undergo negative selection prematurely (Baldwin et al. 2005; Egawa et al. 2008). What is clear is that the nature of TCR

Figure 2. Cell types in central tolerance. (Top) T cells are positively selected in the thymic cortex. Negative selection via clonal deletion can also occur in the cortex, but occurs frequently in the medulla. The thymic medulla is also the site for Treg differentiation. (Bottom) Cortical thymic epithelial cells (cTECs) express several unique genes that relate to proteolysis (cathepsin L, CtSL; thymus-specific serine protease, TSSP; and β -5t proteasome subunit, β 5t), which are essential for positive selection. Tissue-specific antigens (TSAs) can be directly presented by medullary thymic epithelial cells (mTECs) or cross-presented by DC (dotted line with arrow). RANKL, CD40L, and lymphotoxin (LT) expressed by SP thymocytes interact with their receptors on mTEC to promote the development of mTEC.

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and self-antigen expression can dramatically impact the timing of clonal deletion and the molecular mechanism by which apoptosis is induced (McCaughy and Hogquist 2008). It is therefore important to move forward making use of the most physiological tools available. Using a TCR transgenic mouse that recapitulates the appropriate timing of TCR α expression at the DP stage, it was shown that deletion of T cells specific for ubiquitous antigens occurs at the DP-to-SP transition (Baldwin et al. 2005). In terms of physical location, this deletion event was shown to occur in the cortex (McCaughy et al. 2008). Thus, in general, polyclonal thymocytes specific for ubiquitous self-antigens seem to be deleted in the thymic cortex, whereas those restricted to tissue-specific antigens, some superantigens, and circulating antigens all occur in the thymic medulla. The relative proportion of the repertoire specific to each of these types of antigens has not yet been determined.

Multiple aspects of T-cell development in the thymus, including clonal deletion, depend crucially on interactions with other cells in the thymic microenvironment. Although the thymus is composed of predominantly T-cell progenitors, there are small numbers of stromal cells, which include hematopoietic non-T-cell progenitors, such as macrophages and dendritic cells (DCs), and non-hematopoietic cells, such as epithelial cells and fibroblasts. Epithelial cells of the thymic cortex (cTECs) are required for positive selection of thymocytes, whereas medullary thymic epithelial cells (mTECs) and DC are more important for Treg differentiation and clonal deletion (Fig. 2).

THYMIC APCs: mTECs

mTECs play a crucial role in thymic tolerance. They are unique among thymic antigen-presenting cells (APCs) in that they express a large number of tissue-specific self-antigens (TSAs) (Derbinski et al. 2001; Gotter et al. 2004). They also express an interesting nuclear regulatory protein called autoimmune regulator (AIRE). Mutations in the AIRE gene lead to the multi-organ syndrome known as autoimmune polyendocrinopathy candidiasis ectodermal dys-

trophy syndrome (APECED) (Anderson and Su 2011). Like patients with APECED, AIRE-deficient mice also develop spontaneous multi-organ autoimmune disease. In mTECs, the AIRE gene promotes expression of a wide array of TSAs (Anderson et al. 2002; Derbinski et al. 2005). mTECs also express B7 family costimulatory molecules, and perhaps not surprisingly, they can be efficient at inducing clonal deletion of T cells reactive to TSAs (Klein et al. 1998; Liston et al. 2003; Anderson et al. 2005). Although direct presentation of TSAs by mTECs was sufficient to induce clonal deletion of CD8SP thymocytes, it was not for CD4SP thymocytes (Gallegos and Bevan 2004). However, cross-presentation of mTEC-derived TSAs by DC can also occur, and that promotes deletion of both CD4 and CD8 SPs (Gallegos and Bevan 2004; Koble and Kyewski 2009).

AIRE contains a nuclear-localization signal, a proline-rich region, and other domains that are found in other transcription factors. The PHD1 domain of AIRE binds to unmethylated histone H3 at lysine-4 (H3K4me0) (Org et al. 2008). In addition, coimmunoprecipitation experiments showed that AIRE interacts with an unexpectedly large number of binding partners: proteins involved in pre-mRNA processing, transcription, nuclear transport, and chromatin binding and structure (Abramson et al. 2010). AIRE plays a key function in regulating gene expression of TSAs, although how AIRE specifically promotes the presentation of tissue-specific antigens by MHC molecules remains enigmatic.

