Review

Homeostasis of T Cell Diversity

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T cell homeostasis commonly refers to the maintenance of relatively stable T cell numbers in the peripheral lymphoid organs. Among the large numbers of T cells in the periphery, T cells exhibit structural diversity, i.e., the expression of a diverse repertoire of T cell receptors (TCRs), and functional diversity, i.e., the presence of T cells at naïve, effector, and memory developmental stages. Although the homeostasis of T cell numbers has been extensively studied, investigation of the mechanisms underlying the maintenance of structural and functional diversity of T cells is still at an early stage. The fundamental feature throughout T cell development is the interaction between the TCR and either self or foreign peptides in association with MHC molecules. In this review, we present evidence showing that homeostasis of T cell number and diversity is mediated through competition for limiting resources. The number of T cells is maintained through competition for limiting cytokines, whereas the diversity of T cells is maintained by competition for self-peptide-MHC complexes. In other words, diversity of the self-peptide repertoire limits the structural (TCR) diversity of a T cell population. We speculate that cognate low affinity self-peptides, acting as weak agonists and antagonists, regulate the homeostasis of T cell diversity whereas non-cognate or null peptides which are extremely abundant for any given TCR, may contribute to the homeostasis of T cell number by providing survival signals. Moreover, self-peptides and cytokines may form specialized niches for the regulation of T cell homeostasis. *Cellular & Molecular Immunology*. 2005;2(1):1-10.

Key Words: lymphocyte homeostasis, lymphocyte compartment, lymphocyte diversity, lymphopenia-induced proliferation, self-peptide, cytokine

Introduction

Homeostasis refers to the tendency of the body to preserve its internal steady state, allowing it to return to a normal set point following perturbation. The term was first used by the American physiologist Walter Cannon in his seminal work, Wisdom of the Body, in 1932 (1). He emphasized the dynamic nature of homeostasis, stating that while it ensures stability of the organism, homeostasis "does not imply something set and immobile, a stagnation" This dynamism is evident in the homeostasis of the adaptive immune system where rapid fluctuations in the number, diversity, and function of lymphocytes occur during immune responses. Yet, for the efficient function of the immune system, the population and activation states of T cells need to remain relatively stable in the long term (2). The term lymphocyte homeostasis has been used to refer to the maintenance of lymphocyte numbers as well as the maintenance of lymphocyte diversity (3, 4). Increasing evidence suggests that the homeostasis of both T cell number and diversity is regulated through competition for limiting resources, including self-peptide-MHC complexes (spMHC) and cytokines such as IL-7 and IL-15 (5). As the homeostasis of T cell numbers has been reviewed recently (2, 6), this review focuses on the homeostasis of T cell diversity.

Two types of T cell diversity

It is useful to take a closer look at the meaning of diversity. The diversity of a population refers to both the variety and abundance of the constituent individuals or in the case of T cells, the constituent clones. However, there is no universal measure of diversity. Different measures that are used to quantify diversity account for the variety and abundance of constituent species in different ways (Figure 1). We can thus speculate that the immune system monitors diversity in its own unique manner. As will become apparent in this review, the immune system does not always try to maximize the diversity of T cells but rather optimizes their diversity during various rounds of selection and competition that occur during different stages of T cell development.

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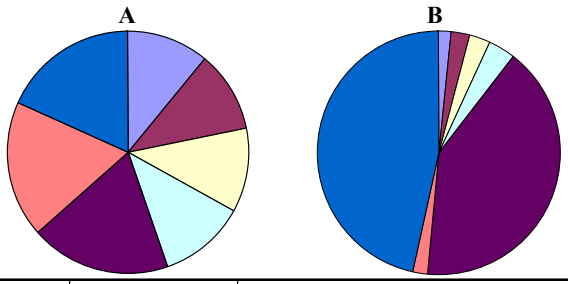

Index of diversity	Expression	Definition
Simpson's index	$D^{s} = 1 - \sum_{i=1}^{N} \frac{1}{s_i^2}$	This index measures the probability that any two individuals in the population are different. It gives greater importance to the more abundant species.
Shannon-Weaver index	$D^{sw} = \sum_{i=1}^{N} s_i \ln(s_i)$	This index is based on information theory and is a measure of information contained. It gives more importance to the rarer species.

Figure 1. Indices used to measure diversity: which population is more diverse? Population A and B are each comprised of seven clones but in different proportions. Which population is more diverse in this case? Population A is considered to be more diverse in terms of both the variety of the clones present as well as their relative abundance. Shannon-Weaver index and Simpson's index are two indices that are commonly used to compare the diversities of populations with varying proportions of comprising species. (s_i is the frequency of an individual clone in the population and N is the total number of clones.)

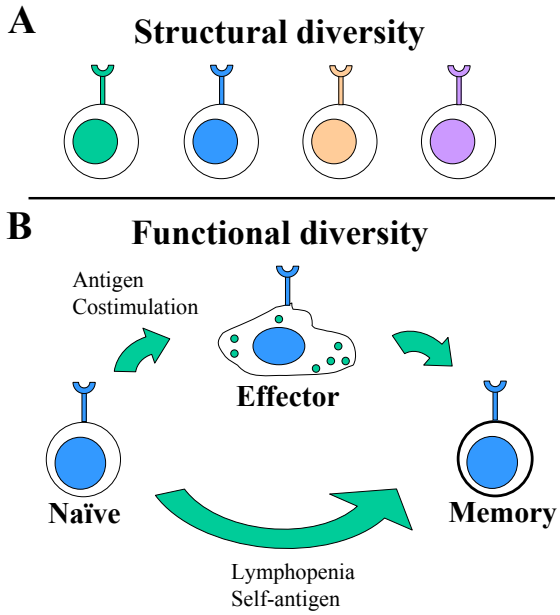

Figure 2. Structural and functional diversity of T cells. (A) Structural diversity of T cells refers to T cells expressing different TCRs. (B) Functional diversity refers to T cells that have the same TCR but are at different stages of development. Both antigen-induced and lymphopenia-induced proliferation and differentiation of naïve T cells are depicted.

