nature immunology

REVIEW

Mechanisms maintaining peripheral tolerance

Daniel L Mueller

The presentation of self-peptide–MHC complexes in the periphery to potentially autoreactive T cells that have escaped negative selection in the thymus poses an important problem to the immune system. In this review, I discuss data that reveal barriers preventing peripheral T cell recognition of self-peptide–MHC complexes, as well as the physiological mechanisms that ensure the elimination or functional inactivation (anergy) of T cells that do come to recognize self-peptide–MHC and threaten the health of the individual.

In the past, one might have assumed that an apparently tissue-specific autoimmune disease such as type I diabetes mellitus (T1D) was the consequence of a breakdown in peripheral self tolerance, whereas systemic inflammatory diseases such as rheumatoid arthritis resulted from some more global defect in central tolerance. In fact, genome-wide association studies indicate that genetic predisposition to T1D is also associated with predisposition to rheumatoid arthritis[1,2]. Unfortunately, one cannot ascertain the relative importance of peripheral tolerance mechanisms in the avoidance of clinically important autoimmunity without a clearer picture of the self-peptide–MHC (self pMHC) complexes that are recognized by pathogenic T cells in individuals with autoimmune diseases. Regardless, a better understanding of peripheral T cell tolerance in animal models offers not only a mechanistic framework for further investigation of human disease pathogenesis, but also the promise of new therapeutic strategies to promote self tolerance in diseased individuals.

The discovery of the nuclear factor called autoimmune regulator (Aire), which controls ectopic expression of 'tissue-restricted' antigens (TRA; for example, insulin) within medullary thymic epithelial cells (mTECs), indicates that thymic negative selection may play the dominant role in the elimination of T cell precursors bearing T cell antigen receptors (TCRs) that bind strongly to widely expressed or tissue-restricted self pMHC complexes[3,4]. However, regardless of the site(s) of self pMHC presentation, it seems that no single control mechanism acting at one particular point in time is sufficient to facilitate the generation of a peripheral T cell repertoire that shows broad specificity for pathogen-derived antigens while maintaining an indifference to the presence of self pMHC. Therefore, we will concern ourselves here with peripheral tolerance mechanisms that control the intrinsic reactivity of mature T cells to one's own tissues. These mechanisms include the anatomical sequestration of some peripheral self pMHC complexes, the development of T cell functional unresponsiveness, and physical deletion of peripheral T cells. In-depth discussions of the roles of central tolerance and peripheral immunoregulation are offered elsewhere in this issue[5,6].

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Published online 17 December 2009; doi:10.1038/ni.1817

Autoreactive T cells escape negative selection

Evidence suggests that thymic negative selection most effectively deletes those T cell precursors that express TCRs having high avidity for self pMHC complexes expressed on medullary dendritic cells (DCs) and mTECs. This then implies that peripheral immune tolerance mechanisms are most important for controlling mature T cells that bear a TCR of relatively low avidity for self pMHC and that escape to the periphery. To explore this idea, Liu *et al.*[7] created TCR-transgenic mice using genes cloned from an MHC class II–restricted $CD4^+$ T cell that recognizes the central nervous system antigen myelin basic protein (MBP) acetylated peptide Ac1-9. This peptide is considered 'encephalitogenic' on the basis of its capacity to induce experimental autoimmune encephalomyelitis (EAE), a model for human multiple sclerosis, when administered to wild-type mice in the presence of adjuvant. Remarkably, these TCR transgenic mice show efficient thymic development of $CD4^+$ T cells expressing the autoreactive TCR, yet show no evidence of autoimmune disease. Perhaps as expected, immunization with MBP Ac1-9 plus pertussis toxin leads to EAE development.

Several additional observations have been made using these transgenic mice[7]. First, infusion of MBP Ac1-9 (without adjuvant) does not cause negative selection of $CD4^+CD8^+$ thymocytes. Moreover, mature naive TCR-transgenic $CD4^+$ T cells recovered from the spleens of healthy mice have a relatively low avidity for native Ac1-9–I-A^u complexes. In contrast, an MBP Ac1-9 peptide analog that has been mutated to increase its avidity for the transgenic TCR by as much as 1,000-fold effectively and rapidly deletes $CD4^+CD8^+$ thymocytes. Finally, chronic and repeated intraperitoneal infusions of this mutant Ac1-9 in the absence of adjuvant leads to the eventual development of tolerance to MBP Ac1-9 in the mature peripheral $CD4^+$ T compartment. Thus, autoreactive T cells escape negative selection in the thymus when their TCR is of sufficiently

low avidity for self pMHC. For these low-avidity autoreactive T cells, as well as T cells bearing TCRs having high avidity for TRAs that are not expressed in sufficient amounts in mTECs, immune tolerance must rely on peripheral mechanisms.