The role of AIRE in promoting tolerance may not be exclusively due to its ability to promote TSA expression. Even when AIRE deficiency did not affect TSA gene expression, it still affected clonal deletion (Anderson et al. 2005). AIRE-deficient mice have a disorganized medulla (Gillard et al. 2007; Dooley et al. 2008; Yano et al. 2008; Milicevic et al. 2009), with fewer medullary DCs (Lei et al. 2011), altered maturation of thymocytes (Sha et al. 1988), and reduced thymic export in the neonatal period (Laan et al. 2009). Interestingly, AIRE expression that was restricted solely to the neonatal period could prevent autoimmunity in AIRE knockout (KO) mice (Guerau-de-Arellano et al. 2009).

Further work will be necessary to fully comprehend how gene deficiency in AIRE causes autoimmunity in humans.

In the thymus, several members of the TNF receptor superfamily, including the receptor activator of NF- κ B (RANK), CD40, and lymphotoxin- β receptor (LT β R), are prominently expressed on mTECs, whereas their ligands, including RANKL, CD40L, and LT, are expressed on hematopoietic cells (Hikosaka et al. 2008). Recent observations indicate that positively selected thymocytes produce these cytokines, which act on mTECs to regulate their proliferation and differentiation to form the microenvironment of the thymic medulla, which is crucial for the establishment of self-tolerance (Akiyama et al. 2008; Hikosaka et al. 2008; Irla et al. 2008; Nitta et al. 2011). Both CD40L and RANKL activate the classical and alternative NF- κ B signaling pathways. In addition, studies of gene-deficient and mutant mice have clearly suggested that these pathways are involved in the development of mTECs, including: REL-B-deficient mice (Weih et al. 1995); Nik^{aly/aly} mice (Kajjura et al. 2004), which carry the alymphoplasia mutation in NF- κ B-inducing kinase (Nik); tumor-necrosis factor-receptor-associated factor 6 (TRAF6)-deficient mice (Akiyama et al. 2005), and NF- κ B2-deficient mice (Zhu et al. 2006). All of these mutant mouse strains show reduced levels of AIRE, which promotes TSA expression by mTECs. mTECs might also contribute to central tolerance through their regulated expression of costimulatory molecules, such as CD40, CD80 (B7-1), and CD86 (B7-2) (Fig. 2).

THYMIC APCs: DENDRITIC CELLS

Like mTECs, the majority of thymic DCs also reside in the medulla, although there are some DCs in the cortex. Both medullary and cortical DCs are potentially important in central tolerance by inducing the apoptosis of self-reactive thymocytes. Thymic DCs are composed of three major subsets: CD11c⁺B220⁺ plasmacytoid DCs (pDCs), CD11c⁺B220⁻CD8a⁻, signal regulatory protein Sirp α ⁺ conventional DCs (cDCs), and CD11c⁺B220⁻CD8a⁺, Sirp α ⁻ (CD8⁺ DCs). It is thought that most CD8⁺

DCs develop intrathymically from a lymphoid pro-thymocyte precursor, whereas the CD8a⁻ DC population, is thought to be of myeloid origin (Wu and Shortman 2005; Schlenner et al. 2010). Given that the majority of thymic DCs are found in the medulla, it is thought that the anatomical location of clonal deletion is generally in the medulla. However, in one model of clonal deletion to ubiquitous self-antigen, cortical DCs were shown to be important (McCaughy and Hogquist 2008; McCaughy et al. 2008). Interestingly, it has been reported that Sirp α ⁺ cDCs are disseminated in the thymic cortex with some of them localized inside perivascular regions and nearby small vessels in the thymus, and it was suggested that they crucially contribute to central tolerance against blood-borne antigens (Baba et al. 2009; Atibalentja et al. 2011).