In normal individuals, T cells are diverse in both structure and function. The diversity among T cells due to the expression of different TCRs is called structural diversity (Figure 2A). Unless otherwise specified, the term "diversity" will be used to refer to structural diversity of T cells in this review. During T cell development in the thymus, a diverse population of T cells each expressing a different T cell receptor (TCR) is generated by random recombination of TCR gene segments. Following positive and negative selection a small fraction of the thymocytes mature and exit the thymus, constituting αβ, γδ, and NK T cells in the periphery. Depending on the co-receptor expression, αβ T cells are further divided into $CD4^+$ and $CD8^+$ T cells. Among the various types of T cells, αβ T cells are the most abundant and therefore will be the focus of this review.

When stimulated with appropriate antigens, naïve $CD4^+$ and $CD8^+$ T cells become activated and undergo clonal expansion. The resulting T cells acquire effector functions and migratory properties that allow them to clear antigens in both lymphoid and non-lymphoid organs. Subsequently, most of the effector T cells die by apoptosis and only a small fraction survive and differentiate into memory T cells. Thus, T cells expressing the same TCR can exist in different functional states: naïve, effector or memory. We refer to this as functional diversity (Figure 2B). Furthermore, studies have shown that memory T cells are heterogeneous in terms of development, effector functions, surface phenotype and trafficking properties (7, 8). "Central" memory T cells are long-lived, reside in the lymphoid organs and expand upon re-stimulation by appropriate antigen. In contrast, 'effector' memory T cells reside in the non-lymphoid organs and exhibit immediate effector function without first undergoing proliferation. Memory cells can also arise directly from naïve T cells without an intermediate effector stage in lymphopenic conditions by a process called lymphopenia-induced proliferation (LIP) (Figure 2B) (9, 10). Thus, T cells are diverse in both structure, i.e., the TCR repertoire, and function, i.e., the different developmental states of T cells.

The structural diversity of different functional T cell compartments can vary significantly. Most T cell diversity in the periphery is evident in the naïve T cell population, which is generated by thymopoiesis and persists throughout life. In contrast, effector T cells are short-lived, pauci-clonal expansions of antigen-specific T cells. The memory T cell repertoire is estimated to contribute less than 1 percent of the total diversity of T cells in young adult humans although the memory population comprises one-third of the total T cells (11). This results probably because only a small proportion of naïve T cells encounter a cognate antigen during an individual's lifetime. In addition, only a small fraction of naïve T cells in normal individuals is likely to undergo direct conversion into memory-like cells by LIP in the face of stiff competition by bystander T cells. Thus, the diversity of T cells present in different T cell compartments is markedly different. Despite fluctuations in cell numbers in different compartments during immune responses, the immune system maintains both the numbers and diversity of T cells in the naïve and memory compartments during an individual's

Table 1. Factors required for the homeostasis of αβ T cells

	Cytokines	Self-peptide MHC
Naïve	IL-7 is needed for survival of both $CD4^+$ and $CD8^+$ T cells. IL-7 is essential for LIP of naïve T cells.	Interaction with spMHC is necessary for survival of naïve $CD4^+$ and $CD8^+$ T cells and for LIP.
Memory	IL-15 is needed for basal proliferation. IL-7 or IL-15 can support acute LIP of memory T cells.	spMHC are not required for memory T cell survival but are necessary for the maintenance of memory cell function.

lifetime. This is accomplished through competition for spMHC and cytokines.

Central theme in lymphocyte homeostasis: Competition for limiting resources

Competition for extraneous and limited resources that are required for the survival and proliferation of T cells emerges as a central theme in the regulation of lymphocyte homeostasis. In contrast to other known biological systems in which population numbers are regulated, such as quorum sensing in bacteria (12) and community effect in *Xenopus* embryonic mesoderm (13, 14), T cell homeostasis does not involve competition for autocrine ligands produced by the T cells themselves but for extraneous factors such as cytokines and spMHC that are produced by dendritic cells and stromal cells in the lymphoid tissues. These limited and limiting resources, which are often referred to as "space", set the limits on the numbers and the diversity of an individual T cell population. Table 1 summarizes the factors for which αβ T cells compete (15). These factors include both pro-survival cytokines as well as spMHC. The major pro-survival cytokines, IL-7 and IL-15, are produced constitutively by dendritic cells and stromal cells in the lymphoid tissues (16, 17). spMHC is also expressed at high levels by dendritic cells, which are believed to be the most important source of spMHC for T cells. Because recognition of particular spMHC depends on TCR specificity, whereas interactions between cytokines and their receptors is independent of T cell specificity, the pro-survival cytokines regulate total T cell numbers while spMHC regulates the T cell repertoire (Figure 3).

The homeostasis of T cell numbers and diversity is brought about through a balance of generation, proliferation, survival, death and differentiation of T cells (18). T cells are generated in the thymus and the naïve T cell compartment is primarily replenished by thymic output. At the extremes of life, i.e., in neonates and in old age, the thymic output is not adequate to fill the peripheral T cell compartment. Under these circumstances, increased survival and proliferation of T cells in the periphery is believed to compensate in a manner that maintains the total number of T cells. This highlights the importance of peripheral mechanisms such as proliferation, survival, death and differentiation in the regulation of T cell homeostasis under normal physiological conditions.

T cells in different compartments turnover at different rates. For example, naïve T cells rarely proliferate while, in contrast, memory T cells undergo a slow but steady proliferation that depends on availability of IL-15 (19). Naïve T cells require both IL-7 and spMHC for survival (20, 21). However, spMHC are not required for the survival of both $CD8^+$ and $CD4^+$ memory T cells (22, 23), but rather are needed for the maintenance of their function (24). The lack of dependence of memory T cells on spMHC probably ensures the long-term maintenance of memory cells that are selected by a particular antigen experience, without being subjected to the skewing effects of the self-peptide repertoire. Pro-survival cytokines such as IL-7 and IL-15 increase the levels of anti-apoptotic factors such as Bcl-2 and Bcl-xL in T cells, while the lack of access to survival cytokines results in their apoptotic death (25). Upregulation of anti-apoptotic