Note that peripheral tolerance mechanisms can fail, and this may be the genesis of some autoimmune diseases. Goverman *et al.* also made TCR-transgenic mice reactive to MBP, and in conventional rodent housing as many as 50% of these mice spontaneously develop EAE[8,9]. However, fewer than 15% of MBP-specific TCR transgenic mice develop EAE when housed in specific pathogen–free facilities. Taken together, the data suggest that EAE development in unimmunized TCR transgenic mice is not spontaneous, but rather depends on pathogen triggering (as, perhaps multiple sclerosis itself may do).

More recent observations[10] extended these principles to polyclonal $CD8^+$ T cells. Double-transgenic mice expressing a TCRβ gene derived from a chicken ovalbumin (OVA)-specific $CD8^+$ T cell together with a rat insulin promoter–driven, membrane-bound OVA transgene (RIP-mOVA) expressed only in the pancreas and in mTECs contain a relatively high number of low-avidity OVA-MHC class I–specific $CD8^+$ cells in the blood and secondary lymphoid organs. Diabetes does not spontaneously develop, but it can be induced by infection with *Listeria monocytogenes* engineered to express the OVA sequence. Interestingly, $CD8^+$ T cells that infiltrate the pancreatic islets after infection continue to show a relatively low avidity for antigen. Thus, low-avidity autoreactive T cells routinely escape negative selection in the thymus and populate the secondary lymphoid organs. If self pMHC complexes become sufficiently abundant (either high density per individual DC or presentation by multiple DCs), one may expect that low-avidity autoreactive T cells will have the opportunity to respond[11].

Ignorance of self pMHC complexes

One barrier to self pMHC complex recognition is the physical separation of potentially autoreactive T cells from the parenchymal cells that express a TRA. Naive T cells circulate from blood to secondary lymphoid organs, to efferent lymph, and then back again to the blood[12]. Guided by the concentration gradients of CCR7 ligands, and through an interaction mediated by the binding of T cell L-selectin (also called CD62L) to complex carbohydrate epitopes on peripheral lymph node addressins, blood-borne naive $CCR7^+CD62L^+$ T cells recognize, bind and migrate through the venule walls of lymph node post-capillary high endothelium to enter the T cell–rich regions of the cortex, where they scan interdigitating DCs for the presence of pathogen-derived pMHC complex that they bind with high avidity[13–15]. If TCR ligation does not occur, desensitization of CCR7 and recognition of efferent lymph sphingosine 1-phosphate eventually drives naive T cells out of the lymph node and back to the blood. Thus, naive T cells are excluded from nonlymphoid peripheral tissues, in which the likelihood of coming in contact with a tissue-resident cell expressing a high density of TRA is higher. Consistent with this conclusion, mature naive TCR-transgenic $CD8^+$ T cells (P14 cells) bearing a high-avidity TCR specific for lymphocytic choriomeningitis viral (LCMV) glycoprotein bound to MHC class I do not elicit autoimmune destruction of the pancreatic islets in mice made transgenic for RIP-glycoprotein, because these P14 $CD8^+$ T cells remain ignorant of the presence of glycoprotein pMHC class I (pMHCI) complexes within the pancreatic tissue[16].

Unlike naive T cells, antigen-experienced T cells naturally adopt an alternative pattern of circulation that allows steady state trafficking through most tissues of the body, preferential homing to local sites of inflammation, retention at sites of pMHC accumulation, and eventual return to the blood by way of the tissue-draining lymph[12,17,18]. The downregulation of CCR7 and CD62L on these effector-memory T cells limits direct reentry into lymph nodes from blood through high endothelial venules. Nonetheless, the upregulation of P- and E-selectin ligands (for example, P-selectin glycoprotein ligand-1 and cutaneous lymphocyte–associated antigen), as well as integrins (for example, CD11a and integrin $\alpha_4\beta_1$), allows efficient egress from post-capillary venules into the interstitium of parenchymal organs and skin, particularly in the setting of local inflammation or infection[19–21]. Therefore, pMHC recognition leading to effector-memory T cell differentiation greatly increases the risk that a potentially autoreactive T cell will gain access to parenchymal tissues with high TRA expression.

