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Peripheral Tolerance of CD8 T Lymphocytes

Review

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Whereas high-avidity recognition of peptide-MHC complexes by developing T cells in the thymus results in deletion and promotes self-tolerance, such recognition by mature T cells in the periphery results in activation and clonal expansion. This dichotomy represents the basis of a dilemma that has stumped immunologists for many years, how are self-specific T cells tolerized in the periphery? There appear to be two important criteria used to achieve this goal. The first is that in the absence of inflammatory pathogens, tolerance is promoted when T cells recognize antigen presented by quiescent dendritic cells (DCs) expressing low levels of costimulatory molecules. A second critical factor that defines "self" and drives tolerance through deletion, anergy, or suppression is the persistence of antigen.

Peripheral Tolerance: What's It Good For?

Elimination of potentially autoreactive T cells is an important part of T cell development in the thymus. Although TCR-mediated recognition of self-peptide-MHC complexes by developing T cells is necessary to signal further maturation (Starr et al., 2003), immature T cells (thymocytes) that exhibit high-affinity recognition of self-peptide-MHC complexes could potentially contribute to autoimmunity and, therefore, are deleted by a process referred to as negative selection or central tolerance (Starr et al., 2003). Thymocytes are exposed to self-antigens presented as peptide-MHC (pMHC) complexes by bone marrow (BM)-derived, antigen-presenting DCs. DCs present endogenously expressed antigens as well as exogenous antigens acquired through endocytic and phagocytic pathways, a process referred to as crosspresentation (Heath and Carbone, 2001; Mellman and Steinman, 2001).

In addition to DCs, recent work has implicated thymic epithelial cells (TECs) as potential mediators of negative selection (Palmer, 2003). Interestingly, TECs are able to constitutively synthesize and express many peripheral tissue-specific antigens that would otherwise be unavailable to induce thymic tolerance, a function that was recently shown to be dependent upon expression of the autoimmune regulator (*AIRE*) gene (Anderson et al., 2002). TEC-dependent expression of self-antigens is an important mechanism to help eliminate autoreactive cells as *AIRE*-deficient humans and mice develop organ-specific autoimmunity (The Finnish-German APECED Consortium, 1997; Anderson et al., 2002; Ramsey et al., 2002). Furthermore, a recent report demonstrated that TEC-mediated central tolerance could occur via recognition of antigen displayed by the TECs themselves or after transfer of the antigens to BM-derived, antigen-presenting cells (APCs) (Gallegos and Bevan, 2004).

Considering that the T cell repertoire is carefully purged of potentially autoreactive T cells in the thymus, the question arises as to whether additional tolerance mechanisms are necessary in the periphery. One reason such mechanisms are required is to avoid the development of an immune response against the myriad of innocuous environmental antigens to which we are continuously exposed, both from our diet and environment (Huang et al., 2000; Mowat, 2003). Furthermore, there are severe limitations on the ability of central tolerance to delete all potentially autoreactive T cells. Despite the activity of AIRE, not all self-antigens are expressed in the thymus at levels sufficient to eliminate all autoreactive cells. Indeed, although negative selection deletes T cells of high avidity for self-antigens, low-avidity T cells that have much less chance of initiating autoimmunity are spared (Liu et al., 1995; Morgan et al., 1998). This cutoff in avidity threshold is an important investment in repertoire diversity at the expense of retaining certain potentially autoreactive cells.

Because the affinity threshold of TCR-pMHC interaction that signals thymic deletion is lower than that for activation in the periphery (Pircher et al., 1991), it is likely that some T cells with low avidity for self-antigens will not be activated in the periphery and, instead, remain "ignorant" of their cognate antigen (Ohashi et al., 1991; Oldstone et al., 1991). In effect, autoreactive T cell responses are prevented by simply avoiding recognition of the self-protein either through physical restriction of the antigen to an immunologically privileged location or due to low TCR avidity for the cognate antigen. However, ignorance of the antigen is an inherently dangerous mechanism and cannot be relied upon to maintain peripheral tolerance because, given the proper stimulatory milieu, such antigens may no longer be ignored and could potentially initiate autoimmune responses. Indeed, it has been shown that viral priming can break CD8 T cell ignorance and promote autoimmunity (Ohashi et al., 1991; Oldstone et al., 1991).

There is, however, one distinct disadvantage to postponing tolerance until after the self-reactive T cell reaches the periphery—thymocytes and mature T cells respond differently to TCR signaling. In contrast to thymocytes, which are deleted by a strong TCR signal without signaling cellular division, a strong TCR signal in mature T cells is hard wired to initiate division (Gett et al., 2003; Kishimoto and Sprent, 1997). For this reason, peripheral tolerance mechanisms must deal not only with the autoreactive T cell but also with its clonal progeny. Therefore, it is advantageous to eliminate as many potentially harmful cells as possible during thymic development.