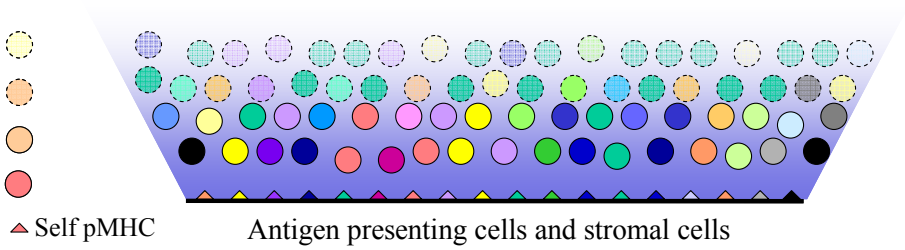

Figure 3. Competition for spMHC regulates the T cell repertoire while competition for pro-survival cytokines regulates the total T cell number. Circles of different colors represent T cells of different specificities and triangles of a particular color represent the set of spMHC displayed in steady-state conditions on the surface of APCs that can be recognized by T cells of the corresponding specificity. The gradient of blue represents the limiting availability of survival cytokines, IL-7 and IL-15, that are constitutively produced by antigen presenting cells and stromal cells in the lymphoid tissues. T cells are driven to occupy the available space and the T cells that do not have adequate access to space consequently die out (represented by broken circles). While interaction with spMHC is TCR specific, signaling by cytokines does not depend on the TCR specificity.

factors such as Bcl-2 is especially important in the long-term maintenance of memory T cells (26). Competition for limiting pro-survival factors, which becomes especially pronounced in densely crowded lymphoid organs, is a key mechanism for maintaining T cell homeostasis.

Rapid proliferation of naïve T cells can occur under two circumstances and in both cases it is accompanied by concomitant phenotypic change or differentiation into effector or memory cells. When stimulated by antigens, naïve T cells are activated and undergo rapid proliferation and concomitant differentiation into effector cells and subsequently into memory cells. Naïve T cells also proliferate in a lymphopenic setting, but in this case they differentiate directly into memory-like cells or "homeostatic" memory cells by a process that is dependent on IL-7 and spMHC (9). These "memory-like" cells are indistinguishable from conventional antigen-induced memory cells based on a variety of functional and molecular assays (27). LIP also occurs under physiological conditions, as in neonates when the thymic output has yet to fill the peripheral space with mature T cells (28). It is also believed to be important in old age when lymphopenic conditions may be present due to a severe decline in thymic output or other physiological changes. LIP is often equated with homeostatic proliferation by some investigators. We prefer not to use this term because LIP of naïve T cells does not result in the replenishment of the naïve T cell compartment even in the long term (29). Rather LIP results in differentiation of naïve T cells into memory cells and therefore contributes to homeostasis of the memory compartment. Thus, T cell numbers and diversity are maintained throughout life through a balance of generation, proliferation, survival, death and differentiation.

Competition among naïve T cells of different specificities

Competition among TCR-different T cells for specific spMHC maintains the structural diversity of T cells. Several studies have examined the competition between T cells of different specificities for both $CD8^+$ and $CD4^+$ lineages. Some examples from studies on $CD8^+$ T cells are outlined in this section and similar conclusions can also be drawn for $CD4^+$ T cells. Naïve polyclonal T cells proliferate when adoptively transferred into TCR transgenic hosts, but not into syngeneic wild-type hosts, which have a much greater TCR diversity. These findings suggest that the proliferation of donor cells depends on the T cell repertoire of the host. $CD8^+$ T cells expressing the P14 TCR proliferated when transferred into OT-1 TCR transgenic mice or vice versa but P14 or OT-1 cells did not proliferate when transferred into mice expressing the same TCR (30). Therefore, it appears that TCR transgenic mice have additional "space" for TCR-different T cells. This is consistent with the observation that TCR transgenic mice have fewer numbers of T cells than the corresponding numbers of $CD4^+$ or $CD8^+$ T cells in wild-type mice. For example, there are approximately 20 million $CD8^+$ T cells in 2C TCR transgenic mice on the $RAG^{-/-}$ background whereas there are about 40 million $CD8^+$ T cells in wild type mice. Thus, competition for spMHC affects both the number and diversity of T cells.

There are dramatic differences in the ability of T cells of different specificities to undergo LIP. For instance, HY T cells do not undergo LIP in syngeneic female lymphopenic hosts (31). F5, 2C and OT-1 T cells undergo increasing degrees of LIP, in that order, when transferred into syngeneic $RAG^{-/-}$ hosts. The ability of T cells to undergo LIP is correlated with the strength of interaction between the TCR and spMHC as indicated by the levels of CD5 (32). LIP of naïve $CD8^+$ T cells of a particular specificity is dramatically inhibited when adoptively transferred into TCR transgenic hosts expressing a TCR whose interaction with spMHC exceeds that of the donor T cells (32). In this particular study, it was shown that 2C TCR transgenic mice have space for the proliferation of OT-1 cells but have little or no space for the proliferation of F5 cells. This is notable because F5, 2C and OT-1 T cells probably compete for different self-peptides. 2C TCR transgenic mice clearly have space for additional TCR-different T cells. But why is it then that only some T cells such as OT-1 proliferate and others such as F5 do not proliferate in the 2C TCR transgenic recipients? One possible explanation is that the spMHC and cytokine signals are spatially and temporally coupled. In such a scenario, the T cells that are better at competing for spMHC, due to a greater interaction with spMHC, could have better access to pro-survival cytokines. This could result in an added dimension of complexity in the competition for resources among T cells such that the ability to access to one resource may ensure better access to a second resource. Consistent with this notion, it has been recently shown that IL-15 is not free *in vivo* but is "presented" by cells that produce it, bound to IL-15Rα on those cells (33). Thus, it is possible that IL-15 may be co-presented by the same APCs that present spMHC to T cells for homeostatic regulation. This mechanism would allow T cells to be signaled by spMHC only on certain specialized cell types such as dendritic cells for the purposes of T cell homeostasis. Co-presentation of cytokines and spMHC in specialized niche areas may also serve to avoid the danger of autoimmunity. Thus, cytokines and spMHC interact in complex ways with a diverse repertoire of T cells in the lymphoid tissues in order to maintain homeostasis of T cell diversity.