In transgenic mice expressing mOVA driven by a keratin-14 promoter (K14-mOVA), OVA expression on epithelial cells of the skin, thymus, tongue and esophagus does not cause overt autoimmune disease[22]. In fact, adoptive transfer of naive OVA-reactive TCR-transgenic $CD8^+$ and $CD4^+$ T cells (called OT-I and OT-II, respectively) into these animals does not elicit autoimmunity, even though the T cells undergo some proliferation and adopt an antigen-experienced phenotype ($CD62L^{lo}CD44^{hi}$E-$selectin^{hi}$) in the skin-draining lymph nodes[22,23]. Despite this strong evidence for TCR engagement by the naive OVA-reactive T cells, the steady-state presentation of OVA does not cause immunopathology, as there is no tissue inflammation or injury. However, tape stripping of the skin—which physically disrupts the cutaneous epithelial barrier—in K14-mOVA mice causes an OVA-dependent infiltration of the injured skin by OVA-specific T cells[22]. Similarly, LCMV infection of the P14 TCR RIP-glycoprotein double transgenic mice described above causes robust priming and cytotoxic differentiation of glycoprotein-reactive P14 $CD8^+$ T cells, and leads to the onset of T1D[16].

Self pMHC–specific effector-memory T cells are most efficiently retained at sites of high TRA expression only when their TCR continues to be ligated under conditions of tissue injury, infection and/or inflammation. Even 'immune-privileged' organs having only limited expression of endothelial P-selectin, E-selectin and/or VCAM-1 (for example, post-capillary venules of the central nervous system blood-brain barrier) are not resistant to the development of immunopathology once inflammation within the organ itself has begun[24].

Thus, the restricted trafficking patterns of naive T cells unaware of self pMHC, as well as of antigen-experienced T cells that have been activated in the absence of an inflammatory stimulus, preserve the state of ignorance to TRA. One caveat in this clonal ignorance theory is the recent observation that certain $CD45^-$ stromal cell elements within lymph nodes can express Aire and cross-present TRA pMHCI complexes in a non-immunogenic fashion[25]. Consequently, an ignorant autoreactive $CD8^+$ T cell circulating between lymph nodes may have only a limited life span before it encounters its TCR ligand on a stromal cell and is tolerized.

Immunogenic antigen-presenting cells

Before their recruitment to the lymph node, immature DCs reside within parenchymal tissues and evaluate these tissues for infection or injury[13]. Through macropinocytosis, DCs constantly process available antigens through MHC class II (MHCII)-rich late endosomal compartments and are poised to deliver a high density of pMHCII complexes—or pMHCI complexes as a result of cross-presentation[26]—to the surface should activation occur. Immature DCs also express various C-type lectin receptors (for example, mannose receptor and DEC-205) as well as Fcγ and Fcε, and are proficient at receptor-mediated phagocytosis. This constitutive uptake and processing of antigens ensures that apoptotic and necrotic cellular debris as well as pathogen-derived proteins can be evaluated by the naive $CD4^+$ and $CD8^+$ T cell repertoires, should DC maturation be triggered.

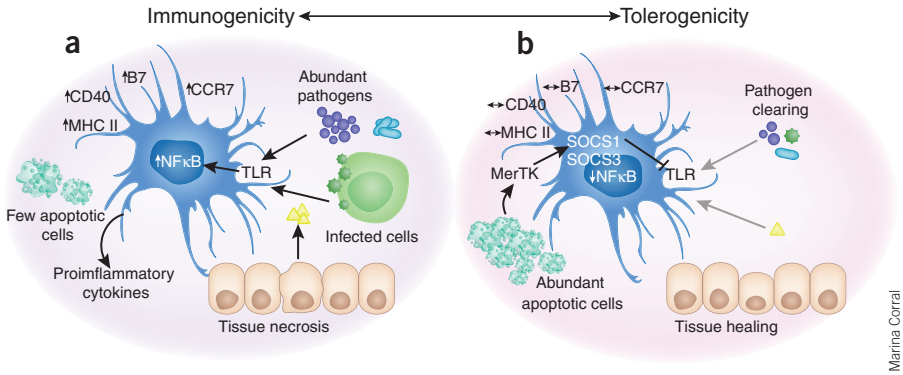


Figure 1 DC maturation model. (**a**) Activation of DCs in the presence of large amounts of pathogens or necrotic cells favors NF-κB-dependent expression of MHCII, CCR7, CD40, B7 and proinflammatory cytokines. (**b**) Activation in the presence of abundant apoptotic cells favors MerTK-dependent expression of SOCS1 and SOCS3 and downregulation of NF-κB-mediated gene expression.

