Homeostasis of αβ TCR+ T cells

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Cytokines contribute to T cell homeostasis at all stages of T cell existence. However, the particular cytokine involved varies as T cells progress from a naïve through an activated to a memory state. In many cases the important cytokines are members of the interleukin 2 subfamily of the short-chain type I cytokines. A case is made for the idea that the evolutionary divergence of the short-chain family allowed for concurrent divergence in leukocytes.

Multicellular organisms sacrificed the simplicity of protists to gain the advantages that cellular specialization affords. However, at the time that they gained the selective advantage of specialized cells, multicellular organisms had to develop many specialized systems that were not required by their unicellular ancestors. Among these was the ability to regulate the numbers of cells in the organism devoted to any particular task. Theoretically the numbers of any particular type of cell can be regulated at one or more points. By their rates of production and death of course, but also by events that might occur once they are produced that do not necessarily lead to cell death, for example, by their rates of division once created. Within the immune systems of higher vertebrates numbers of T cells are probably controlled by all these phenomena.

Evidence that T cell homeostasis exists

The idea that the numbers of T cells in mice and human are well controlled is commonplace. So much so, in fact, that peripheral T cell counts are used in human patients to monitor certain diseases, such as progression to AIDS. But there is plenty of other evidence that T cell numbers are controlled. For example, T cell numbers in the spleens and lymph nodes of mice are remarkably consistent within members of a given strain at a given age. Sublethal irradiation greatly reduces T cell counts in mice and humans, although the number of cells slowly returns to normal after the insult. Virus infection dramatically increases T cell counts in mice and humans, although these increases rapidly subside to normal levels as the infection is rejected[1,2–4]. Mice trapped in the wild contain about the same numbers of T cells as laboratory strains despite the fact that wild mice have presumably been exposed to many more antigens than the laboratory strains[5].

The example of mice transgenic for rearranged genes encoding the T cell receptor (TCR) is particularly dramatic. In mice expressing the right major histocompatibility complex proteins every thymocyte is a candidate for maturation into a T cell. However, such mice do not contain more T cells than normal animals[6–11].

Finally there are several examples of the fact that mature T cells can somehow detect the absence of their own kind in animals and respond by rapid division. In neonatal mice many of the mature T cells are dividing[12] and after thymectomy T cell numbers still increase in young animals[13]. In adult animals a subset of T cells divide every 24–48 h[14–18]. It is possible, however, that some of the cells in these latter studies were dividing in response to antigen and, as others have reported, that most peripheral T cells are not in constant division[19,20]. Whether naïve T cell division is driven by homeostasis rather than outright challenge with antigen remains uncertain.

Whatever the situation in normal animals, it is clear that mature T cells can detect the absence of their fellows because such cells divide rapidly after transfer to T cell–deficient mice but not after transfer to normal mice[17,21–26]. Such division is not caused by obvious exogenous antigens, the division does not lead to animals that contain more T cells than animals in which no proliferation is apparent, and the phenomenon does not appear to discriminate between $CD4^+$ and $CD8^+$ T cells. That is, $CD4^+$ T cells do not divide unexpectedly if transferred to an animal containing $CD8^+$ cells but no CD4 cells and *vice versa* (J. Bender and P. Marrack, unpublished observations). It is not known what T cells are sensing in these experiments. Perhaps the signal is increased access to dendritic cells or increased availability of cytokines, which (in T cell–sufficient animals) are normally consumed.

Despite this last point, there is evidence that CD4 and CD8 T cells are controlled to a certain extent independently. In CD4-deficient mice $CD8^+$ T cells compensate for the lack of $CD4^+$ T cells by increasing their numbers, but the reverse is not true in CD8-deficient animals[27]. The ratio of $CD4^+$:$CD8^+$ T cells is very consistent in any given strain of mouse, but varies markedly between strains. For example, BALB/c and DBA/2 animals have CD4:CD8 ratios of about 4:1, whereas the ratio in MHC-identical B10.D2 animals is closer to 2:1. In the thymus this difference in ratios is controlled by genes mapping to the *Tcra* locus[28], but in the periphery it is controlled by genes mapping to other loci in addition or instead[29] (and see C. Myrick *et al.*, unpublished observations).

Only a few papers stand in apposition to this notion that mature T cell numbers are tightly regulated. One of these was published in the late 1970s[30]. These authors changed T cell numbers in mice by injecting large numbers of mature T cells into syngeneic animals. The authors calculated, from dilution and percentages, that the transferred cells remained at stable numbers in the recipients and that they increased the total number of T cells in the host by more than 60%. However, the authors did not actually count the numbers of T cells in their animals directly, so it is possible that errors arose due to the indirect methods of measurement used.