In summary, although central tolerance is a critical

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step in purging the repertoire of autoreactive T cells, the additional layers of protection provided by peripheral tolerance mechanisms are required to maintain self-tolerance in vivo. This review focuses on peripheral tolerance mechanisms that act directly on CD8 T cells, as opposed to dominant tolerance mechanisms that involve inhibition by regulatory T cells, as may occur in immunologically privileged sites (Sakaguchi, 2004). It is likely that many of the same mechanisms discussed below for CD8 T cells also relate to CD4 T cell peripheral tolerance. Moreover, the word tolerance is used in a broad sense to include all situations in which TCR recognition and activation fail to stimulate a protective immune response.

The Role of DCs in Defining Self/Nonself Discrimination in the Periphery

Newly minted T cells express high levels of CD62L, a molecule that promotes the entry of T cells from blood to lymph nodes (Weninger et al., 2001). This confines naive T cells exported from the thymus to a pattern of circulation between blood and secondary lymphoid tissues (Mackay, 1993). It may be argued that with such a restricted range of movement, T cells would never encounter peripheral antigens. However, DCs are charged with the task of acquiring antigens from peripheral tissue and traveling to draining lymph nodes where the antigen is crosspresented to naive CD8 T cells (Heath and Carbone, 2001). This is a constitutive process that allows CD8 T cells in lymph nodes to become acquainted with all antigens expressed in the peripheral tissue. Tissue-resident DCs are believed to acquire antigens during phagocytic clearance of apoptotic cells arising from normal cell turnover and then migrate to the lymph nodes that drain the tissue (Albert et al., 1998; Belz et al., 2002; Iyoda et al., 2002). After processing and presenting these acquired antigens as peptides associated with MHC class I molecules, the DCs are able to stimulate naive CD8 T cells (Ingulli et al., 1997).

In this way, all systemically and peripherally expressed antigens can be presented to naive CD8 T cells circulating through the secondary lymphoid tissue. This provides an opportunity for CD8 T cells that recognize peripheral self-antigens with high avidity to be activated. In the absence of pathogens, however, DCs are quiescent and express low levels of costimulatory molecules, such as B7.1 and B7.2 (Steinman et al., 2003). These molecules interact with CD28 on T cells to enhance T cell responsiveness and survival (Boise et al., 1995). As a result, high-avidity recognition of antigen in the absence of costimulation through CD28 results in T cell activation characterized by only a brief period of proliferation and suboptimal development of effector cell function (Hernandez et al., 2001; Kearney et al., 1994). The end result is the induction of T cell tolerance, which can occur by one of two mechanisms, deletion or anergy. Anergy represents a state of functional unresponsiveness that is actively supported by the presence of antigen, as discussed further below.

In contrast, upon infection by a pathogen, tissue-resident DCs are activated by inflammatory cytokines and through pattern recognition molecules, such as toll-like receptors, that are designed to detect conserved bacterial and viral products and alert the innate immune system of the presence of the invading microbes (Iwasaki and Medzhitov, 2004). These are, in effect, the natural adjuvants that promote an immune response. This process of DC activation results in enhanced antigen presentation, production of inflammatory cytokines, and importantly, increased levels of expression of B7.1 and B7.2 costimulatory molecules. Such DCs are now "licensed" to activate T cells. Although a strong TCR signal is sufficient to initiate cell division, additional interactions between costimulatory molecules and their ligands, such as CD28-B7, as well as the presence of proinflammatory cytokines are critical to support T cell survival and optimize the development of effector functions (Lenschow et al., 1996). Signaling through CD28 results in the enhanced expression of antiapoptotic molecules, such as Bcl-xL (Boise et al., 1995), which promote the survival of clonal progeny and signals expression of a cascade of downstream costimulatory molecules that further promote cell survival (Croft, 2003). Costimulatory signals also modulate the expression of molecules that permit activated effector CD8 T cells to leave the lymphoid tissue and migrate into peripheral tissues. This includes the downregulation of CD62L and CCR7 and upregulation of adhesion molecules, such as LFA-1 and VLA-4, which facilitate extravasation of the activated CD8 T cells into peripheral tissues, thus allowing the cells to participate in the clearance of pathogens from peripheral tissues (Sallusto et al., 1999).

Other mechanisms are also capable of licensing DCs for CD8 T cell activation (Smith et al., 2004). These include activating DCs through costimulatory molecules, such as CD40 and B7. Activated CD4 T cells express high levels of CD40L that can stimulate DC maturation through interaction with CD40 on the DC (Bennett et al., 1998; Ridge et al., 1998; Schoenberger et al., 1998). This is an important way in which CD4 T cells provide help for a CD8 T cell response. In addition to CD40, it appears that CD28 on activated CD8 T cells can transmit stimulatory signals to DCs through CD28-B7 interactions (Orabona et al., 2004). The existence of such a "back-stimulation" pathway helps to explain previous reports in which it was found that large numbers of activated CD8 T cells are capable of inducing DC activation (Mintern et al., 2002; Ruedl et al., 1999; Schuler and Blankenstein, 2002; Wang et al., 2001). This observation also sheds light on how large numbers of cognate CD8 T cells supply their own "help" that can result in tissue destruction, eventually leading to diabetes (Kurts et al., 1997a) or tumor eradication (Hanson et al., 2000; Lyman et al., 2004; Nguyen et al., 2002).