Microbial products such as toll-like receptor (TLR) ligands and mechanical trauma, necrosis and proinflammatory cytokines induce DC maturation[13]. This leads to a downregulation of macropinocytosis and antigen processing, and upregulation of MHCII expression as a consequence of a decreased rate of pMHCII turnover. Thus, pMHC complexes present at the time of maturation are retained within the DC as it migrates to the lymph node. Mature DCs upregulate expression of the B7 costimulatory molecules CD80 and CD86, as well as CCR7 and CD40, migrate into the T cell–rich regions of the lymph node, and provoke T cell activation. Finally, full DC maturation is associated with the synthesis of proinflammatory cytokines, which can amplify the immunogenicity of pMHC complexes as well as regulate the differentiation of the responder T cells (**Fig. 1a**).

Tolerogenic antigen-presenting cells

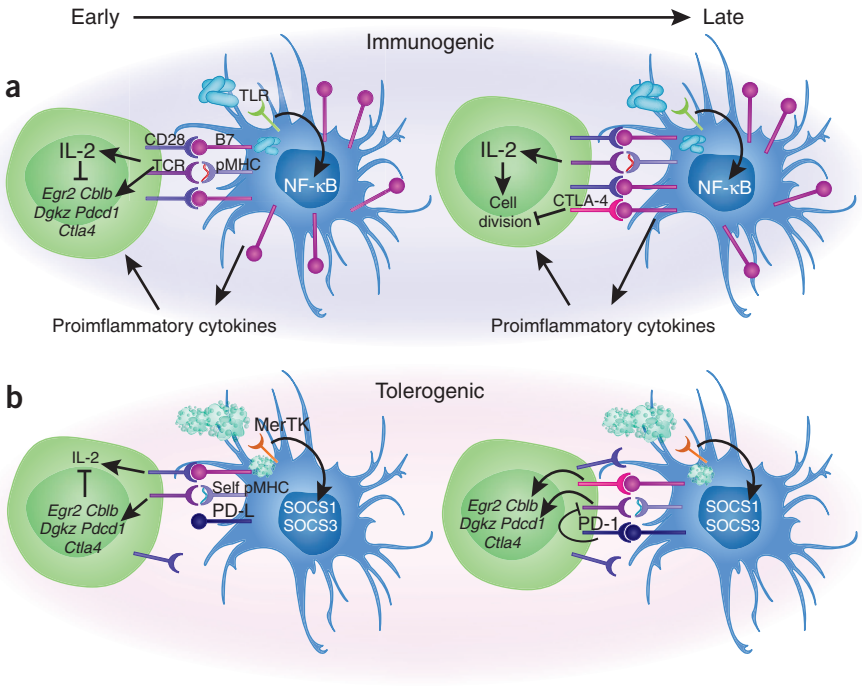
Considerable evidence now suggests that an incomplete form of DC maturation generates a tolerogenic antigen-presenting cell. Hawiger *et al.*[27] directly targeted protein antigen to the endocytic compartment of lymphoid DCs using a DEC-205–specific monoclonal antibody (mAb) conjugated to the experimental antigen hen egg lysozyme (HEL). Administration of anti-DEC-205–HEL led to no detectable change in the expression of MHCII or CD80 on peripheral lymph node–resident DCs. Nevertheless, these incompletely matured lymph node DCs stimulated population expansion of naive HEL-specific 3A9 TCR-transgenic $CD4^+$ T cells. Remarkably, the T cell proliferative response was not sustained, nor was it associated with differentiation into interferon (IFN)-γ producing effector T cells. In fact, most of the responder 3A9 T cells eventually disappeared from the mice, and the remainder were unresponsive to further HEL stimulation. Similar functional unresponsiveness (also known as clonal anergy) was observed in naive antigen-specific $CD8^+$ T cells after encounter with splenic lymphoid DCs that were exposed to dying cells loaded with cognate antigen by osmotic shock[28]. Collectively, these results suggest that, in the absence of inflammation, lymph node and spleen resident DCs induce tolerance in naive T cells that bear a TCR with high avidity for self pMHC complexes presented by the DCs. Furthermore, the data indicate that certain dead or dying cells can reinforce a tolerogenic DC phenotype (**Fig. 1b**).