Competition between naïve and memory T cells

T cells in the periphery exist in two pools: naïve and memory. Unlike naïve T cells, memory T cells undergo significant basal proliferation in the periphery in the absence of cognate or non-cognate spMHC. Since this process occurs without displacing the naïve T cells, it was initially believed that naïve and memory T cells are independently regulated for the purposes of T cell homeostasis (34). However, more recent studies have suggested that this is only partially correct. Although the sizes of the naïve and memory T cell compartments may be independently regulated because they

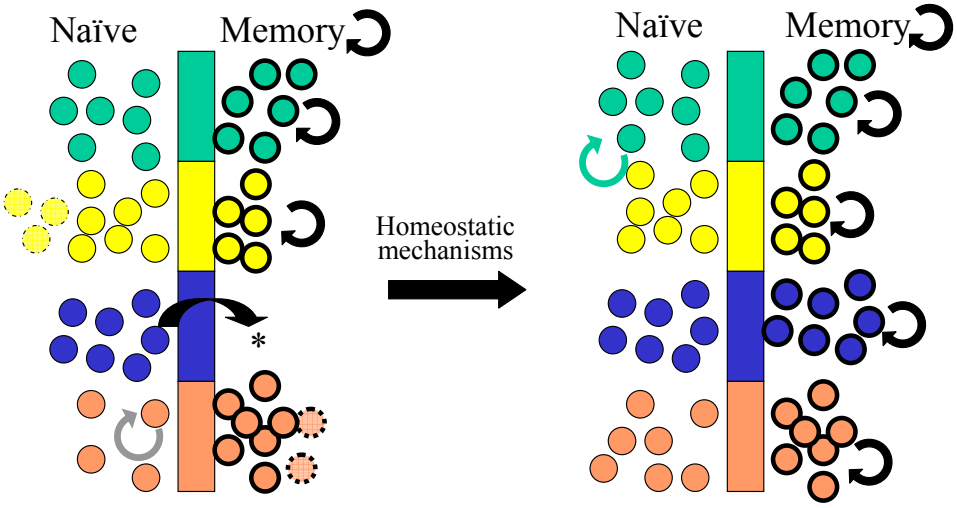

Figure 4. Homeostasis of T cell diversity in the naïve and memory T cell pools. Both naïve and memory T cells compete for spMHC. In the steady state, naïve T cells divide occasionally in the periphery while memory T cells undergo significant basal proliferation. However, there is a hole in the repertoire created by the absence of memory cells of a certain specificity, it can be filled up by endogenous proliferation of naïve T cells in the absence of foreign antigen analogous to the process of LIP. The circles represent T cells of a particular specificity and the rectangles represent the corresponding cognate spMHC that are displayed on the surface of APCs. The circular arrows represent the cycling or basal proliferation of cells.

compete for different cytokines, the naïve and memory T cell repertoires are interdependent and homeostasis of naïve and memory T cell diversity is not independently regulated (32, 35). For example, naïve 2C T cells do not undergo LIP when transferred into lymphopenic mice containing a small population of memory 2C cells generated either by LIP or antigen-induced proliferation (32). Although memory T cell survival does not require TCR-spMHC interaction, these cells do engage spMHC in order to maintain their functional reactivity. In addition, both naïve and memory T cells express the IL-7 receptor. Thus, naïve and memory T cells having the same TCR compete for spMHC as well as for cytokine. Competition for the same spMHC prevents naïve T cells from undergoing LIP and thus keeps them naïve. Competition for cytokines limits the total number of T cells of a given T cell specificity. TCR-different memory T cells also compete for the same cytokine, thus keeping the total number of memory cells steady.

Competition for spMHC between naïve and memory T cells can also occur under steady state conditions and shape their respective repertoires. It has been shown that infusion of polyclonal T cells into $RAG^{-/-}$ mice inhibits the proliferation of additional newly transferred polyclonal T cells in a way that depends upon the repertoire of the pre-existing T cells (35). The second population of transferred polyclonal T cells was shown to proliferate, as if to fill up the available "holes in the repertoire". While LIP is generally studied in the extreme case of complete lymphopenia, it is entirely possible that an analogous process occurs under steady state conditions allowing spontaneous conversion of certain naïve T cells into memory cells. It is possible that the conversion of naïve to memory cells by LIP occurs at low levels under physiological conditions whenever there is a "hole" in the memory repertoire of the same specificity as the proliferating naïve T cells. In such a case, although there is no total lymphopenia, a hole in the repertoire can be viewed as a selective lymphopenia that is restricted to T cells of a particular specificity (Figure 4). Thus, the spontaneous conversion of naïve to memory cells may contribute to the diversity of memory T cells.

Diversity of self-peptides in the periphery

The immune system uses a diverse array of spMHC, not only to select a diverse TCR repertoire in the thymus, but also to keep a diverse population of naïve T cells alive and poised to respond in the periphery (36, 37). It has been estimated that in both mice and humans, more than 10^{15} different TCRs can be generated in the thymus but only about 1 to 30 in a 100 (i.e., ~10^{13}) can survive thymic selection (38, 39). However there is space in the periphery of a mouse for only about 10^{8} T cells and in humans for about 10^{12} T cells (11, 40). Thus, selection for certain TCR specificities is likely to occur in the periphery due to competition for specific spMHC. The homeostasis of T cell numbers and diversity is interrelated in the sense that both processes involve competition for resources. While the homeostasis of T cell numbers involves competition for resources that are shared among all T cells, the maintenance of T cell diversity requires competition for access to a diverse set of peripheral self-peptides.

The diversity of the self-peptide repertoire has been estimated and some quantitative estimates are presented below for the class I spMHC repertoire and similar arguments can be made for the class II spMHC repertoire. From a total of 30,000 distinct proteins present in the mouse or human with an average length of 400 amino acids, it can be calculated that nearly 10^{7} peptides of 8-9 amino acids in length can be generated. This estimate assumes no preference for specific protease cleavage sites. For a peptide to be presented on class I MHC it needs to be 1) generated by proteases, 2) transported by TAP into the endoplasmic

Table 2. Classification of peptide MHC based on T cell response

Type of peptide MHC complex	Induced T cell response	Specificity for a given TCR
Agonist	Full activation of T cell; cytokine secretion; T-cell proliferation; CTL effector function	++++
Antagonist	Inhibits activation by agonist; does not induce activation on its own	++
Weak agonist	Same as agonist, but requires a higher dose for the same degree of activation; slower activation kinetics	++
Partial agonist	Induces some, but not all, effects of agonist (e.g., cytokine secretion but not proliferation)	++
Null	No detectable activation	Not TCR specific