If animals can support more than normal numbers of mature T cells then one might predict that increasing the number of thymi in the animal would increase the numbers of mature T cells. In fact that is exactly

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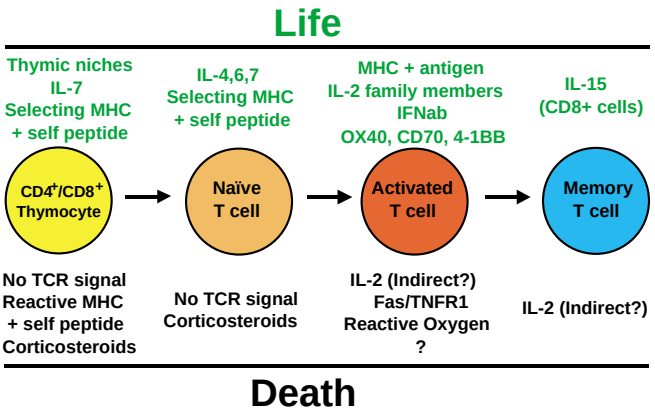

Figure 1. Factors that control cell numbers at different stages of αβ T cell maturation.

what Berzins *et al.* found when they increased the numbers of thymus lobes in animals from two (the normal quota) to 11 in total[31]. The results of this radical increase showed that, in mice, the number of one particular subset of T cells (presumably the naïve cells) increased linearly with thymus number, whereas the number of another subset (memory perhaps?) was unaffected. At first sight this experiment suggests that in animals the number of naïve T cells is governed entirely by production rates rather than by death rates or by factors required for their survival once made. However, it is worth noting that thymus grafts themselves produce at least one of the factors, interleukin 7 (IL-7), which it is thought is needed for survival of mature T cells[32–36]. Therefore, when the authors of this paper increased the numbers of thymi in their animals they increased not only mature T cells production but also IL-7 concentration, and consequently affected survival of the cells after they had left the thymus.

Overall, therefore, the evidence favors the idea that animals control their number of mature T cells. The factors that allow this control are discussed below (and see **Fig. 1**).

Control of homeostasis

There are, of course, many types of T cells bearing αβ receptors, distinguished by previous exposure to antigen (naïve *versus* memory), responsiveness (resting *versus* activated *versus* anergic), specificity for MHC+ peptide (CD4 *versus* CD8) and activities (helper *versus* suppressor *versus* cytotoxic; T_H1 *versus* T_H2, CTL1 *versus* CTL2). We will focus here on homeostasis of different kinds of T cells defined by their previous exposure to antigen and consider the evidence dealing with this problem for naïve, activated and memory T cells separately.

Homeostasis of naïve T cells

Most naïve T cells are created in the thymus and move to the periphery where, if they do not encounter antigen, they eventually die with some defined half-life[37–39]. (Although in young animals, within a few months of thymectomy this disappearance may not be apparent, perhaps because it is counteracted by T cell expansion[20].) The equally naïve observer might infer that product feedback–inhibition of thymus production would be the most obvious and straightforward way to control mature naïve T cell numbers. But there is little evidence for such control. Mice given more thymus lobes contain more mature T cells[31], depletion of peripheral T cells does not increase thymus output in mice or in men[40,41]. Thymus output does increase under certain circumstances, however. For example, sublethal irradiation regenerates the activity of the apparently nonfunctional thymi of old mice. HAART (highly active retroviral therapy) therapy increases the thymic activity of HIV-infected patients[42]. However, even though in both these cases the activity of the thymus increases in animals with a deficiency of peripheral T cells, the treatments may affect the thymus directly.

On the whole, therefore, thymus output seems to be regulated by processes that are internal to the thymus itself. These processes may include the availability of sites in the thymus that are suitable for positive selection and thymocyte maturation. Thus, it has been shown that the thymus contains a limited number of niches in which T cells can mature to a state in which they are fit for export[11] and that some similar limitation, which controls the number of CD4+ CD8+ thymocyte precursors the thymus can contain, limits thymocyte maturation in TCR transgenic and—by extension—normal mice[10].