The prevailing paradigm that explains tolerance versus immunity is as follows: Invading pathogens announce their presence through activation of a variety of innate immune signals, including recognition by innate immune receptors on DCs, which lead to DC activation and a vigorous immune response to cognate antigens. In contrast, self-antigen that is constitutively presented on quiescent DCs leads to a quantitatively and qualitatively different type of response, specifically the induction of tolerance. Furthermore, there have been numerous examples in which the same soluble antigen can be shown to be tolerogenic or immunogenic to CD8 T cells, depending upon whether it is delivered with adju-

vant (Lefrancois et al., 2000; Schmidt and Mescher, 1999). Therefore, it may be concluded that an important component of self/nonself discrimination in the periphery is the activation status of the antigen-presenting DC.

The Role of Antigen Persistence in Tolerance Induction

Experiments using several different CD8 TCR transgenic models have lent support to another explanation for self/nonself recognition that places the responsibility for this decision on the persistence of antigen rather than on the presence of adjuvant (Aichele et al., 1995; Rocha and von Boehmer, 1991). Rocha and coworkers performed a particularly elegant experiment using the HY (male antigen)-specific model. They produced chimeras expressing different numbers of HY-bearing male cells and then introduced HY-specific CD8 T cells (Rocha et al., 1995). In all cases, the T cells initially proliferated and exhibited some effector function. In mice that contained few HY-expressing cells, this initial activation resulted in complete elimination of the antigen-bearing cells, and the activated T cells went on to become memory cells. In contrast, in mice that contained a larger number of HY cells, the T cells were gradually tolerized (Rocha et al., 1995). These studies demonstrated that it was possible to develop an effector T cell response in the absence of adjuvant. The variable that appeared to determine whether the activation conditions led to the development of memory T cells or tolerance induction was the persistence of antigen.

Zinkernagel and coworkers obtained analogous results by using a different CD8 TCR transgenic specific for a peptide from the glycoprotein of the lymphocytic choriomeningitis virus (LCMV). They found that delivery of a single dose of peptide to mice containing viral glycoprotein (GP)-specific CD8 T cells resulted in T cell priming and memory cell formation, as defined by further protection from infection with LCMV, whereas multiple doses resulted in tolerance (Aichele et al., 1995). In this case, CD8 T cell tolerance appeared to occur by deletion. Based upon these results, Zinkernagel proposed a tolerance model in which antigen localization, dose, and persistence are the critical factors that determine tolerance induction, rather than just the delivery of costimulating signals by the antigen-presenting cells (Zinkernagel, 2000).

Surprisingly, it has also been found that CD8 T cells activated in the persistent presence of a pathogen can undergo tolerance. Zajac et al. examined CD8 T cell responses during a viral infection and showed that persistent infection with LCMV led to an initial CD8 T cell response that was followed by the induction of anergy in the remaining cells (Zajac et al., 1998). This effect appeared to be antigen dependent, as use of an acute infecting strain of LCMV that was rapidly cleared did not cause CD8 T cell nonresponsiveness.

The above experiments support a model of self/nonself recognition that is based upon antigen persistence, as the same activation conditions that led to an immune response and memory cell development could also result in tolerance. In fact, the critical difference between these divergent outcomes was the persistence of antigen rather than the activation status of the APC.

Comparing the Relative Importance of Antigen Persistence and DC Activation in Tolerance Induction

To further assess the relative contribution of DC activation and antigen persistence in CD8 T cell tolerance induction, we performed an experiment in which each of these variables was considered (Redmond et al., 2003). Clone 4 TCR transgenic CD8 T cells, specific for the influenza hemagglutinin (HA), were adoptively transferred into mice in which HA was crosspresented by activated (anti-CD40 mAb or influenza virus-treated mice) or quiescent (nontreated mice) DCs. After 4 days, the cells were recovered, and the effector status of the activated clone 4 T cells was examined. As expected, the activation status of the DC was important in terms of affecting the differentiation of naive CD8 T cells into effector CTL (Figure 1) as effector functions were exhibited by clone 4 T cells recovered from virus or anti-CD40 mAb-treated mice. In contrast, clone 4 T cells activated by quiescent DCs exhibited weak effector functions.

Next, we addressed whether the activation status of the DCs affected survival of the CD8 T cells. Day 4 after activation, clone 4 T cells were removed from the host in which they were activated and then transferred into antigen-free recipients. After several weeks, the antigen-free hosts were challenged with influenza virus to reveal the presence of antigen-responsive clone 4 T cells. Surprisingly, regardless of the initial stimulatory conditions, a portion of the activated CD8 T cells survived and responded fully to virus. In contrast, if the cells were transferred into antigen-bearing hosts, then regardless of the activation status of the antigen-presenting DCs, the clone 4 T cells were tolerized (Figure 1). These results demonstrate that the effector status of a CD8 T cell does not affect its decision to undergo tolerance or deletion, as effectors can be tolerized (in antigen-bearing hosts), and CD8 T cells that have no effector activity can become memory cells (in antigen-free hosts).