To establish whether peripheral antigen presentation is critical in the regulation of bona fide autoreactive $CD4^+$ T cells, Laufer *et al.*[29,30] created mice with MHCII expression limited to the thymic cortical epithelium, to allow for positive selection of developing $CD4^+$ T cells in the absence of negative selection. Adoptive transfer of mature $CD4^+$ T cells from these mice into irradiated wild-type syngeneic hosts leads to the rapid demise of the recipient mice as a consequence of graft-versus-host–like disease, proof of self pMHC reactivity within this abnormal peripheral $CD4^+$ T cell repertoire. Nevertheless, transfer of these autoreactive $CD4^+$ T cells into wild-type syngeneic unirradiated mice leads to the generation of anti-nuclear antibodies but no overt clinical disease. Thus, polyclonal $CD4^+$ T cells expected to have high avidity to self pMHC complexes can be controlled by peripheral tolerance mechanisms.

But what should one expect of potentially autoreactive naive T cells that escape normal thymic negative selection simply because they bear TCRs with low avidity for TRAs? One TRA, the gastric pump H^+/K^+-ATPase, physically associates with both gastric tissue-resident and draining lymph node DCs that express increased MHCII but only modest amounts of CD80, CD86, and CD40 (ref. 31). Interestingly, these DCs take up, process and present even more H^+/K^+-ATPase after the induction of autoimmune gastritis. Working under the hypothesis that apoptotic cell uptake and cross-presentation of TRAs by lymphoid DCs promotes peripheral tolerance induction, Luckashenek *et al.*[32] interfered with this putative 'cross-tolerance' pathway. To this end they used transgenic mice expressing a dominant-negative mutant of Rac1 (N17-Rac) in $CD11c^+$ DCs; this construct blocks uptake of apoptotic cellular materials and cross-presentation[33]. Consistent with a defect in self tolerance, a proportion of polyclonal $CD8^+$ T cells isolated from N17-Rac1 mice proliferate after adoptive transfer into syngeneic non-transgenic hosts. Interestingly, these polyclonal $CD8^+$ T cells cause autoimmune disease in *Rag1*$^{-/-}$ but not wild-type recipients, presumably because of their relatively low avidity for endogenous self pMHC class I complexes and their susceptibility to peripheral tolerance mechanisms.

Apoptotic cells, unlike necrotic cells, are insufficient to trigger DC maturation[34]; the uptake of apoptotic cellular material suppresses TLR signaling (**Fig. 1b**). Gas6 and Protein S, two ligands for the Tyro, Axl and MerTK (TAM) family of receptor tyrosine kinases, are ubiquitously present in apoptotic cell membranes. These ligands trigger an association of TAM protein with type I IFN receptors in DCs, which leads to the expression of SOCS1 and SOCS3, two proteins that interfere with TLR and cytokine receptor–mediated activation of NF-κB[35]. Consistent with a role for apoptotic cell uptake and TAM activation in the maintenance of self tolerance, mice lacking TAM receptors develop massive lymphoproliferation and systemic autoimmunity in association with hyperactivation of their DCs[36]. Furthermore, MerTK-deficient mice expressing the BDC2.5 islet cell antigen-specific TCR transgene show earlier-onset T1D than wild-type BDC2.5 TCR-transgenic counterparts[37].

It is conceivable that as an immune response to infection proceeds to completion, diminishing quantities of TLR ligands may remain sufficient to stimulate MHCII and CCR7 upregulation, as well as migration of DCs to the draining lymph node, whereas the increasing uptake of apoptotic debris may simultaneously inhibit MyD88- and NF-κB-dependent proinflammatory cytokine synthesis[38]. The end result may be DCs that mature to a more tolerogenic phenotype. This $CCR7^+MHCII^{hi}$ tolerogenic DC phenotype can also develop in the absence of a pathogen, such as after the physical disruption of E-cadherin-dependent cell adhesion within peripheral tissues[39]. There are likely additional molecular mechanisms by which DC maturation to a $CCR7^+$ $MHCII^+$ tolerogenic phenotype can occur in the absence of infection[40].