A unidirectional transfer of antigens was shown to occur in which thymic DCs specifically acquire self-antigen for cross-presentation from mTECs and not from hematopoietic sources (Gallegos and Bevan 2004; Koble and Kyewski 2009). A question arises as to how such antigens can load into the MHC Class II presentation pathway. A recent study has implicated autophagy as a means of antigen transfer, because mTECs appear to have autophagosomes and blocking autophagy in the thymus is associated with autoimmunity (Nedjic et al. 2008). However, further study is needed to establish the precise roles of distinct thymic DC subsets.

MOLECULAR MECHANISMS OF CLONAL DELETION

Thymocytes that recognize self-peptide-MHC with low affinity induce positive selection, whereas those with high affinity undergo negative selection (Fig. 1). This model, for which there is extensive experimental support, is known as an “affinity model” of selection (Starr et al. 2003). Thus a key question for understanding clonal deletion is how a TCR can discriminate between low- and high-affinity ligands. A simple model would be that low- and high-affinity signals are essentially the same, except that the threshold for positive selection is lower than for negative

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selection. This seems not to be the case, because preventing cell death after high-affinity signaling in the thymus was not sufficient to yield positive selection (Hu et al. 2009; Kovalovsky et al. 2010). Rather, the evidence suggests that low- and high-affinity interactions trigger qualitatively different responses. A “zipper” model was recently proposed, which suggests that high-affinity TCR-peptide–MHC interaction induces a stable “zippering” between the membrane-proximal domain of CD8 β and the connecting peptide motif of the α -chain of the TCR (α -CPM), which then causes signal transduction leading to negative selection. Conversely, a low-affinity peptide–HC ligand that interacts with a TCR-coreceptor induces incomplete zippering and partial CD3 ITAM phosphorylation, consequently triggering positive selection (Palmer and Naehre 2009).

With respect to the downstream signals, the strength and kinetics of both Ca²⁺ and extracellular-signal-regulated kinase (ERK) signaling are important in positive versus negative selection. For example, negative selection induces a rapid and robust ERK activation that is associated with death, whereas positive selection stimulates a lower intensity but sustained ERK activation (McNeil et al. 2005). Further evidence suggests that the cellular location of ERK activation is also different under conditions of positive and negative selection (Daniels et al. 2006). A key protein, called Themis (thymocyte expressed molecule involved in selection), seems to be involved in determining the strength and kinetics of Ca²⁺ influx and phosphorylation of ERK (Fu et al. 2009; Johnson et al. 2009; Kugawa et al. 2009; Lesourne et al. 2009; Patrick et al. 2009). Its deficiency markedly impairs positive selection of thymocytes. Interestingly, thymocytes seem to have a unique biochemical pathway that prevents them from undergoing death in response to low-affinity signals, which involves the protein Schnurri-2 (Staton et al. 2011). More work is needed to understand how these differences in proximal TCR signals lead to unique gene expression patterns and the distinct outcomes of life versus death.

Although important, TCR affinity is clearly not the only parameter that decides the life/

death fate of a thymocyte. cTECs are crucial for positive selection, although the reasons for this remain a mystery (Hogquist and Xing 2010). cTECs do express several unique genes that relate to proteolysis (Ctsl, TSSP, and β 5t) and are essential for positive selection. These genes likely contribute to a unique display of self-peptide ligands by cTECs, compared with other thymic APCs (Nakagawa et al. 1998; Honey et al. 2002; Murata et al. 2007; Gommeaux et al. 2009; Nitta et al. 2009; Viret et al. 2011). The fact that cells that support positive selection display distinct self-ligands compared with those that support negative selection may be a crucial part of the process, or it may simply enhance the numbers of progenitors that survive the gauntlet of thymic selection (Fig. 2).