Importantly, for at least several days after their initial activation by quiescent DCs, the progeny of the activated CD8 T cells had not fully committed to undergo clonal deletion, as at least a portion of the cells survive and can regain responsiveness if removed from the presence of antigen. Rather, further exposure to antigen appears to be required in order to induce all CD8 T cells and their clonal progeny to undergo tolerance. This requirement for sustained antigenic exposure to promote complete clonal elimination ensures that only those autoreactive CD8 T cells that encounter persistently expressed self-antigens undergo clonal elimination while sparing from deletion CD8 T cells that may recognize a transiently expressed antigen, such as an environmental or food-derived antigen.

Does Tolerance to a Persistently Expressed Antigen Occur if the Antigen Is Presented by an Activated APC?

Several groups have demonstrated that chronic infections can lead to the induction of T cell anergy (Bronstein-Sitton et al., 2003; Zajac et al., 1998), suggesting that tolerance can occur even if antigen is presented in the presence of a pathogen. However, recent

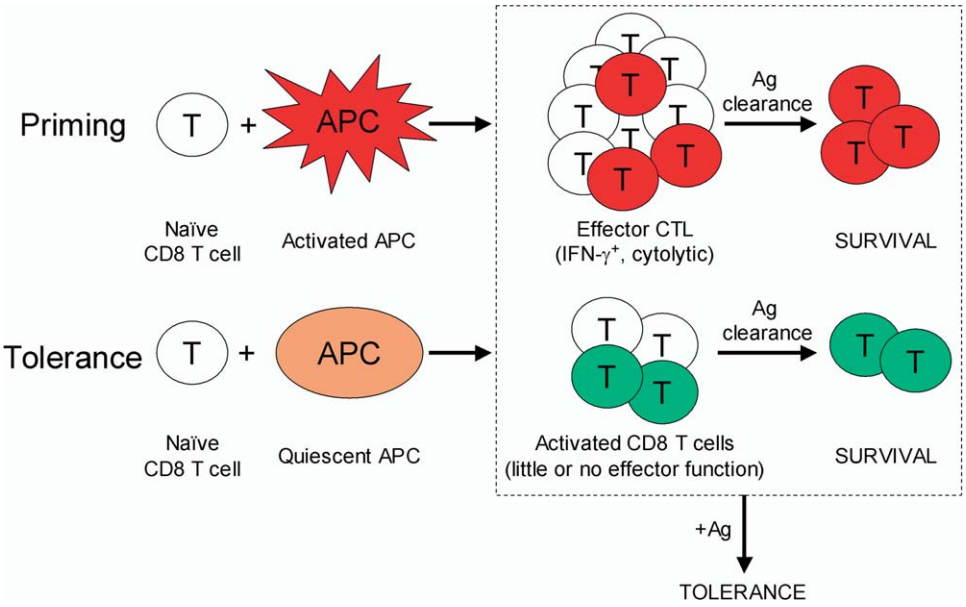

Figure 1. Activated APCs Program the Acquisition of CD8 T Cell Effector Functions, but Chronic Exposure to Antigen Is Required for Tolerance Induction

Stimulation of naive CD8 T cells by peptide-MHC (pMHC) on activated DCs expressing high levels of costimulatory molecules leads to CD8 T cell proliferation and differentiation into IFN-γ producing cytolytic effector cells. Costimulation also increases levels of antiapoptotic molecules, which promote survival of the effectors. In contrast, activation of naive CD8 T cells by quiescent DCs does not promote increased levels of antiapoptotic molecules and, therefore, leads to an abbreviated proliferative response. Additionally, the cells acquire little or no effector functions. Importantly, in both cases, antigen clearance promotes CD8 T cell survival and production of memory cells, whereas chronic antigenic stimulation (see +Ag) leads to tolerance.

work has demonstrated the ability of certain pathogens to downmodulate the activation status of the APC (Sevilla et al., 2004) and thereby inhibit the normal proinflammatory response that would promote naive CD8 T cell differentiation into effector CTL. As a result, it becomes difficult to isolate the relative contribution of antigen persistence versus the activation status of the APC in the induction of CD8 T cell tolerance. Furthermore, the presence of a pathogen leads to the involvement of numerous factors that influence the outcome of the response, including the activation of multiple components of the innate and adaptive immune system, such as CD4 helper and regulatory T cells.

Mayerova et al. have demonstrated that even in the absence of pathogen, it may not be possible to achieve tolerance to a persistently expressed antigen that is presented by activated APCs (Mayerova et al., 2004). They examined CD8 T cell responses to self-antigen presented by Langerhans cells, a unique epidermal APC that is constitutively activated. Under these conditions, priming, rather than tolerance, of naive CD8 T cells was induced, which ultimately led to the onset of autoimmunity. Interestingly, when the same antigen was also expressed elsewhere in the animal, resulting in crosspresentation by both activating as well as tolerogenic APCs, tolerance was the dominant outcome, suggesting a possible regulatory mechanism to prevent such autoimmune responses in a wild-type (wt) host.