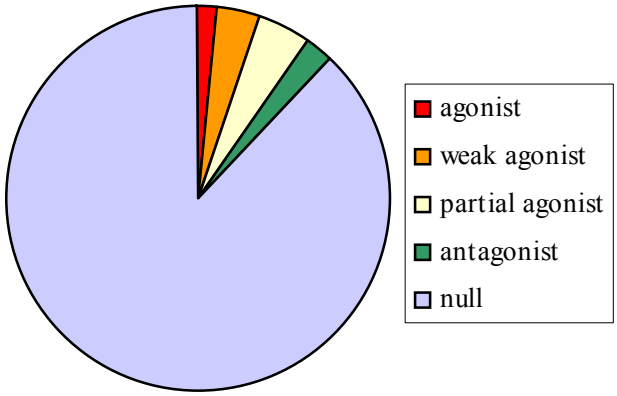

Figure 5. Hypothetical proportions of different self-ligands in the periphery seen from the perspective of a single TCR and vice versa. From a single TCR perspective, null peptides are likely to be the most abundant since they are not TCR specific. High affinity agonist peptides among spMHC are likely to be the least abundant because T cells that strongly recognize self-peptides are negatively selected in the thymus. For any given TCR found in the periphery, an intermediate fraction of the self-peptide repertoire could be low affinity ligands such as weak or partial agonists or even antagonists because there is likely to be higher cross-reactivity among low affinity ligands for a given TCR than for high affinity ligands. A similar argument can be made from a single spMHC perspective that majority of the TCR specificities will see it as a null peptide; a very small fraction of TCRs will recognize it as an agonist and an intermediate fraction of TCRs will recognize any particular spMHC complex as a low affinity ligand such as a weak agonist, partial agonist or an antagonist.

reticulum, and 3) bound by an MHC molecule. When a peptide sequence corresponding to a naturally occurring epitope is inserted anywhere into a protein, it is nearly always displayed on the cell surface but at widely varying levels depending on its flanking sequences unless the epitope carries an obligate protease cleavage site (41). There may be some selection for transport of particular peptides occurring at the level of TAP (42) but the major bottleneck for display of a peptide on the surface lies in its ability to form stable peptide MHC complexes. Evidence suggests that the *a priori* probability for any random peptide to bind relatively stably to MHC is about 1% (43). This leaves only around 10^5 of a total of 10^7 peptides that can be generated in the animal to be presented on the surface of APCs as peptide-MHC complexes per MHC allele.

Experiments suggest that majority of the self-peptides on class I MHC are derived from abundant housekeeping proteins such as ribosomal proteins, N-terminal leader sequences, histones and heat shock proteins (44). The ~30,000 distinct proteins expressed in the mouse or human are present in widely varying quantities in the body. Since there are only around 100,000 MHC class I molecules expressed by each APC, equal representation of peptides on MHC class I molecules from each of these proteins is not possible (45). It has been found that the population of MHC class I molecules on uninfected cells simultaneously displays about 1,000 to more than 10,000 different ‘‘naturally processed’’ peptides with a broad range of copy number. A few of these peptides are present at 10,000 copies per cell, whereas the remaining are presented at less than ten to a couple of hundred copies per cell (46). Thus, assuming a mean of 100 copies per peptide, a single APC presents about 10^3 different self-peptides albeit in widely varying quantities. In comparison, the total number of distinct spMHC complexes in an animal can be estimated to be about 10^5 (see above) which is many orders of magnitude smaller than the TCR diversity. This disparity implies that the spMHC molecules are shared among different TCRs, highlighting the degree of competition for the same spMHC that can occur among T cells expressing different TCRs.

From a single TCR perspective, self-peptides can be divided on the basis of their biological activity into agonist peptides, weak agonists, partial agonists, antagonists or null peptides (Table 2). However, these are not all present in identical proportions (Figure 5). Peptides that are null (non-cognate) are likely to be the most abundant. Agonist peptides for a given TCR are likely to be the least abundant because they are most TCR specific and because T cells that strongly recognize self-peptides are negatively selected in the thymus. It seems counterintuitive that αβ T cells specific for any agonist self-peptides can survive negative selection under the physiological conditions. Not all self-antigens are expressed in the thymus at adequate levels to allow strict negative selection. Such T cells could recognize self-antigens as agonists in the periphery and are potentially autoreactive but are kept in check by various mechanisms of peripheral tolerance (47). Nevertheless, in the self-peptide repertoire, the fraction of agonist peptides for a given TCR is likely to

be extremely small. For any given TCR found in the periphery, a slightly larger fraction of the self-peptide repertoire could be intermediate affinity ligands such as weak or partial agonists or even antagonists. They are likely to be more abundant than agonist peptides not only because T cells are positively selected on such low affinity ligands in the thymus which are presumably also be found in the periphery but also because there is a higher cross-reactivity among low affinity ligands for a single TCR. From a single spMHC perspective, a similar argument can be made that any given spMHC will be seen as a null peptide by a majority of the TCR specificities. A very small fraction of TCRs will recognize a given spMHC as an agonist and an intermediate fraction of TCRs will recognize any particular spMHC complex as a low affinity ligand such as a weak agonist, partial agonist or an antagonist. Although it is interesting to speculate on the nature of single TCR-spMHC interactions, it should be emphasized that when a T cell interacts with a single dendritic cell, ~10^5 TCRs on the T cell interact with a diverse set of spMHC on the surface of the APC, which in combination, give rise to a TCR signal.

Identity of the self-peptides that regulate T cell homeostasis

The identity of self-peptides that regulate the homeostasis of T cell diversity in the periphery is still not clear. Competition between clones expressing different TCRs during LIP suggests that self-peptides are present in limiting amounts in the periphery. The low affinity self-peptides, rather than agonist peptides, are likely to drive LIP because of the lack of observable T cell activation. Studies with $CD4^+$ cells selected in H2-$M^{-/-}$ mice, which express mostly the CLIP peptide (class II-associated invariant chain peptide) in the periphery, suggest that the positively selecting ligands in the thymus are responsible for LIP in the periphery (48). However, this does not entirely rule out the possibility that agonist self-peptides play a role in LIP. Weak agonist peptides may be able to drive LIP without resulting in conventional activation either because they are present in extremely low amounts or because they stimulate T cells in the absence of costimulation or by cooperating with other (null) spMHC. Regardless whether agonist peptides or low affinity peptides or both support lymphocyte homeostasis, given that the amount of these peptides is small, it is natural that competition for these peptides contributes to the homeostasis of TCR diversity in the periphery.