Marina Corral

Figure 2 Control of T cell responsiveness by DCs. (**a**) Pathogen-derived pMHC complexes on immunogenic DCs trigger early autocrine growth factor (for example, IL-2) production that prevents the accumulation of anergy factors (for example, those encoded by *Egr2, Cblb, Dgkz, Pdcd1* and *Ctla4*) and promotes late cell cycle progression. CTLA-4 brings about proliferative arrest of T cells at the end of the response. (**b**) Self pMHC complexes on tolerogenic DCs induce the synthesis of anergy factors including CTLA-4 and PD-1. CTLA-4 function is necessary to achieve an anergic state. PD-1 later maintains T cells in an unresponsive state, in part by inhibiting stable interactions with antigen-presenting cells and preventing further TCR engagement.

TCR-specific control mechanisms

TCR engagement by self pMHC complexes on tolerogenic DCs therefore eliminates potentially dangerous responder cells, while sparing other naive T cells with potentially protective TCR specificities. This requirement for self pMHC specificity complicates the investigation of peripheral tolerance mechanisms, as one must be able to distinguish these autoreactive T cells from among the background of normal unaffected and protective naive lymphocytes. This requires that one know the identity of important self pMHC complexes and possess advanced technologies that facilitate the monitoring of cells that bear TCRs specific for that particular self pMHC complex.

One advance in this field was use of tissue-restricted promoters to drive expression of model antigens in peripheral tissues[41]. A second major advance was the adoptive transfer of TCR-transgenic T cells of known antigen-specificity into normal hosts[42]. The combination of these technologies has proven to be a powerful approach for studying peripheral self tolerance. We now understand that influenza hemagglutinin–specific TCR-transgenic 6.5 donor CD4$^+$ T cells develop an antigen-experienced phenotype (CD44^{hi}CD45RB^{lo}), yet do not undergo a sustained clonal population expansion after their adoptive transfer into C3-hemagglutinin recipient mice, in which transgenic hemagglutinin expression is driven by a prostatic steroid-binding protein promoter[43]. Bone marrow chimeras confirm that hematopoietic cells pick up hemagglutinin and present it to T cells in a nonimmunogenic fashion. Notably, 6.5 CD4$^+$ T cells recovered from these mice do not proliferate or produce interleukin 2 (IL-2) in response to hemagglutinin rechallenge. OT-I CD8$^+$ T cells similarly proliferate when transferred into RIP-mOVA hosts as a result of OVA cross-presentation by hematopoietic cells, yet few mice develop T1D and, in most cases, the OT-I T cells are eventually deleted[44,45]. Thus, the recognition of a TRA on tolerogenic mature DCs by autoreactive T cells leads to a functional inactivation and/or peripheral deletion in secondary lymphoid organs that precludes their pathogenic recognition of self pMHC complexes within the peripheral tissues (**Fig. 2**).

However, tolerogenic DCs do not work in isolation to downregulate T cell responsiveness. In lymphopenic *Rag2*$^{-/-}$ OVA-transgenic recipients, DO11.10 CD4$^+$ T cells expand to large numbers, maintain their functional responsiveness, and eventually kill a substantial fraction of the recipient mice as a consequence of immunopathology[46]. DO11.10 T cells subjected to repeated infusions of soluble OVA in athymic nude mice also resist tolerance induction[47]. Notably, prior reconstitution of these nude mice with a CD25$^+$CTLA-4$^+$ Foxp3-expressing CD4$^+$ T cell population controls chronic antigen-induced DO11.10 T cell proliferation and promotes DO11.10 T cell anergy. Polyclonal CD8$^+$ T cells specific for poorly immunogenic tumor antigens also avoid anergy induction in lymphopenic *Rag2*-deficient hosts[48]. Thus, the lymphopenic environment presents a barrier to clonal anergy induction, at least in part as a consequence of the absence of Foxp3$^+$ T regulatory cells. Note, however, that even in the lymphopenic environment, T cells that recognize widely expressed self pMHC complexes may eventually undergo a desensitization of TCR signaling pathways known as adaptive tolerance[49].