Two genes that are consistently up-regulated in cells undergoing clonal deletion and can promote apoptosis are Nur77 and bim (Baldwin and Hogquist 2007). Nur77 is an orphan nuclear receptor that was originally suggested to be a key effector molecule in apoptosis (Woronicz et al. 1994). Overexpression was sufficient to induce apoptosis in DP thymocytes, and inhibition blocked clonal deletion in TCR transgenic models (Calnan et al. 1995). Nur77 can act as a transcriptional regulator, but recent work suggests that the influence of Nur77 in deletion may be via its ability to interact with BCL-2 family member proteins in the mitochondria (Thompson and Winoto 2008), and not via inducing pro-apoptotic genes. Although strongly expressed after high-affinity signaling in thymocytes, Nur77 is also expressed at low levels in positively selected T cells, suggesting a complex role in survival.

Bim is a pro-apoptotic member of the BCL-2 family that mediates cell death by an intrinsic apoptotic pathway. Bim-deficient mice show impaired deletion of T cells reactive for superantigen (Bouillet et al. 2002; Villunger et al. 2004), tissue-specific antigen (Moran et al. 2011), and ubiquitous self-antigen (Hu et al. 2009; Kovalovsky et al. 2010). Interestingly, in models of ubiquitous self-antigen, where deletion occurs at the DP $\rightarrow$ SP stage in the thymic cortex, elimination of Bim reduced the apoptosis of self-reactive thymocytes, but did not rescue their differentiation. The cells remained at

the immature $CD4^{lo}CD8^{lo}$ stage of development (Hu et al. 2009; Kovalovsky et al. 2010). These data suggest a mechanistic distinction between positive and negative selection as discussed above. One implication of this fact is that failure of tolerance to ubiquitous antigens does not necessarily present a threat to the health of the animals, because thymocytes that receive high-affinity signals, but do not die, fail to be positively selected and do not become mature autoaggressive T cells. This is not the case for tissue-specific antigens, where deletion occurs at the medullary or SP stage. Thymocytes specific for TSA have already survived the positive selection checkpoint, and when they fail to be deleted (in this case, because of bim deficiency), autoreactive T cells accumulate (Moran et al. 2011). Whether this explains the prevalence of tissue-specific over systemic autoimmune diseases in humans remains to be determined. Although Bim induction is key to clonal deletion, it is not yet clear what TCR signals are required for its expression and why it is uniquely up-regulated after high-affinity TCR signaling. Furthermore, other molecules induced by TCR signaling in thymocytes, such as Nur77, may control the efficacy of BCL-2 family members such as Bim (Thompson and Winoto 2008).

Another class of molecules that contribute to clonal deletion is TCR costimulatory molecules. Experiments that used antibody-mediated costimulation through CD5, CD28, or CD43 in vitro strongly support this idea (Punt et al. 1994; Kishimoto and Sprent 1999). Yet, surprisingly, analysis of individual gene-deficient mice showed no effect or a mild effect on clonal deletion. It is possible that multiple costimulatory molecules act in a redundant fashion to promote apoptosis. More recent data suggest that CTLA-4 signaling in thymocytes may diminish the efficacy of clonal deletion (Buhlmann et al. 2003; Takahashi et al. 2005).

CLONAL DIVERSION

Foxp3-expressing CD4 T cells have been well characterized as regulatory T cells (Tregs). These cells suppress immune responses through nu-

merous mechanisms including the production of anti-inflammatory cytokines, direct cell–cell contact, and by modulating the activation state and function of APCs (Shevach 2009). Mutations in the Forkhead box P3 (FOXP3) gene cause human immunodysregulation, polyendocrinopathy and enteropathy, X-linked syndrome (IPEX). In mouse models, Foxp3 is required for Treg development and maintenance of suppression function. Recognition of self-reactive TCR ligands appears to be a key event to initiate the Treg developmental program. Studies of transgenic mice with TCR specific to foreign antigens show that Treg develop only when the foreign antigen is also expressed in the thymus (Itoh et al. 1999; Jordan et al. 2001). Furthermore, studies using transgenic mice produced with TCR genes cloned from naturally occurring Tregs show that self-reactive TCR specificity is required for the instruction of Treg thymic differentiation in the thymus (Bautista et al. 2009; Leung et al. 2009). Thus, clonal deletion eliminates self-reactive clones from the repertoire, whereas clonal diversion imprints self-reactive clones with suppressive or regulatory function (Fig. 1). Thus, the former is sometimes referred to as “recessive” and the latter as “dominant” tolerance mechanisms. Because both clonal deletion and clonal diversion require TCR interaction with self-MHC ligands in the thymus, an interesting question is what distinguishes these processes?