Once they have emerged from the thymus there is plenty of evidence that naïve peripheral T cells somehow measure the number of T cells that are already present in the animals and respond to a deficiency by dividing. T cells that are newly emerged from the thymus proliferate in very young normal animals[12,14–16,18] (but also see[19,20,43]). Naïve T cells transferred to T cell–deficient animals divide rapidly[17,21–26,44]. This proliferation requires that the T cells interact with the MHC protein on which they were positively selected. However, the nature of the peptide bound to the MHC during this interaction is uncertain. Some suggest that it must be the peptide on which the T cells were positively selected[25,26], others indicate that it must be some other peptide, perhaps one that, when bound to MHC, has slightly higher affinity than the selecting peptide for the T cells[24].

Although naïve T cells do not divide in mature animals with a full complement of T cells[19,20,43] they still require exogenous factors to stay alive. These factors include interaction of TCRs with their selecting MHC on presenting cells[45–47] and cytokines. As far as the cytokines are concerned, IL-6 is certainly a survival factor for naïve T cells in tissue culture[48] but there is little evidence in normal or thymectomized animals that IL-6 affects the life expectancy of naïve T cells *in vivo*. On the other hand, there is plenty of evidence that IL-7 and perhaps IL-4 contribute to the survival of live naïve T cells, both in tissue culture[33,49] and in intact animals[32,34,35,50]. T cells that have lost the ability to respond to IL-7 once they have left the thymus do not survive well[32] and naïve T cells die rapidly in IL-4–deficient thymectomized mice treated with anti–IL-7 reagents[34]. Admittedly, mice that lack IL-7, its receptor, or the ability to signal through IL-7 and IL-4 receptors do contain some mature T cells but these cells behave abnormally[32,51–53], perhaps because they have been selected by their circumstances to resort to unusual methods for survival.

In summary, survival of naïve T cells depends on engagement of receptors by selecting MHC protein and on availability of the constitutively made cytokine, IL-7. IL-4 may substitute for IL-7 but its ability to do this may depend on its availability within the animal, which is controlled perhaps by the antigenic experience of the host and the ability of the extracellular matrix to retain IL-4[54,55].

Homeostasis of activated T cells

During response to antigen the numbers of activated cells increase rapidly and dramatically. The increase is driven by engagement of TCRs and costimulatory proteins such as CD28, OX40, 4-1BB, LFA-1 and CD2 on the surface of antigen-engaged T cells[56–63]. Signals transduced by these engagements combine to move the cell into cycle, make it receptive to stimulatory signals delivered through IL-2 receptors (for example) and induce production of factors such as IL-2[64], which act in an autocrine fashion to induce further division of the activated cells.

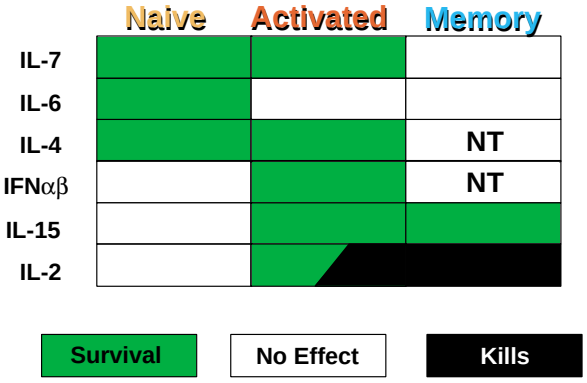

Figure 2. CD8⁺ T cells have different needs for cytokines at different stages in their lives. Cytokines involved in stimulating survival (green), death or number reduction (black) or have no effect (white) on naïve, activated and memory T cells. The effects of cytokines on CD4⁺ T cells at different stages of their lives are similar with the exception of IL-15, which is probably not a survival factor for memory CD4⁺ T cells.

As predicted by the clonal selection theory, antigen specificity ensures that naïve T cells that cannot recognize the antigen are not stimulated by this phenomenon in T cell–sufficient animals (although these cells do divide in the absence of overt antigen in T cell–deficient mice). Memory T cells may be affected by activation of bystander T cells, a phenomenon we discuss later.

Once created, activated T cells are short lived and little is known about the factors that prolong their life expectancy *in vivo*. *In vitro* these cells can be kept alive by members of the IL-2 family[65–67] and also by type I interferons[68] but not by IL-6[36]. *In vivo*, however, type I interferons have not been shown to improve the survival of activated T cells[68]. Also the fact that Bcl-2, a protein induced by IL-2–related cytokines[69], is only briefly increased in activated cells suggests that prolonged survival of T cells in the activated state is not caused by IL-2 family members[70,71].