A pair of peripheral deletion mechanisms

In the periphery, autoreactive T cells chronically engaged by self pMHC complexes die by apoptosis as a result of a combination of molecular events: Fas receptor engagement by FasL and Bim-dependent triggering of a Bcl-2 and Bcl-xL–regulated mitochondrial death pathway[50]. Great interest in Fas developed when it was discovered that the spontaneous T cell lymphoproliferative disease and autoimmunity observed in *Fas*^{lpr} MRL-strain mice was in part the result of a mutant allele of *Fas* that fails to transmit a death-inducing signal[51]. Cells in mice made deficient for Bim also resist apoptosis, and with age these mice spontaneously develop immune complex–mediated glomerulonephritis[52]. Bim is thought to function as a natural antagonist of the survival protein Bcl-2, and both Bim-deficient and Bcl-2 transgenic OT-I CD8$^+$ T cells fail to undergo peripheral deletion after their adoptive transfer into RIP-OVA mice[53]. Mice expressing both the *Fas*$^{lpr/lpr}$ genotype and a Bcl-2 transgene develop T cells that cannot be deleted during chronic and repeated superantigen stimulation *in vivo*[54]. Bim-deficient *Fas*$^{lpr/lpr}$ mice also show defective peripheral tolerance induction, with massive lymph-node and spleen accumulations of CD44^{hi} central and effector-memory T cells, as well as spontaneous production of autoantibodies and immune complex–mediated glomerulonephritis[55–57]. Thus, for at least some self pMHC complexes, peripheral deletion of autoreactive T cells is essential for maintaining peripheral tolerance.

DO11.10 CD4$^+$ T cells made deficient for Bim undergo a typical proliferative burst when transferred into syngeneic soluble OVA–transgenic

hosts and assayed 4 days later[58]. By 8 days, however, Bim-deficient DO11.10 T cells are retained in significantly higher numbers than wild-type DO11.10 T cells. Despite the obvious differences in survival, both wild-type and Bim-deficient T cells eventually lose their capacity to produce IL-2, consistent with the development of clonal anergy. Although enhanced cell survival in the absence of Bim clearly distinguishes the role of apoptosis from that of clonal inactivation in the development of peripheral tolerance following chronic self pMHC recognition, clonal elimination and clonal anergy are likely to be highly related at the molecular level. OT-I $CD8^+$ T cells undergoing deletional tolerance in RIP-OVA mice show a gene expression profile not unlike that seen in $CD4^+$ T cells undergoing clonal anergy, with notably increased expression of a number of genes thought to be important to the functional inactivation of T cells, including *Egr2*, *Cblb*, *Ctla4*, *Dgkz* and *Pdcd1*[59–62].

Costimulatory ligands control T cell responsiveness

The development of clonal anergy in antigen-experienced T cell lines and clones *in vitro* is clearly antagonized by CD28 signaling, which enhances production of IL-2 and facilitates subsequent IL-2R- and mTOR-dependent anergy reversal[62–66]. Nonetheless, the role of CD28 ligands CD80 and CD86 in the regulation of peripheral tolerance is complex. Disease progression and death in the NZBWF1 hybrid mouse model of lupus can be interrupted by neutralization of CD80 and CD86 using the cytotoxic T lymphocyte–associated Ag-4 (CTLA-4)–immunoglobulin fusion protein[67]. Results in the EAE disease model are more confusing, as anti-CD80 mAb monotherapy or a single infusion of CTLA-4–immunoglobulin reduces demyelination, but anti-CD86 mAb or repeated CTLA-4–immunoglobulin infusions exacerbates disease[68,69]. NOD mice doubly deficient for CD80 and CD86 develop accelerated diabetes[70]. Differentiation of T helper type 1 (T_H1) and T_H2 effector cells is indeed defective in these double knockout mice; however, the number of $CD25^+CD4^+$ regulatory T cells is also markedly reduced. In summary, it is clear that CD80 and/or CD86 are necessary for the optimal population expansion and effector cell differentiation of naive responder T cells; however, their roles in the induction of clonal anergy *in vivo* remain uncertain.

CTLA-4, a structural homolog of CD28 and higher avidity binder of CD80 and CD86 expressed at late times after T cell activation, plays a critical role in the counter-regulation of cell cycle progression[71,72]. Animals made deficient for CTLA-4 show spontaneous T cell lymphoproliferation and autoimmunity[73,74]. Likewise, NOD mice made transgenic for an agonistic single chain CTLA-4–specific antibody show decreased islet cell infiltration and a delay of T1D development[75]. Remarkably, $Ctla4^{-/-}$ $CD4^+$ T cells, as well as wild-type T cells treated with CTLA-4–specific mAb, resist anergy induction following soluble antigen administration in the absence of infection or adjuvant[76–79]. Although a portion of the counter-regulatory actions of CTLA-4 relates to its role in mediating the suppressive effects of $Foxp3^+$ $CD4^+$ regulatory T cells[80], OVA-specific $Ctla4^{-/-}$ DO11.10 $CD4^+$ T cells cause severe T1D in regulatory T cell-deficient $Rag^{-/-}$ RIP-mOVA mice, whereas $Ctla4^{+/+}$ DO11.10 T cells do not[81].