Given that null peptides are most abundant, it is possible that they could play a role in lymphocyte homeostasis. It should be emphasized that the definition of a null peptide-MHC complex (pMHC) is only related to its lack of potential to induce any grade of activation in a T cell. It does not mean that null pMHCs are biochemically null in terms of interacting with a TCR. A TCR contacts pMHC at its three complementarity determining regions (CDR1, CDR2 and CDR3). The CDR1 and CDR2 regions of the TCR primarily interact with the MHC portion of the pMHC complex and the CDR3 interacts with the presented peptide. If the peptide in the pMHC complex is a null peptide it is conceivable that a stable TCR-pMHC complex is not formed but the CDR1 and CDR2 can still interact weakly with the MHC alone, perhaps giving rise to short-lived "TCR-null pMH" complex. Such transient interactions, wherein the peptide plays a minimal contributory role, may become cumulatively significant because of the abundance of null peptides and may signal into T cells. This may contribute to what has been previously called "TCR tickling" required for naïve T cell survival. Since null pMHC are the most abundant self ligands, they could regulate the homeostasis of total lymphocyte numbers while the less abundant low affinity self ligands could regulate the homeostasis of TCR diversity. Thus, the homeostasis of lymphocyte numbers and diversity are intimately related to the repertoire of the self-peptides in the periphery.

Competition-diversity paradox

One of the unavoidable consequences of stiff competition for limiting resources is that T cell clones that are better able to survive and proliferate can be expected to dominate and thus reduce the diversity of the total T cell pool. This is often referred to as the competition-diversity paradox (49). In some cases, the immune system has evolved means to minimize the loss of diversity resulting from competition. This is exemplified by the adaptation of the immune system to minimize the severity of the competition for IL-7 *via* regulation of IL-7Rα (50). T cells which have a fortuitous access to high amounts of IL-7 by virtue of being closer to cells producing IL-7 or because they express a high amount of the IL-7 receptor may have an advantage and may preferentially survive and proliferate while the rest may die of neglect. One of the ways by which the immune system overcomes this potential problem is by downregulating the expression of IL-7Rα upon exposure to IL-7. The importance of this limiting mechanism is highlighted by the fact that transgenic mice that over-express IL-7Rα contain fewer peripheral T cells than their littermate controls, indicating too much signaling through IL-7R may be counter-productive. In contrast, T cell numbers are expanded in IL-7 transgenic mice that have an increased level of IL-7. This finding shows that IL-7 is present in limiting amounts and the total amount of IL-7 in the periphery determines the size of the lymphocyte pool. Furthermore, signaling by pro-survival cytokines IL-2, IL-4, IL-6, and IL-15, which are produced by antigen-activated T cells, also decreases IL-7Rα expression, while increasing the expression of their own receptors. As a result, T cells that undergo acute proliferation in response to foreign antigens downregulate IL-7Rα expression and therefore do not compete for IL-7 with naïve T cells. This ensures the survival of the rest of the T cells in the naïve compartment by making IL-7 available to naïve T cells. Thus, the downregulation of IL-7Rα on T cells, which is triggered by IL-7 signaling, at least partly resolves the competition-diversity paradox for IL-7 competition.

In some situations, the competition for limiting resources may indeed lead to the survival of T cell clones that are more useful for the immune system. For instance, although there may be loss of diversity of naïve T cells in the periphery due to competition for specific self-peptides which are necessary for T cell survival, this may indeed lead to physiological selection of naïve T cells that are better suited for specific functions. T cells selected by spMHC may maintain the TCR signaling cascade at an optimal threshold for responding to foreign antigen with the highest sensitivity. It has been shown that naïve and memory T cells of the same specificity compete for the same spMHC. But neither naïve nor memory T cells dominate the competition in the organism and a relatively stable ratio of naïve to memory T cells is maintained so that the host may be adequately prepared against both novel and previously encountered pathogens. Although all memory T cells compete for the same cytokine (i.e., IL-15), it may be advantageous for an organism to retain the most recently generated memory T cell fraction at higher proportions, as the risk of re-infections by the most recently encountered pathogens is high soon after the initial exposure due to the continuous circulation of the pathogen in the immediate environment. Thus, the competition-diversity paradox may indeed be a feature of the immune system that does not maximize but rather optimizes the diversity of T cells for the efficient function of the immune system.

Defects in lymphocyte homeostasis and autoimmunity

In a very broad sense, autoimmunity is a case of disturbed lymphocyte homeostasis in which there is an increased number of autoreactive clones. It is speculated that clonal competition for spMHC among T cells in a lymphoreplete individual results in the maintenance of T cell diversity and also retards the outgrowth of autoimmune clones (51). Lymphopenia can reduce the severity of clonal competition, allowing the dominance of autoreactive T cells in the repertoire and the onset of autoimmune disorders is often related to episodes of lymphopenia (52). Many viruses that are known to induce transient lymphopenia in humans are also associated with autoimmunity (53). Naïve T cells differ in their competitive fitness to undergo LIP and clones with the highest capacity for LIP, which is determined by their degree of interaction with spMHC, dominate the repertoire following lymphopenia (54). Thus, lymphopenia results in the skewing of the repertoire towards T cells that have a greater autoreactivity and are functionally more reactive because of their conversion into memory-like cells. Unlike naïve T cells, these memory-like cells can rapidly produce IFN-γ without the need for proliferation and can be activated by antigen independent of CD28 costimulation. Therefore, it is speculated that LIP can lead to autoimmunity. There are several animal models that link lymphopenia and a consequent restricted T cell repertoire to autoimmunity. For example, when neonatal mice are thymectomized between day 2 and 4, they develop autoimmunity in multiple organs (55). In mice, the number of lymphocytes reaches a plateau in the lymph nodes by day 7 and in the spleen by day 14 (56). Thymectomy performed after day 7, when the peripheral compartment has been filled with diverse T cells by thymic output, does not result in autoimmunity. But if thymectomy is performed in adult mice in addition to depleting lymphocytes with cyclophosphamide treatment, autoimmune gastritis ensues (57). Transfer of small polyclonal populations of $CD4^+$ T cells into $RAG^{-/-}$ mice also results in autoimmunity. This was most dramatic when 2×10^5 polyclonal naïve $CD4^+$ $CD45RB^{hi}$ T cells were transferred, while transfer of 2×10^7 $CD4^+CD45RB^{hi}$ T cells did not result in disease. In all the examples cited above, introduction of $CD4^+CD25^+$ cells into the mice can protect them from autoimmunity (58). But majority of these observations can also be explained by the lack of clonal competition. For instance, cotransfer of an equal number of AND $CD4^+$ T cells protects $RAG^{-/-}$ mice that are infused with 2×10^5 polyclonal naïve $CD4^+CD45RB^{hi}$ T cells. Transfer of OT-1 $CD8^+$ T cells also offers partial protection because the space between $CD4^+$ and $CD8^+$ cells is shared, i.e., they compete for spMHC on the same APCs as well as for the same cytokines. Both AND and OT-1 T cells are $CD5^{hi}$, which reflects their ability to effectively compete for spMHC. Since AND $CD4^+$ T cells or OT-1 $CD8^+$ T cells lack suppressive activity *in vitro* unlike $CD4^+CD25^+$ regulatory T cells, the inhibition of autoimmunity is thought to result from increased clonal competition that may reduce the degree of LIP among the cotransferred polyclonal $CD4^+$ cells. These findings highlight the importance of clonal competition for spMHC in preventing autoimmune disorders.

