Immunology and Cell Biology (2008) 86, 54–56

www.nature.com/icb

REVIEW

Self/non-self discrimination and the problem of keeping T cells alive

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Burnet appreciated the requirement for self/non-self discrimination and presciently postulated elimination of auto-aggressive cells during early life. He could not have known that, for T cells, a limited degree of autoreactivity is beneficial for the immune system. Here, we review evidence that recognition of self-components keeps T cells alive and regulates self-tolerance.
Immunology and Cell Biology (2008) **86,** 54–56; doi:10.1038/sj.icb.7100139; published online 11 December 2007

Keywords: cytokines; T cells; IL-2; IL-7

When Burnet's paper was first published in 1957, information about the cells of the immune system was minimal.[1] Most lymphocytes were regarded as short-lived cells of unknown function although there were quaint ideas that lymphocytes might act as 'trephocytes', that is food for other cells.[2] Typical small lymphocytes were especially enigmatic, and the presence of huge numbers of these cells in the thymus was a total mystery. Then came the 1960s and the pivotal discovery of Miller that the thymus acts as a source of immunocompetent cells[3] and the demonstration of Gowans and others that typical small lymphocytes form a pool of long-lived recirculating cells able to mount immune responses to foreign antigens;[4] the dichotomy of T and B cells came a little later.[5] But the stimulus for this body of work was Burnet's suggestion that 'lymphocytes' represent clones of antigen-specific cells.

SELF/NON-SELF DISCRIMINATION AND THE ROLE OF Tregs

A key feature of Burnet's model is that, to avoid autoimmunity, clones of autoreactive cells have to be eliminated during early development. In fact, deletion (or receptor editing) of self-reactive T and B cells following contact with antigen in the primary lymphoid organs is now dogma.[6] Although Burnet was clearly right about clonal deletion, his view that the immune system distinguishes cleanly between self and non-self needs modification, especially for T cells. Thus, despite the wealth of evidence that many self-reactive cells are destroyed in the thymus, it is now well accepted that self-tolerance is heavily dependent on the presence of a population of $CD4^+$ $CD25^+$ T regulatory cells (Tregs), which function by inhibiting responses of normal T cells, both to self and foreign antigens. As discussed elsewhere, Tregs are generated in the thymus in response to self-major histocompatibility complex (MHC) II molecules, their avidity for these ligands being just below the threshold required for clonal deletion.[7–9] In the post-thymic environment, contact with self-MHC ligands plus a γ_c cytokine, interleukin (IL)-2, maintains Tregs in a state of chronic activation. Because of the expression of an intracellular inhibitor, Foxp3, however, Tregs do not display injurious effector function but instead have a suppressive function on other cells.

THE THYMUS AND POSITIVE SELECTION

Well before the discovery of Tregs, it was shown that the generation of normal T cells in the thymus requires positive selection to self-MHC ligands.[6] This process leads to selective survival of a very small fraction (1–2%) of thymocytes that have low but significant affinity for various peptides bound to self-MHC molecules. Recognition of these ligands induces a covert form of T-cell receptor signalling that causes the cells to survive (avoid 'death by neglect') and form the pool of post-thymic T cells.

Despite much speculation, the raison d'être of positive selection is still poorly understood. Originally, selecting T cells for covert self-reactivity was thought to enhance reactivity to foreign antigens. Although various studies on bone marrow chimeras and thymus-grafted mice are consistent with this idea, envisaging how selection for self-MHC molecules could potentiate responsiveness to complexes of foreign peptides plus self-MHC has proved difficult. Later, studies on homeostasis of T cells have provided an alternative explanation for positive selection.

HOMEOSTATIC PROLIFERATION

In recent years, it has become apparent that the survival of mature naïve T cells in the post-thymic environment requires continuous TCR contact with self-MHC ligands plus joint contact with IL-7, another member of the γ_c family of cytokines.[10–12] Through weak signalling, these two stimuli keep T cells alive in interphase, apparently by maintaining expression of antiapoptotic molecules such as Bcl-2. The fact that naïve T cells are maintained in interphase is presumed to reflect that signalling is covert. On this point, it is notable that naïve T cells move out of interphase and enter cell cycle when total cell number falls to low levels.[10–12] Under these conditions, IL-7 levels rise:

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Received 3 October 2007; accepted 11 October 2007; published online 11 December 2007

intracellular signalling becomes overt and T cells begin to proliferate and expand slowly, thus returning the T-cell pool to near-normal size. Such 'homeostatic' proliferation of naïve T cells is heavily dependent on a combination of TCR and IL-7 signals. Thus, proliferation ceases and the cells gradually die when T cells are transferred to MHC- or IL-7-deficient hosts.