The Fate of Tolerized CD8 T Cells In Vivo: Anergy versus Deletion?

Peripheral tolerance of CD8 T cells can occur through anergy or clonal deletion (Kundig et al., 1996; Kurts et al., 1997b; Kyburz et al., 1993; Mamalaki et al., 1993; Rocha et al., 1993). Anergy is an active process that is dependent upon the continuous presence of antigen as the cells regain their ability to respond once they are allowed to rest in the absence of antigen (Rocha et al., 1993; Schwartz, 2003). Numerous questions remain unresolved regarding the underlying mechanisms that regulate this decision and the biological significance of each mechanism in vivo. At least some of the confusion may be due to the fact that, regardless of whether tolerance occurs through anergy or complete clonal deletion, the initial period of proliferation in response to tolerogen is followed by a period of contraction in which most of the cells undergo apoptosis, just as during a response to pathogen (Badovinac et al., 2002). Thus, at issue is the fate of a relatively small number of surviving cells.

There is increasing evidence that in the presence of persistent antigen, the fate of naive CD8 T cells during peripheral tolerance may be determined by the strength of interaction between the TCR and pMHC. As described earlier, studies from Rocha et al. demonstrated the ability of high doses of chronic antigen to promote CD8 T cell anergy, whereas lower doses resulted in deletion (Rocha et al., 1995). In our own studies, we compared the response of clone 4 T cells to continuous provision of high versus low doses of peptide tolerogen in vivo, and we too have found that continuous exposure of naive clone 4 T cells to high doses of soluble peptide induced anergy in a portion of the responding cells, whereas continuous exposure to low doses resulted in clonal deletion (Redmond et al., 2005, and Fig-

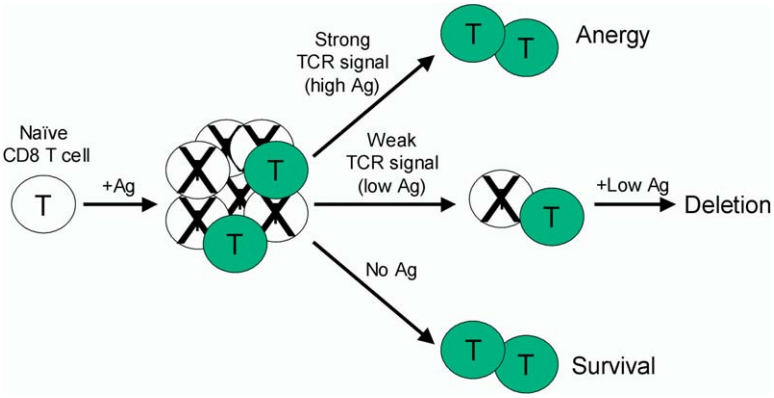

Figure 2. Strength of TCR-pMHC Interaction in CD8 T Cell Peripheral Tolerance

Activation of naive CD8 T cells under tolerogenic conditions promotes their initial proliferation followed by the induction of apoptosis in most of the responding CD8 T cells. Chronic exposure to strong TCR signals (high doses of antigen) renders the CD8 T cell anergic, or nonresponsive, to additional stimulation. Persistence of a strong TCR signal is required to maintain anergy. In contrast, persistence of a weak TCR signal (low doses of antigen) leads to complete clonal deletion. Finally, the induction of CD8 T cell anergy or deletion is dependent upon chronic antigenic stimulation as a portion of the tolerized CD8 T cells can survive long term in vivo once they are removed from the presence of antigen.

ure 2). Thus, anergy appears to be an effective way to preserve cells via an underlying mechanism that relates to the strength of TCR-pMHC interaction.

TCR-induced activation of Ras and down-stream intracellular signaling molecules, such as ERK, are inhibited in anergic T cells (DeSilva et al., 1996; Fields et al., 1996; Li et al., 1996). Phospho-ERK is a tyrosine kinase that regulates the activation of down-stream molecules that are critical for optimal proliferation and differentiation of naive T cells (Cantrell, 1996; Dong et al., 2002). To determine whether the strength of TCR-pMHC interaction relates to the decision to undergo deletion versus anergy, clone 4 T cells were first exposed to varying doses of HA peptide in vivo. After several days, the cells were recovered, and their ability to upregulate phospho-ERK in response to stimulation with peptide was assessed in vitro. We found that clone 4 T cells activated with high doses of HA peptide in vivo were unable to upregulate phospho-ERK in response to antigen and that this correlated with their inability to respond to further stimulation through their TCR (Redmond et al., 2005). Furthermore, as the level of peptide declined with time, the anergized clone 4 T cells regained their ability to produce phospho-ERK and to respond to antigen.

In contrast to these results, chronic exposure of clone 4 T cells to low doses of peptide that could successfully induce clonal deletion in vivo was associated with continuous production of phospho-ERK. These results suggest that clonal deletion in the periphery requires continuous signal transduction through the Ras-ERK pathway and that this can only occur when the strength of TCR-pMHC interaction is below a certain threshold. This may occur either as the result of exposure of the T cell to low amounts of antigen, a situation that may be common for crosspresented self-antigens in vivo, or high amounts of an antigen that is recognized with low avidity. Before considering what determines the threshold that is responsible for deletion versus anergy, it is informative to first consider the mechanism of anergy.