In theory, distinct APCs might instruct the two processes. Because most Foxp3 expression is localized to the medulla, interest has focused on SP thymocytes and medullary APCs. Antigen presentation on mTECs was sufficient for the generation of antigen-specific Tregs, and antigen presentation on DCs, which could acquire and cross-present mTEC-derived antigen, was dispensable (Aschenbrenner et al. 2007). Other evidence showed that antigen expressed on thymic DCs could elicit Treg differentiation (Proietto et al. 2008; Hanabuchi et al. 2010). Thus, how thymic DCs and mTECs manage to elicit clonal deletion as well as clonal diversion remains to be determined and may reflect a complex interplay between both cell types (Lei et al. 2011).

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**FACTORS IN THE DISTINCTION
BETWEEN CLONAL DIVERSION
AND CLONE DELETION**

TCR engagement is essential for both clonal deletion and Foxp3 induction during thymic differentiation of Tregs. TCR signals of distinct strength and duration have been proposed to explain this paradox, with stronger signals leading to deletion and weaker signals leading to diversion (Fig. 1). Experiments in a recently generated mouse model showed that reduction of MHC class II levels on mTECs through transgenic expression of a CIITA-specific microRNA diminished the efficacy of negative selection and led to the increased emergence of Tregs (Hinterberger et al. 2010). Furthermore, a TCR signal strength reporter mouse showed that cells undergoing clonal deletion showed greater ex-

pression of the fluorescent reporter than Tregs (Moran et al. 2011). However, in addition to TCR signals, CD28 costimulation signals have an essential cell-intrinsic role in inducing the differentiation of Tregs (Tai et al. 2005; Lio et al. 2010), and IL-2 signaling is required for the survival of Tregs (Fig. 3) (Burchill et al. 2008; Lio and Hsieh 2008; Vang et al. 2008). It has long been appreciated that the cytokine TGF- β can convert mature T cells into the so-called adaptive Treg lineage in the periphery, whereas its role in thymic commitment to the Treg lineage is less well studied. However, the combined absence of both TGF- β and IL-2 signaling pathways led to a complete absence of thymic Treg (Liu et al. 2008). Interesting recent work suggests that TGF- β might provide a protective effect to Tregs that are experiencing strong TCR signals from self-MHC, and thus be

Figure 3. Differential TCR signaling in clonal deletion and clonal diversion. TCR engagement of low-affinity self-peptide MHC ligands presented by cTECs transduces a signal that promotes survival and differentiation. TCR engagement with high-affinity self-peptide–MHC results in expression of Nur77 and Bim and apoptotic cell death. However, cytokines (TGF- β and IL-2) can prevent Treg progenitors from undergoing apoptosis and promotes the differentiation of Tregs.

another key signal in the discrimination between clonal deletion and clonal diversion (Ouyang et al. 2010). Clearly developing thymocytes integrate information from multiple inputs when deciding cell fate in the thymus.

PERIPHERAL TOLERANCE

Although central-tolerance mechanisms are efficient, they cannot eliminate all self-reactive lymphocytes, in part because not all self-antigens are expressed at the primary site of lymphocyte development—the thymus. Therefore, peripheral-tolerance mechanisms exist, and these are crucial to control tolerance of lymphocytes that first encounter their cognate self-antigens outside of the thymus—such as in the case of food antigens, developmental antigens, and antigens displayed during chronic infection. Both anergy and deletion of self-reactive T cells can occur in the periphery (Fig. 4).