The rapid disappearance of activated T cells does not affect the number of naïve or memory cells. The death of these massively expanded activated cells is probably very important to the animal as it restores T cells to reasonable numbers and removes cells that might otherwise continue to secrete toxic cytokines. Therefore, to make sure that these cells die, the process is driven by several different pathways including one which results from Fas or tumor necrosis factor α receptor (TNF-αR) engagement[72,73] and another triggered by reactive oxygen species generated within the activated cell[74]. Other pathways may also exist. Different death pathways dominate under different experimental systems. For example, some laboratories find that all activated T cell death can be accounted for by Fas engagement, whereas others, including our own, find that activated T cell death in mice is almost completely independent of Fas or TNFRs[74,75].

Hence the numbers of activated T cells in animals are normally well controlled by mechanisms that include cytokines such as IL-2. In most cases the processes that control the number of activated cells do not also affect naïve or memory cells.

Homeostasis of memory T cells

The control of memory T cells, and indeed their very existence, has been hotly contested for some years. Opinions range from the idea that there are no such cells and that all immunological memory is caused by antibody and long lived plasma cells, to the notion that memory T cells do exist and that they can be as long-lived as their host itself.

Experiments using limiting dilution, TCR transgenic T cells or MHC⁺ antigenic peptide staining have made it clear that after antigenic stimulation, particularly in the presence of an adjuvant, the animal contains more antigen-specific T cells than it did before exposure[76–80]. Some of the newly created cells may be relatively unresponsive but others definitely respond to antigen with faster kinetics and more vigor than their naïve precursors. Therefore in our view, antigen does generate memory T cells.

Despite many years' research we still do not know how memory T cells are created. Exposure to antigen in the presence of adjuvant probably has something to do with their production as adjuvants definitely increase the number of antigen-specific T cells that survive antigen exposure[81,82]. The effects of the adjuvants involve inflammatory cytokines such as TNF-α and probably induction of costimulatory ligands such as OX40 ligand, 4-1BBL and CD70 on antigen presenting cells[58–60,82]. Activation of proteins that are involved in the creation of memory in the brain, such as calcium-calmodulin kinase II, may also be important[83]. Somehow these signals combine to convert a certain percentage of antigen-activated T cells into memory cells.

Several authors have made the case that continuous exposure to antigen, which may be long lived in the body, is needed for the survival of memory T cells[78,84]. Recent experiments have shown, however, that memory cells can certainly survive in the absence of antigen[85–89]. This is not to say that continuous antigen may not, in addition, help to keep the number of these cells at high frequency. To some extent the survival of memory cells may be controlled by their location. In this regard the recent discovery that chemokines may selectively affect various types of memory cells[90,91] is particularly relevant and in fact the whole subject of physical position, chemokines and the fates of various types of T cells will undoubtedly be of great interest in the future.

What is the nature of these long-term surviving memory T cells? Previous authors noted that the cells appeared to be dividing very slowly in their hosts[87,88,92]. Recently this observation has been confirmed and it has also been shown that the division of CD8⁺ memory T cells is driven in large part by IL-15, which is made constitutively by the stromal cells of their hosts[43]. Such an idea was predicted by earlier experiments which showed that bursts of extra IL-15 led to bursts of proliferation by CD8⁺ memory T cells[92]. It was also found that the proliferation of these CD8⁺ memory cells was inhibited by IL-2[43]. Subsequent experiments suggested that the effects of IL-2 are indirect rather than due to direct cytotoxicity or the cytokine inhibiting the CD8⁺ memory T cells themselves. Perhaps IL-2 operates via CD25⁺-regulatory cells[93–95]. CD4⁺ memory cells may be controlled by similar processes as IL-2 inhibits their cycling to some extent (C.C. Ku and P. Marrack, unpublished observations). The nature of the material that stimulates CD4⁺ memory cells to remain in cycle is unclear, however.

We therefore believe that CD8⁺ memory T cells are preserved by continuous division in response to IL-15, constitutively made by their host and kept in continuous check by IL-2 made principally by T cells during immune responses but preserved even in the immunologically "quiet" host, bound to extracellular matrix[55]. If memory T cells are in continuous, albeit slow, cycle perhaps this accounts for the fact that it is difficult to distinguish memory from effector T cells and, indeed to actually define memory cells themselves at all. The recent discovery of a few cell surface markers that distinguish the two cell types[96,97] and the realization that memory T cells may never be in G_0[43], may help to clear the problem up.