Programmed cell death-1 (PD-1), a second counter-regulatory molecule with limited structural similarity to both CD28 and CTLA-4, has been implicated in peripheral tolerance induction and maintenance based on the development of systemic autoimmunity following deletion of the gene encoding PD-1, or the genes encoding its two ligands PD ligand 1 (PD-L1) and PD-L2 (refs. 82–84). Low-avidity islet antigen-specific BDC2.5 $CD4^+$ T cells previously activated with the high avidity mimic peptide 1040-31 cause T1D when transferred into NOD hosts, but their pathogenicity can be abrogated by treatment of the recipient mice with 1040-31 peptide–coupled spleen cells to induce clonal anergy[85]. Anti-PD-L1 mAbs prevents anergy development in this system, and even established tolerance can be broken at later times with infusion of anti-PD-L1. PD-1 appears to play a unique role in maintaining T cells in an anergic state in part by blocking tissue migration 'stop signals' that are necessary for productive TCR engagements, since treatment with PD-L1–specific mAb, but not CTLA-4–specific mAb, allows anergic BDC2.5 T cells to significantly slow their rate of movement within pancreatic islets[86] (**Fig. 2**).

Tolerant polyclonal $CD8^+$ T cells show similar counter-regulation by PD-1. Seven days after the induction of a tamoxifen-inducible transgene encoding LCMV $glycoprotein_{33–42}$ and $nucleoprotein_{396–404}$ peptides in DCs, acute LCMV infection could no longer trigger proliferation and differentiation of wild-type polyclonal $glycoprotein_{33–42}$ and $nucleoprotein_{396–404}$ tetramer-binding $CD8^+$ T cells[87]. In contrast, PD-1–deficient $CD8^+$ T cells were resistant to tolerance development and responded well to LCMV challenge. In fact, in the absence of PD-1, viral peptide–specific $CD8^+$ T cells in tamoxifen-treated animals spontaneously underwent proliferation and differentiation to an effector cell phenotype that protected against LCMV infection. Interestingly, PD-1 ligand expression does not appear to be required on the DC itself to achieve self tolerance. Deletions of both PD-L1 and PD-L2 from the parenchymal tissues alone within NOD–severe combined immunodeficiency (SCID) mice accelerate the development of T1D following an adoptive transfer of polyclonal $CD4^+$ T cells from diabetic NOD mice[84]. Taken together, these data are most consistent with a model wherein CTLA-4–B7 interactions terminate proliferation and promote anergy induction during the primary response to self pMHC recognition, whereas PD-1–PD-1 ligand interactions restrain previously tolerized autoreactive T cells that enter the peripheral tissues and find self pMHC, maintaining them in an anergic state.

Conclusions

The existence of ectopically expressed 'tissue-restricted' antigens in the thymus blurs the line between central and peripheral tolerance and challenges the utility of the TRA concept. Nevertheless, several mechanisms are in place to cope both with the rare T cell that escapes central tolerance despite having a high avidity TCR to self pMHC and with the larger number of lower-avidity self-reactive T cells that escape and have the potential to cause harm. Although TRAs may be expressed within primary and secondary lymphoid organs, their increased abundance in certain tissues still poses a challenge to the immune system. Tolerogenic DCs seem to be the default mechanism for avoiding autoimmunity, perhaps as the result of constant recognition and uptake of apoptotic cells, and provide self-reactive T cells with the opportunity to take themselves out of the functional repertoire through physical elimination or functional inactivation (**Fig. 1**). Finally, autoreactive T cells rely on their own expression of counter-regulatory receptors such as CTLA-4 and PD-1 to develop and maintain, respectively, a state of functional unresponsiveness to peripheral self pMHC presentation (**Fig. 2**).

ACKNOWLEDGMENTS

Supported by the US National Institutes of Health (P01 AI35296 and R01 AI080764).

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