ANERGY

T cells become activated in the presence of a TCR signal and a costimulatory signal mediated by CD28 ligation, and will then secrete cytokines

such as IL-2. Subsequent signaling through the IL-2R complex can fully activate the PI3K/AKT-mTOR pathway. However, T-cell activation in the absence of a second signal induces a state of long-term hyporesponsiveness in T cells, termed “anergy,” which is characterized by an active repression of TCR signaling and IL-2 expression.

Initial studies using the mTOR inhibitor rapamycin have shown that blocking mTOR activation is sufficient to induce anergy in T cells following full activation with anti-CD3 and anti-CD28. Recent studies suggest that several energy and nutrient-sensing pathways may be involved in the promotion of T-cell anergy through inhibiting mTOR activation (Powell and Delgoffe 2010). These include nutrient deprivation or activation of AMPK, a direct sensor of ATP deprivation and hypoxia, or mTOR-independent pathways, such as the GCN2 amino acid-sensing pathway and adenosine signals through the A2A receptor (A2AR). Tregs have recently been postulated to promote a hypoxic environment via their expression of both the 5'-ectonucleotidase CD73 and the ATPase/ADPase CD39 (Sitkovsky 2009; Chappert and Schwartz 2010). CD39 hydrolyzes ATP to AMP,

Figure 4. Peripheral tolerance. T-cell anergy is induced by inhibiting mTOR pathways or can be induced by tolerogenic DCs. The expression of *Egr2*, *Cblb*, *Ctla4*, *DgkZ*, and *Pdcd1* genes is important in T-cell anergy. Lymph node stromal cells (LNSCs) express tissue-specific antigens (TSAs) and can mediate the deletion of self-reactive naive T cells.

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and CD73 converts AMP into adenosine. Tregs may thus regulate T-cell activation by anergy.

Costimulatory pathways provide second signals that promote T-cell activation as discussed above. However, they can also provide negative second signals that inhibit T-cell responses, mediate T-cell tolerance, and prevent autoimmunity. Key among these is the programmed death 1 (PD-1) receptor and its ligands PD-L1 and PD-L2 (Keir et al. 2008). Ligation of TCR and PD-1 leads to the recruitment of phosphatases SHP-1 and SHP-2, which dephosphorylate proximal signaling molecules and effectively attenuate the activation of the PI3K and Akt pathways. The first evidence that PD-1 plays a critical role in control of self-reactivity came from the phenotype of mice lacking PD-1 (*Pdcd1*^{-/-}), which develop a lupus-like autoimmune disease (Nishimura et al. 1999). Subsequent studies suggested that PD-1 interactions with its ligands PD-L1 and PD-L2 inhibit T-cell effector functions in an antigen-specific manner. This pathway can limit the initial phase of activation, the expansion of self-reactive T cells (Probst et al. 2005), or restrict self-reactive T-cell effector function and target organ injury, possibly via regulating dynamic T-cell motility and the TCR stop signal (Fife and Pauken 2011). PD-1 signaling can also mediate the conversion of naive T cells to Tregs.

The other costimulatory molecule important in anergy is CTLA-4. CTLA-4 is induced late after T-cell activation and binds B7 family costimulatory molecules with high avidity. However, it transduces a negative signal and prevents cell cycle progression. CTLA-4-deficient mice show spontaneous autoimmunity (Tivol et al. 1995; Waterhouse et al. 1995), and *Ctla4*^{-/-} CD4 T cells resist anergy induction (Perez et al. 1997). Like PD-1, part of the role of CTLA-4 in peripheral tolerance relates to its role in regulatory T cells. Clinical approaches that focus on costimulatory molecules are discussed in Bluestone (2012).

Several other genes are uniquely induced in anergic cells (Macian et al. 2002) and are functionally important for the anergic state, including *Cbl-b*, *p27Kip1*, *Dgkz*, *Egr2/3*, *Itch*, *NFAT1*, *Tob1*, and *Grail*. Interestingly, microarray anal-

ysis showed significant similarity in the gene expression profiles of cells undergoing peripheral deletion and anergy induction (Parish et al. 2009).