Future directions

Interactions between TCR and pMHC are of fundamental importance throughout T cell development. T cells are positively selected in the thymus on self-peptides and interaction with spMHC is critical for T cell homeostasis in the periphery. Both naturally occurring and synthetic positively selecting peptides have been identified and characterized for various TCRs because of the availability of *in vitro* models of positive selection (59). This has led to a mechanistic understanding of the process of positive selection. Similarly, development of *in vitro* models of lymphocyte homeostasis could help identify the self-peptides as well as the molecular details of the signaling mechanisms involved (20, 60). Identifying the self-peptides that regulate the homeostasis of T cell numbers and diversity may well provide a key to understanding the precise relationship between lymphopenia and autoimmunity. Competition for survival signals and growth factors in localized environments can create specialized niches for subpopulations of T cells, such as naïve T cells. Co-presentation of self-peptides and cytokines, such as IL-15, on specialized populations of antigen presenting cells could provide a niche for memory T cells. Although there have been rapid advances in the field of T cell homeostasis over the past few years, many questions remain to be investigated.

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References

1. Cannon WB. The wisdom of the body. New York: W.W. Norton & Co.; 1932.
2. Jameson SC. Maintaining the norm: T-cell homeostasis. Nat Rev Immunol. 2002;2:547-556.
3. Tanchot C, Rosado MM, Agenes F, Freitas AA, Rocha B. Lymphocyte homeostasis. Semin Immunol. 1997;9:331-337.
4. Freitas A, Chen J. Introduction: regulation of lymphocyte homeostasis. Microbes Infect. 2002;4:529-530.
5. Grossman Z, Min B, Meier-Schellersheim M, Paul WE. Concomitant regulation of T-cell activation and homeostasis. Nat Rev Immunol. 2004;4:387-395.
6. Khaled AR, Durum SK. Lymphocide: cytokines and the control of lymphoid homeostasis. Nat Rev Immunol. 2002;2:817-830.
7. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature. 1999;401:708-712.
8. Woodland DL, Dutton RW. Heterogeneity of $CD4^+$ and $CD8^+$ T cells. Curr Opin Immunol. 2003;15:336-342.
9. Cho BK, Rao VP, Ge Q, Eisen HN, Chen J. Homeostasis-stimulated proliferation drives naïve T cells to differentiate directly into memory T cells. J Exp Med. 2000;192:549-556.
10. Goldrath AW, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. J Exp Med. 2000;192:557-564.
11. Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human αβ T cell receptor diversity. Science. 1999;286:958-961.
12. Miller MB, Bassler BL. Quorum sensing in bacteria. Annu Rev Microbiol. 2001;55:165-199.
13. Gurdon JB. A community effect in animal development. Nature. 1988;336:772-774.
14. Gurdon JB, Lemaire P, Kato K. Community effects and related phenomena in development. Cell. 1993;75:831-834.
15. Sprent J, Surh CD. Cytokines and T cell homeostasis. Immunol Lett. 2003;85:145-149.
16. Grabstein KH, Eisenman J, Shanebeck K, et al. Cloning of a T cell growth factor that interacts with the β chain of the interleukin-2 receptor. Science. 1994;264:965-968.
17. Waldmann T, Tagaya Y, Bamford R. Interleukin-2, interleukin-15, and their receptors. Int Rev Immunol. 1998;16:205-226.
18. Ge Q, Hu H, Eisen HN, Chen J. Naïve to memory T-cell differentiation during homeostasis-driven proliferation. Microbes Infect. 2002;4:555-558.
19. Goldrath AW, Sivakumar PV, Glaccum M, et al. Cytokine requirements for acute and Basal homeostatic proliferation of naive and memory $CD8^+$ T cells. J Exp Med. 2002;195:1515-1522.
20. Tan JT, Dudl E, LeRoy E, et al. IL-7 is critical for homeostatic proliferation and survival of naïve T cells. Proc Natl Acad Sci U S A. 2001;98:8732-8737.
21. Kirberg J, Berns A, von Boehmer H. Peripheral T cell survival requires continual ligation of the T cell receptor to major histocompatibility complex-encoded molecules. J Exp Med. 1997;186:1269-1275.
22. Murali-Krishna K, Lau LL, Sambhara S, Lemonnier F, Altman J, Ahmed R. Persistence of memory CD8 T cells in MHC class I-deficient mice. Science. 1999;286:1377-1381.
23. Seddon B, Tomlinson P, Zamoyska R. Interleukin 7 and T cell receptor signals regulate homeostasis of CD4 memory cells. Nat Immunol. 2003;4:680-686.
24. Kassiotis G, Garcia S, Simpson E, Stockinger B. Impairment of immunological memory in the absence of MHC despite survival of memory T cells. Nat Immunol. 2002;3:244-250.
25. Akbar AN, Borthwick NJ, Wickremasinghe RG, et al. Interleukin-2 receptor common γ-chain signaling cytokines regulate activated T cell apoptosis in response to growth factor withdrawal: selective induction of anti-apoptotic (bcl-2, bcl-xL) but not pro-apoptotic (bax, bcl-xS) gene expression. Eur J Immunol. 1996;26:294-299.
26. Grayson JM, Zajac AJ, Altman JD, Ahmed R. Cutting edge: increased expression of Bcl-2 in antigen-specific memory $CD8^+$ T cells. J Immunol. 2000;164:3950-3954.
27. Goldrath AW, Luckey CJ, Park R, Benoist C, Mathis D. The molecular program induced in T cells undergoing homeostatic proliferation. Proc Natl Acad Sci U S A. 2004;101:16885-16890.
28. Min B, McHugh R, Sempowski GD, Mackall C, Foucras G, Paul WE. Neonates support lymphopenia-induced proliferation. Immunity. 2003;18:131-140.
29. Ge Q, Hu H, Eisen HN, Chen J. Different contributions of thymopoiesis and homeostasis-driven proliferation to the reconstitution of naive and memory T cell compartments. Proc Natl Acad Sci U S A. 2002;99:2989-2994.
30. Troy AE, Shen H. Cutting edge: homeostatic proliferation of peripheral T lymphocytes is regulated by clonal competition. J Immunol. 2003;170:672-676.
31. Tanchot C, Lemonnier FA, Perarnau B, Freitas AA, Rocha B. Differential requirements for survival and proliferation of CD8 naive or memory T cells. Science. 1997;276:2057-2062.
32. Ge Q, Bai A, Jones B, Eisen HN, Chen J. Competition for self-peptide-MHC complexes and cytokines between naive and memory $CD8^+$ T cells expressing the same or different T cell receptors. Proc Natl Acad Sci U S A. 2004;101:3041-3046.
33. Burkett PR, Koka R, Chien M, Chai S, Boone DL, Ma A. Coordinate expression and trans presentation of interleukin (IL)-15Rα and IL-15 supports natural killer cell and memory $CD8^+$ T cell homeostasis. J Exp Med. 2004;200:825-834.
34. Goldrath AW. Maintaining the status quo: T-cell homeostasis. Microbes Infect. 2002;4:539-545.
35. Min B, Foucras G, Meier-Schellersheim M, Paul WE. Spontaneous proliferation, a response of naïve CD4 T cells determined by the diversity of the memory cell repertoire. Proc Natl Acad Sci U S A. 2004;101:3874-3879.
36. Bender J, Mitchell T, Kappler J, Marrack P. $CD4^+$ T cell division in irradiated mice requires peptides distinct from those responsible for thymic selection. J Exp Med. 1999;190:367-374.
37. Surh CD, Lee DS, Fung-Leung WP, Karlsson L, Sprent J. Thymic selection by a single MHC/peptide ligand produces a semidiverse repertoire of $CD4^+$ T cells. Immunity. 1997;7:209-219.
38. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. Nature. 1988;334:395-402.
39. Nikolich-Zugich J, Slifka MK, Messaoudi I. The many important facets of T-cell repertoire diversity. Nat Rev Immunol. 2004;4:123-132.
40. Doherty PC, Riberdy JM, Belz GT. Quantitative analysis of the $CD8^+$ T-cell response to readily eliminated and persistent viruses. Philos Trans R Soc Lond B Biol Sci. 2000;355:1093-