Mechanisms of CD8 T Cell Anergy

The characteristics of CD8 T cells undergoing anergy are diverse. By using the HY TCR Tg system, Rocha et al. have shown that the induction of CD8 T cell anergy was associated with downregulation of both TCR and CD8 molecules (Rocha and von Boehmer, 1991). Interestingly, TCR downregulation occurs in some (Schonrich et al., 1991), but not all (Dubois et al., 1998), situations involving the induction of anergy during chronic CD8 T cell activation in vivo. We observed minimal TCR downregulation during peptide-induced clone 4 T cell anergy (Redmond et al., 2005). Because TCR downregulation in naive CD8 T cells is dependent upon the TCR affinity for cognate peptide-MHC class I molecules (Cai et al., 1997), it is possible that the cells we examined may not have possessed sufficient affinity for their cognate antigen to promote TCR downregulation during tolerance induction. In addition to TCR downregulation, anergic CD8 T cells have been shown to exhibit decreased expression of many TCR-associated signaling molecules, such as ZAP-70, and exhibit defective calcium responses in response to TCR ligation in vitro (Guillaume et al., 2003; Tanchot et al., 1998). Furthermore, as mentioned previously, defects in Ras activation and phosphorylation of down-stream TCR signaling molecules, including ERK, have been reported for chronically activated CD4 and CD8 T cells, suggesting that this may be a common mechanism of T cell anergy in vivo (Dubois et al., 1998; Li et al., 1996; Schwartz, 2003).

Recent studies have shed light on the underlying mechanism for this plethora of signaling defects. First, anergy is an active process that occurs as the result of TCR signaling in the absence of costimulation (Macian et al., 2002). Such activation results in mobilization of free calcium and activation of the calcium-sensitive protein phosphatase calcineurin. Calcineurin dephosphorylates the transcriptional activator NFAT, thereby allowing it to translocate to the nucleus where it induces the transcription of a number of anergy-specific genes, including Itch, Cbl-b, and GRAIL, ubiquitin ligases that are required for CD4 and CD8 T cell anergy in vivo (Gronski et al., 2004; Jeon et al., 2004). The absence of Cbl-b lowers the threshold for T cell activation, as Cbl-b-deficient mice are susceptible to the development of autoimmune disease (Bachmaier et al., 2000; Jeon et al., 2004). Cbl-b is thought to promote the induction and maintenance of T cell anergy by reducing the level of calcium mobilization that occurs in response to TCR signaling, a process that is regulated

by PLCγ-1. The basis for reduced activity of PLCγ-1 has been reported to be both reduced phosphorylation (Jeon et al., 2004) and increased degradation (Heissmeyer et al., 2004) of the molecule. Ubiquitination by Cbl-b tags the protein for lysosomal degradation. This appears to be a general method of reducing a number TCR signaling molecules in anergic cells, including TCR, PLCγ-1, and PKC-θ (Heissmeyer et al., 2004). Collectively, these data support a model of T cell anergy in which persistent engagement of the TCR promotes continuous downregulation of TCR signaling pathways through activation of anergy-inducing genes.

Negative regulatory molecules have also been implicated in the induction and maintenance of CD8 T cell anergy. Some reports have shown increased expression of the negative regulatory molecule CD5 on the surface of chronically stimulated CD4 and CD8 T cells (Hawiger et al., 2004; Stamou et al., 2003). CD5 is thought to moderate TCR-mediated signaling in both thymocytes and mature T cells, and its expression has been found to increase on high-avidity or chronically activated T cells (Azzam et al., 2001; Azzam et al., 1998; Tarakhovsky et al., 1995). Surprisingly, we have not observed increased expression of CD5 in anergized CD8 T cells (our unpublished data). The reasons underlying the apparent involvement of CD5 in some, but not all, models of peripheral tolerance are unknown, but may reflect differences in TCR avidity among the various model systems examined. Indeed, several groups have shown that the upregulation of CD5 is related to the extent of TCR signaling and avidity for cognate antigen (Azzam et al., 1998; Tarakhovsky et al., 1995).

It should also be noted that another negative regulatory molecule, cytotoxic T lymphocyte antigen 4 (CTLA-4), which plays a key role in the induction of tolerance and anergy of CD4 T cells (Greenwald et al., 2001; Perez et al., 1997), appears to be dispensable for CD8 T cell anergy (Deeths et al., 1999; Frauwirth et al., 2001).