Interleukin-7-dependent homeostatic proliferation is typically slow and causes minimal differentiation into effector cells. More recently, it has been found that a much more intense form of proliferation occurs when naïve T cells are exposed to high levels of certain other γ_c cytokines, namely IL-2 or IL-15, *in vivo*.[13,14] Under these conditions, T-cell proliferation is as marked as with responses to foreign antigens and, as with the latter, the proliferating cells display prominent effector function. Later, when cytokine levels are reduced, the stimulated T cells differentiate into typical central memory cells. As for IL-7-dependent homeostatic proliferation, the rapid expansion of naïve T cells induced by high levels of IL-2 or IL-15 is much more pronounced for $CD8^+$ cells than for $CD4^+$ cells.

Since slow proliferation of naïve T cells by IL-7 is MHC-restricted, the question arises whether the same applies to the rapid pattern of proliferation elicited by IL-2 and IL-15. In initial studies, we were surprised to find that for naïve T cells responding to IL-2 on adoptive transfer, responses were almost as high in MHC $I^{-/-}$ hosts as in normal hosts.[13] However, subsequent studies showed that responses to IL-2 in MHC $I^{-/-}$ hosts were almost undetectable when the number of $CD8^+$ cells injected was kept to a low level. This finding suggested that the responses seen with higher doses of T cells might reflect T-cell signalling as the result of T–T interaction, that is TCR recognition of MHC ligands on neighboring T cells. In favor of this idea, minimal responses to IL-2 occurred when the transferred T cells lacked MHC I molecules; these cells were obtained by reconstituting irradiated normal mice with MHC $I^{-/-}$ bone marrow cells.[13]

To sum up these data, the above findings indicate that T cells are maintained *in vivo* by a combination of TCR/MHC interaction plus contact with one or more γ_c cytokines. Under physiological conditions, levels of only one γ_c cytokine, IL-7, are sufficient to provide a significant signal to naïve T cells. Being only poorly mitogenic, IL-7 at background concentrations provides only a weak signal that, together with similarly weak signalling from TCR/MHC interaction, keeps naïve T cells alive in interphase. With raised levels of IL-7 or other γ_c cytokines such as IL-2 or IL-15, signalling increases and naïve T cells are driven to proliferate and differentiate, the tempo of this 'anti-self' response being determined by the stimulatory properties of the cytokine concerned, weak for IL-7 and strong for IL-2 and IL-15.

The implication from this model is that, at least in terms of proliferation, naïve T cells can be induced to respond to self simply by raising the level of γ_c cytokines. On this point, it was mentioned earlier that one of these cytokines, IL-2, drives constitutive self-MHC-restricted proliferation and activation of Tregs. An obvious question here is why background levels of IL-2 are sufficient to control Treg survival and proliferation but do not seem to affect naïve T cells. The likely explanation is that Tregs differ from resting T cells in expressing high-affinity receptors for IL-2. Naïve T cells do express a low level of receptors for IL-2 (and IL-15), but these receptors are of low affinity.

WHY DOES MAINTENANCE OF NAÏVE T CELLS REQUIRE TWO SIGNALS?

The observation that naïve T cells need two different signals, one from TCR ligation and the other from cytokines, to survive and proliferate implies that these signals are qualitatively distinct. The precise effects of these two signals, however, are still unclear. In the case of γ_c cytokines, signalling via these ligands seems to be especially important for maintaining Bcl-2 expression.[15,16] Thus, for TCR transgenic $CD4^+$ cells, crossing the donor cells to a $\gamma_c^{-/-}$ background leads to poor generation and survival of naïve T cells, the few cells generated having low levels of Bcl-2 and abnormal mitochondrial membrane potential.[15] These abnormalities can be largely overcome by crossing $\gamma_c^{-/-}$ mice to a Bcl-2 transgenic background. Nevertheless, enforced Bcl-2 expression does not fully restore T-cell numbers and the cells remain below normal in size. This finding might reflect the requirement for additional antiapoptotic molecules or the need for a TCR signal.