1101.
41. Rammensee HG, Falk K, Rotzschke O. Peptides naturally presented by MHC class I molecules. Annu Rev Immunol. 1993;11:213-244.
42. Koopmann JO, Hammerling GJ, Momburg F. Generation, intracellular transport and loading of peptides associated with MHC class I molecules. Curr Opin Immunol. 1997;9:80-88.
43. Bongrand P, Malissen B. Quantitative aspects of T-cell recognition: from within the antigen-presenting cell to within the T cell. Bioessays. 1998;20:412-422.
44. Jardetzky TS, Lane WS, Robinson RA, Madden DR, Wiley DC. Identification of self peptides bound to purified HLA-B27. Nature. 1991;353:326-329.
45. Yewdell JW, Reits E, Neefjes J. Making sense of mass destruction: quantitating MHC class I antigen presentation. Nat Rev Immunol. 2003;3:952-961.
46. Engelhard VH. Structure of peptides associated with class I and class II MHC molecules. Annu Rev Immunol. 1994;12:181-207.
47. Piccirillo CA, Thornton AM. Cornerstone of peripheral tolerance: naturally occurring $CD4^{+}CD25^{+}$ regulatory T cells. Trends Immunol. 2004;25:374-380.
48. Ernst B, Lee DS, Chang JM, Sprent J, Surh CD. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. Immunity. 1999;11:173-181.
49. Freitas AA, Rocha B. Population biology of lymphocytes: the flight for survival. Annu Rev Immunol. 2000;18:83-111.
50. Park JH, Yu Q, Erman B, et al. Suppression of IL-7Rα transcription by IL-7 and other prosurvival cytokines: a novel mechanism for maximizing IL-7-dependent T cell survival. Immunity. 2004;21:289-302.
51. Stockinger B, Barthlott T, Kassiotis G. The concept of space and competition in immune regulation. Immunology. 2004;111:241-247.
52. Gleeson PA, Toh BH, van Driel IR. Organ-specific autoimmunity induced by lymphopenia. Immunol Rev. 1996; 149:97-125.
53. Hernan MA, Zhang SM, Lipworth L, Olek MJ, Ascherio A. Multiple sclerosis and age at infection with common viruses. Epidemiology. 2001;12:301-306.
54. La Gruta NL, Driel IR, Gleeson PA. Peripheral T cell expansion in lymphopenic mice results in a restricted T cell repertoire. Eur J Immunol. 2000;30:3380-3386.
55. Penhale WJ, Farmer A, McKenna RP, Irvine WJ. Spontaneous thyroiditis in thymectomized and irradiated Wistar rats. Clin Exp Immunol. 1973;15:225-236.
56. Garcia AM, Fadel SA, Cao S, Sarzotti M. T cell immunity in neonates. Immunol Res. 2000;22:177-190.
57. Barrett SP, Toh BH, Alderuccio F, van Driel IR, Gleeson PA. Organ-specific autoimmunity induced by adult thymectomy and cyclophosphamide-induced lymphopenia. Eur J Immunol. 1995; 25:238-244.
58. Barthlott T, Kassiotis G, Stockinger B. T cell regulation as a side effect of homeostasis and competition. J Exp Med. 2003;197: 451-460.
59. Santori FR, Kieper WC, Brown SM, et al. Rare, structurally homologous self-peptides promote thymocyte positive selection. Immunity. 2002;17:131-142.
60. Ge Q, Palliser D, Eisen HN, Chen J. Homeostatic T cell proliferation in a T cell-dendritic cell coculture system. Proc Natl Acad Sci U S A. 2002;99:2983-2988.