A Model of CD8 T Cell Fate during Peripheral Tolerance

It is of interest to consider that regardless of the ultimate fate of an activated CD8 T cell, initially, all immune responses follow a similar pattern—TCR engagement signals T cell activation and proliferation. If the cell also receives a costimulatory signal, this ensures a robust proliferative response that is followed by a contraction phase during which up to 95% of the clonal progeny die through an apoptotic mechanism (Dutton et al., 1998). This contraction occurs independently of the presence of antigen (Badovinac et al., 2002). In the absence of costimulation, the amount of proliferation and time to contraction is reduced, and again, the majority of cells undergo apoptosis. When viewed in this light, the qualitative difference between tolerance and immunity comes down to the fate of the cells that were capable of surviving the contraction phase: the memory cell precursors. What happens to these memory cell precursors during tolerance induction? Are they never formed or, alternatively, are these the cells that must be convinced to undergo deletion or anergy in order to achieve tolerance?

Before addressing this question, it is necessary to first consider the signals that contribute to the life and death of an activated CD8 T cell during tolerance induction. There is increasing evidence that the survival of naive T cells in the periphery is largely determined by a balance between endogenous pro- and antiapoptotic molecules that regulate mitochondrial integrity (Zhu et al., 2004). In particular, the level of the antiapoptotic molecule Bcl-2 is maintained in resting T cells by signaling through the IL-7Rα (Schluns et al., 2000). T cell activation leads to downregulation of IL-7Rα, resulting in decreased expression of these antiapoptotic molecules. At the same time, metabolic activity associated with T cell activation and proliferation results in depletion of existing Bcl-2. Because Bcl-2 is required to inhibit the activity of Bim, a proapoptotic molecule that has been implicated in both thymic and peripheral deletion (Bouillet et al., 2002; Hildeman et al., 2002; Mintern et al., 2002), the result is clonal deletion.

It should be noted that activation induced cell death involving TNFR family members, such as Fas-FasL, does not appear to be involved in CD8 T cell peripheral tolerance induced by quiescent DCs (Davey et al., 2002). This may be because the induction of FasL requires increased activation of NF-κβ, which is unlikely to occur in the absence of costimulation (Teixeiro et al., 2004).

Several reports have demonstrated that during priming, a subpopulation of naive CD8 T cells that expresses IL-7Rα and CD8αα preferentially survive and differentiate into memory cells (Kaech et al., 2003; Madakamutil et al., 2004). It has been proposed that CD8αα serves to reduce the strength of signaling to the T cell during stimulation, which may inhibit Fas-mediated cell death that can occur when the cell receives a strong signal involving both the TCR and CD28 (Teixeiro et al., 2004). In addition, CD8αα and IL-7Rα signaling would enhance production of antiapoptotic molecules and allow memory cells to survive.

Given our current knowledge regarding the fate of naive CD8 T cells after chronic antigenic exposure, we propose the following model governing the fate of naive CD8 T cells activated under tolerogenic conditions in the absence of costimulation in vivo: in the absence of cognate antigen, naive CD8 T cells express high levels of the IL-7Rα and their survival and homeostatic maintenance is regulated via signaling by endogenous IL-7 (Figure 3). We propose that during activation under conditions of weak TCR-pMHC interaction, the amount of free calcium released is insufficient to activate "anergy" genes. However, the cells downregulate IL-7Rα (Schluns et al., 2000), resulting in decreased expression of antiapoptotic Bcl-2. As yet, no pathway has been identified that specifically links TCR signaling with activation of proapoptotic molecules such as Bim. However, activation of the Ras-signaling pathway under conditions in which the PKC-signaling pathway is inhibited, as may occur in the absence of costimulation, is known to promote apoptosis through a Bcl-2-inhibitable pathway (Denis et al., 2003). The increased metabolic activity associated with T cell activation and division increases production of reactive oxygen species and increases the need for antiapoptotic molecules such as Bcl-2, thereby further depleting their level in the cell (Denis et al., 2003; Hildeman et al., 2003). This

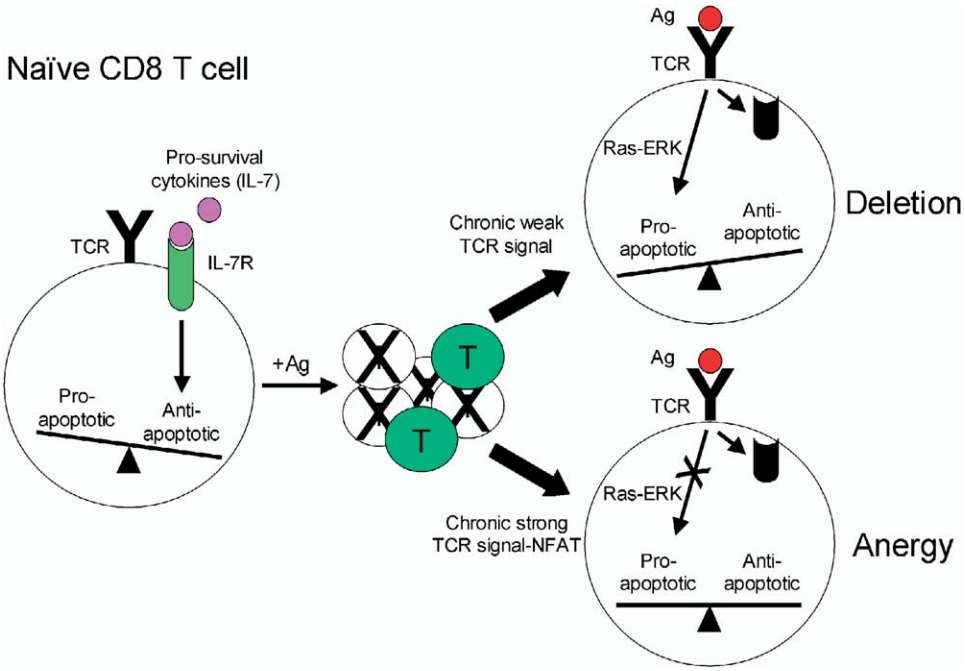

Figure 3. Model of CD8 T Cell Fate during Peripheral Tolerance: Anergy versus Deletion