The idea that memory cells may be slowly but continuously dividing leads to several thoughts. First the fact that, unlike naïve cells, memory cells may never be in G_0 could account in part for their more rapid response to antigen. Second, memory cells bear receptors for IL-15 on their surfaces[98]. One of the signals that create memory T cells must lead to the permanent expression of the genes for these proteins. Perhaps it is

Cytokine	SCF	M-CSF	IL-3	GM-CSF	IL-5	IL-9	IL-7	IL-15	IL-2	IL-4	IL-13
Receptor chains			IL-3Rα	GM-CSFRα	IL-5Rα	IL-9Rα	IL-7Rα	IL-15Rα	IL-2Rα	IL-4Rα	IL-4Rχ or IL-13Rα2
	c-kit	CSF-R1	βc	βc	βc			IL-2Rβ	IL-2Rβ		
						γc	γc	γc	γc	γc IL-13Rα1	IL-13Rα1 /2γc
Stem cell											
Macrophage-like											
Basophil/mast											
Eosinophil											
Neutrophil											
Erythroid											
Megakaryocyte											
B cell											
Natural killer											
Thymocyte											
γδ T cell											
αβ T cell						?					

Figure 3. Different members of the short-chain type 1 cytokine family are devoted to homeostasis of different types of leukocytes[101–110].

this signal itself that creates memory cells? Perhaps the distinction between activated T cells that go on to die and those that survive and become memory cells is the fact that the latter were exposed to a signal which permanently induced *IL15R* genes and thus allowed the cells to continue to live, maintained by host IL-15, which they can (unlike their unlucky dying counterparts) continue to detect.

Other ideas concern the effects of bystander infections on memory T cell numbers. Infection increases IL-15 production by the host. Memory $CD8^+$ T cells should therefore increase in number during infection whether or not they are specific for the invading organism. However these cells are regulated by IL-2, a cytokine that is also produced during infection. The net result of infection on $CD8^+$ memory T cells specific for other antigens will, therefore, be controlled by the relative concentrations of IL-15 and IL-2 the infection induces. How these ideas also apply to $CD4^+$ memory T cells yet remains to be discovered. IL-15R–deficient mice contain a normal number of CD4 memory T cells[99], so molecules other than IL-15 must substitute for, or act redundantly with, this cytokine to stimulate cycling of these cells.

Whether or not these ideas turn out to be correct, it is clear that memory $CD8^+$ T cell numbers are increased by IL-15 and decreased by IL-2. Competition for IL-15 may therefore be the overriding controlling factor. The total number of $CD8^+$ memory T cells that can be maintained by any given host will depend upon the amount of IL-15 the host can produce. Perhaps this explains why successive infections and successive memory cells for different antigens reduce the numbers of $CD8^+$ memory cells for preceding infections[4].

Coda: IL-2 family members and T cell homeostasis

Cytokines affect T cell homeostasis at many points and T cells have different requirements for cytokines and different stages of their lives (**Fig. 2**). For example, naïve T cells are maintained by IL-7, IL-4 and IL-6 whereas activated T cells can be sustained by any member of the IL-2 subfamily of cytokines: IL-2, IL-4, IL-7, IL-9 (if the cell bears receptors for IL-9) and IL-15, but not IL-6. Activated T cells, but not naïve T cells, can be kept alive (at least in tissue culture) by type I interferons. $CD8^+$ memory T cells are supported by IL-15 but not, for example, IL-4 and memory T cells may actually be killed by IL-2, a lethal hit that may also apply to some activated T cells. The changing needs of T cells for cytokines may have evolved to allow separate but related control of different T cell populations. For example, the number of naïve cells may be controlled by the animal's ability to produce IL-7 and IL-4, whereas $CD8^+$ memory T cell numbers are certainly affected by levels of IL-15 and IL-2.

Finally, it is striking that many of the cytokines that affect T cell homeostasis are members of the short-chain branch of the type I cytokine family. Type I cytokines are distinguished by their structure, which includes a four-helix barrel. Some years ago they were divided into two subfamilies based on the length of two of the helices and the relative arrangement of two of the loops between the helices[100]. Members of the long-chain family are functionally very diverse, and include proteins such as IL-6, growth hormone, leptin and cardiotropin-1. Members of the short-chain family are much less diverse and appear to be devoted mainly to control of various members of the hematopoietic lineage of cells (**Fig. 3**)[101–109]. In fact the IL-2 subfamily of the short-chain type I cytokines is almost entirely involved with control of lymphocytes. From these observations we predict that the short-chain cytokines actually evolved from a member of the older long-chain family. Moreover it was the recurring duplication and divergence of the genes of the short-chain family that allowed, in part, the development of diversity of leukocytes in vertebrates.

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