TOLEROGENIC APCs

Peripheral DCs are inducers of immune responses, but are also crucial regulators of tolerance induction and maintenance. Tolerogenic DCs present antigen to antigen-specific T cells, but fail to deliver adequate costimulatory signals (or deliver net coinhibitory signals) for T-cell activation and proliferation (Gallucci et al. 1999). DC tolerogenicity is not specific to a single DC subset. Evidence suggests that tolerogenic DCs are generated by incomplete maturation. For example, apoptotic cells, unlike necrotic cells, are insufficient to trigger DC maturation (Hawiger et al. 2001) through suppressing the activation of NF- κ B pathways mediated by TLR and cytokine receptor. PD-L1 and PD-L2 can be expressed on tolerogenic DCs, providing a means to control the decision between T-cell activation and tolerance. Tolerogenic DCs can also be induced and maintained by various anti-inflammatory and immunosuppressive agents in vitro and in vivo, including IL-10, TGF β 1, corticosteroids, and rapamycin (Morelli and Thomson 2007). Targeting and manipulating tolerogenic DCs has been shown to suppress experimental autoimmune disease and promote improved outcomes of transplantation; this is discussed in greater detail in Reizis (2012).

Lymph node stromal cells (LNSCs) were once thought to function solely as parenchymal support to lymphocytes. However, new evidence shows that all types of LNSCs—including fibroblastic reticular cells (FRCs), follicular DCs (FDCs), and lymphatic endothelial cells (LECs)—express TSA (Fletcher et al. 2011). Recently, an extrathymic AIRE-expressing stromal cell population (eTAC) in lymph nodes was identified that lacks expression of CD80 and CD86 (Gardner et al. 2008). Interestingly, microarray experiments show that AIRE-dependent TSAs in peripheral eTACs and thymic mTECs are largely distinct. The ability of eTACs (Gardner et al.

2009; Fletcher et al. 2010) and other LNSCs (Cohen et al. 2010) to promote tolerance has been shown primarily in transgenic mouse model systems. Although the mechanisms need to be more fully explored, in these models, LNSCs could directly present antigens to CD8⁺ T cells, which were subsequently deleted.

PERIPHERAL DELETION

Deletion of self-reactive lymphocytes in both the thymus and the periphery is achieved through apoptotic cell death. Both Fas- and Bim-mediated apoptosis pathways appear to be important for self-reactive lymphocytes in the periphery. Although Fas-mediated “death receptor” signaling and “BCL-2-regulated” apoptosis signaling are mechanistically distinct, these pathways are coordinated and cooperate in the killing of T lymphocytes chronically stimulated by self-antigens in vivo.

Fas (CD95) is a death-domain-containing receptor, and can be activated by its corresponding ligand FasL (CD178) or agonistic antibodies. Mice lacking either of these proteins (Fas-deficient *lpr* or *lpr^{cg}* mice or FasL-deficient *gld* mice) develop progressive lymphadenopathy and splenomegaly (Cohen and Eisenberg 1991). T cells express Fas, and the expression of FasL is induced on T cells after activation by antigen and IL-2. Fas was shown to be critical for the deletion of T cells that had been stimulated repeatedly by their cognate or foreign antigen in vivo (Kawabe and Ochi 1991; Strasser and Pellegrini 2004). The activation of Fas triggers formation of an intracellular “death-inducing signaling complex” (DISC); in turn, active Caspase-8 and effector caspases consequently promote apoptosis. This pathway of apoptosis is called activation-induced cell death (AICD). Bim is able to directly activate Bax/Bak to cause permeabilization of the outer mitochondrial membrane and induce apoptosis. In contrast to AICD, the killing of antigen-activated T cells during shutdown of an immune response to an acute infection requires Bim, but not Fas (Hildeman et al. 2002; Pellegrini et al. 2003). Bim-deficient aging mice spontaneously develop immune complex-glomerulonephritis (Bouillet et al. 2002).

CONCLUDING REMARKS

In summary, tremendous progress has been made in understanding the cellular and molecular basis of central and peripheral tolerance. This research has led to an understanding of the pathogenesis of at least two human inherited autoimmune diseases and many promising therapeutic strategies.

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