In the absence of cognate antigen, the prosurvival cytokine IL-7 promotes continuous production of antiapoptotic molecules that assure survival of naive CD8 T cells. Exposure to antigen under tolerogenic conditions promotes CD8 T cell proliferation coupled with downregulation of the IL-7Rα. After this initial clonal burst, most of the proliferating cells are eliminated through an apoptotic mechanism initiated by the downregulation of the IL-7Rα. A portion of the cells can survive this initial encounter with antigen and, in the presence of chronic antigenic stimulation, are faced with two fates—deletion or anergy. Weak TCR stimulation is associated with the maintenance of TCR-mediated signaling pathways, which leads to depletion of antiapoptotic molecules, probably through accumulation of reactive oxygen species. This promotes the activity of the proapoptotic molecule Bim, resulting in CD8 T cell deletion. In contrast, strong TCR stimulation leads to the production of anergy genes, which subsequently inhibit further TCR-mediated Ras-ERK signaling and, instead, induce CD8 T cell anergy.

tips the balance towards death, with involvement of proapoptotic molecules such as Bim (Hildeman et al., 2002; Mintern et al., 2002). As long as antigen is present and TCR signaling continues, IL-7Rα levels remain low, and the cells continue to die until clonal deletion is complete. Alternatively, if during this period of time antigen is removed, then a population of cells emerges that is capable of reexpressing IL-7Rα, which in turn promotes the expression of antiapoptotic molecules. These cells would represent the "memory" cells that have been observed when cells undergoing deletion are removed from antigen (Redmond et al., 2003). It is likely that these would be more akin to central rather than effector memory cells (Sallusto et al., 1999), as they may not have developed effector functions when first activated.

In contrast, a different scenario emerges when considering the activation of naive CD8 T cells that experience strong signaling during peripheral tolerance. Initially, the events that occur after activation through strong or weak TCR engagement are similar. The initial exposure to antigen leads to a proliferative response, which is soon followed by the deletion of the majority of the responding cells (Figure 3). As described above, this initial activation leads to downregulation of IL-7Rα, and either through the decrease in antiapoptotic molecules or through a direct TCR-mediated proapoptotic signal, most (but not all) of the CD8 T cells are eliminated. However, with sufficient strength of TCR-antigen interaction, the levels of free calcium produced are adequate to trigger anergy, which occurs in a portion of the responding CD8 T cells. This is associated with reduced Ras-ERK signaling and cessation of the TCR-mediated proapoptotic signals. It is interesting to speculate that the anergized cells that survive may represent the population of memory precursors, although it is not known if they regain expression of IL-7Rα or perhaps utilize another prosurvival cytokine, such as IL-15. Importantly, the anergized cells survive and remain nonresponsive to antigen, although paradoxically, the maintenance of anergy requires the continuous presence of antigen, thus some level of signaling through the TCR must persist in order to sustain the anergized state. Indeed, if the CD8 T cells are allowed to rest in the absence of antigen, they recover their antigenic responsiveness. Furthermore, they also regain their ability to undergo tolerance, and multiple cycles of exposure to high-dose antigen interspersed with periods of rest to regain responsiveness appear to be another effective approach to ultimately achieve clonal deletion (Redmond et al., 2005).

Concluding Remarks

Peripheral tolerance of self-reactive CD8 T cells remains an attractive goal to prevent autoimmunity and allograft rejection. Exposure of CD8 T cells to antigen

in the absence of costimulation has been one approach used to achieve this goal (Wekerle et al., 2002). However, in the case of allograft rejection, the conditions that have been used generally promote anergy, rather than deletion, of the undesired cells. This results in the requirement for persistent treatment with costimulatory blockade or immunosuppressive drugs to prevent graft rejection. Further understanding of the requirements that result in deletion rather than anergy would be of value in developing a more durable solution to this important problem.

There is evidence that in the absence of inflammation caused by pathogens or activated CD4 cells, CD8 T cells representing all stages of development, naive, effector, and memory, can be deleted (Kreuwel et al., 2002). Developing protocols for antigen presentation that can affect such deletion in the clinical setting represents a major challenge. This is further complicated by the fact that variation in the strength of TCR-pMHC interaction among T cells specific for the same antigen results in a diversity of response characteristics. Understanding the essential features required for successful tolerance of CD4 and CD8 T cells is necessary to achieve this goal.

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