Currently, very little is known about how continuous TCR interaction with self-MHC ligands contributes to maintaining T-cell viability. For $CD4^+$ cells, contact with MHC II ligands *in vivo* induces a small but significant degree of CD3ζ phosphorylation, implying that the signalling induced by TCR/MHC is of quite low intensity.[17] But how this type of signalling affects cell viability is unclear. To our knowledge, there is no evidence that a weak TCR signal *in vitro* can mimic the capacity of IL-7 to keep T cells alive in interphase. So, how does a weak TCR signal operate?

Although there is still no clear answer to this question, we are intrigued by recent evidence on the capacity of TCR/MHC contact *in vivo* to influence the response of naïve $CD8^+$ cells to IL-2 *in vitro*. As mentioned above, naïve $CD8^+$ cells from MHC $I^{-/-}$ bone marrow chimeras are unable to proliferate in response to IL-2 after transfer to MHC $I^{-/-}$ hosts. *In vitro*, however, MHC $I^{-/-}$ $CD8^+$ cells do respond to IL-2, though only transiently and only at very high concentrations (J Sprent, J-H Cho and H-O Kim, unpublished data). Curiously, such proliferation is almost undetectable with naïve T cells from the HY transgenic line; by contrast, $CD8^+$ cells from other TCR transgenic lines respond quite well to IL-2 *in vitro*. These data are of interest because naïve $CD8^+$ cells from the HY line differ from other TCR transgenic lines in being unable to undergo homeostatic proliferation *in vivo*, either to IL-7 or IL-2.[18,19] The lack of homeostatic proliferation by HY cells is taken to indicate that the TCR affinity of these cells for self-MHC ligands is 'below average'. Why then are HY cells unresponsive to IL-2 *in vitro*?

The possibility we are exploring is that responsiveness of naïve T cells to cytokines is somehow dependent upon low-level TCR signalling. In the case of HY cells, these cells might fail to receive a TCR signal because their self-MHC affinity is too low. By the same token, the significant responsiveness of chimera MHC $I^{-/-}$ $CD8^+$ cells to IL-2 *in vitro* might reflect that these cells did receive a TCR signal *in vivo* through recognition of self-MHC ligands on host stromal cells. To assess this model, we are exploring the effects of parking normal $CD8^+$ cells in MHC $I^{-/-}$ hosts, thereby depriving the cells of TCR/MHC contact. Preliminary work suggests that parking the cells in MHC $I^{-/-}$ hosts for several days does substantially reduce their responsiveness to IL-2 *in vitro*, implying that the response to IL-2 does indeed require a TCR signal. Whether these findings are also applicable to IL-7 responses is under investigation.

CONCLUDING COMMENTS: THE BENEFITS OF SELF-RECOGNITION

As outlined above, TCR recognition of self-components plays a crucial role in maintaining T-cell viability, both for normal T cells and Tregs. Precisely how TCR interaction with self-ligands promotes the survival of these cells is still unclear. For Tregs, the avidity of TCR interaction with self-MHC II ligands is presumed to be quite high (relative to normal $CD4^+$ cells) and sufficient to keep Tregs in a state of chronic activation and rapid turnover. A simple model here is that continuous TCR ligation by self-ligands signals the cells to maintain high expres-

sion of CD25 (a characteristic feature of Tregs), thereby making the cells highly sensitive to the stimulatory effects of IL-2. Thus, TCR signalling might be needed mainly and perhaps solely to maintain Treg sensitivity to cytokines. Could this model also apply to normal T cells? For the latter, it was mentioned above that TCR interaction of normal T cells with self-ligands is relatively weak and the cells remain in interphase for prolonged periods with no signs of activation. Cell viability is probably maintained largely by high Bcl-2 expression induced by the stimulatory effects of IL-7. As for Tregs responding to IL-2, responsiveness of normal T cells to IL-7 might be a consequence of TCR/self-MHC interaction. This idea is appealing because of our preliminary finding that preventing $CD8^+$ cells from being in contact with MHC I *in vivo* reduces their sensitivity to IL-2 *in vitro*. Whether preventing TCR ligation also reduces responses to IL-7, however, is unclear because, unlike IL-2, IL-7 is poorly stimulatory for T cells *in vitro*, thus making it difficult to test the model. Also, the influence of TCR signalling on responsiveness to IL-2 and IL-7 could be distinctly different. Thus, whereas TCR ligation can enhance IL-2 responses by augmenting IL-2Rα (CD25) expression, TCR signalling is not known to increase expression of IL-7R. Hence, resolving the issue of how TCR signals promote T-cell viability will have to await further study.

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