Class II essential for CD4 survival

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In a recent article, Dorfman *et al.*[1] investigated whether basal T cell receptor (TCR)ζ chain phosphorylation depends on TCR interaction with self–major histocompatibility complex (MHC) molecules. Although these data are interesting in the context of peripheral T cell survival, the authors concluded differently and ended up stating that lymphocyte survival is not dependent on self-MHC expression. This was because in their experiments donor CD4$^+$ T cell numbers declined in both normal and class II MHC–deficient (Aβ-KO) recipients. These conclusions are in marked contrast to those of previous reports[2–7], which addressed the same question but in different experimental systems. However, the inability of Dorfman *et al.*[1] to determine an influence of peripheral self-MHC on CD4$^+$ T cell survival might be explained by the inappropriate experimental system they used.

Peripheral T cell pool sizes are limited by, as yet, unknown factors (ref. 8 and references therein). Dorfman *et al*. compared lymphocyte survival after transfer into normal and Aβ-KO mice. This is not appropriate because in the latter case donor CD4$^+$ T cells do not have to compete with recipient cells, whereas in the former case they have to compete with a full preformed pool of host CD4$^+$ cells. In addition, the continuous thymic output of CD4$^+$ T cells in normal mice (but not Aβ-KO mice) will gradually replace peripheral CD4$^+$ T cells, including those derived from the donor, as is the case for CD8$^+$ T cells[9]. For these reasons, normal mice cannot be used as controls for experiments with Aβ-KO mice. It is like comparing apples to oranges.

The authors observed proliferation of donor CD4$^+$ T cells in Aβ-KO recipients. Proliferation ceased once the mice were infused with antibodies to class II MHC, which indicated the presence of residual class II MHC on host- or donor-derived cells. Regardless of the cell type, the antibody-blocking studies were performed to exclude the contact of CD4$^+$ T cells with (residual) self-MHC. Although the antibodies were sufficient to block proliferation of donor CD4$^+$ T cells in Aβ-KO recipients, we do not know whether the dose was adequate to block the contact to self-MHC that is required for CD4$^+$ T cell survival. This is because proliferation could well require stronger stimuli than those needed for survival[10] and, thus, would result in a slower decline of CD4$^+$ T cells. Here, it is irrelevant that the authors observed an immediate down-regulation of TCRζ chain phosphorylation when blocking self-MHC because we do not know how much phosphorylation is required for survival. The differing kinetics between T cell survival in the absence of self-MHC ($t_{1/2}$ of approximately three weeks[2,5,7], J. Kirberg, unpublished data) and diminished TCRζ chain phosphorylation (within 36 h), as seen by Dorfman *et al.*[1], might suggest that this is actually the case.

In summary, the similar decline of CD4$^+$ T cells in the different mice may occur for very different reasons in the experiments by Dorfman *et al.*[1]: competition and replacement in normal recipients and death due to the absence of TCR contact with self-MHC molecules in Aβ-KO recipients. We find no basis to interpret the data as Dorfman *et al.*[1] have, questioning the concept of TCR "tickling" by self-MHC as a prerequisite for peripheral survival. Finally, basic TCRζ chain phosphorylation might still mediate survival downstream of the TCR "tickling" but this cannot be concluded from the data either.

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Response

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As Kirberg *et al*. and we[1] point out, several past reports[2–8] concluded that self-MHC molecule recognition is required for normal survival of mature näive T cells. Believing this model to be correct, we undertook our biochemical analyses in order to understand the signaling processes that underlie this effect. Our survival experiments were conducted as controls that were meant to verify that the CD4$^+$ T cells we had analyzed showed the expected reduction in lifespan after MHC class II was eliminated from their environment. Only upon obtaining the unexpected results reported in our paper did we begin to question our underlying assumptions on this issue. We then put a substantial amount of effort into considering possible artifactual explanations, including the ones raised by Kirberg *et al*.

Kirberg *et al.* maintain that our experimental approach led us to miss the survival effect of MHC recognition they believe many groups have documented. However, this perspective does not incorporate new data reported after papers on "survival" first appeared, which deal with the role of MHC recognition in the "homeostatic" proliferation of naïve T cells in lymphopenic hosts[9–17]. Naïve T cell populations proliferate in T cell–deficient (that is, lymphopenic or "empty") environments. This proliferation requires the presence of the selecting MHC class II molecule for naïve CD4$^+$ cells and the selecting MHC class I molecule for naïve CD8$^+$ T cells. All the publications claiming that normal CD4$^+$ T cell survival depends on MHC class II recognition involve conditions in which the cells in the selecting MHC$^+$ environment are likely to proliferate much more extensively than in the nonselecting or putatively MHC$^-$ host. In contrast, the reports that show no effect of MHC expression on CD4$^+$ T cell survival for at least 1 month[1,18] use mice in which such proliferation is minimized. Thus, rather than assessing only the relative rates of cell death in nondividing cell populations, the experiments in papers that argue for MHC-dependent survival actually compare the combined effect of death and proliferation in MHC$^+$ hosts to death alone in the MHC$^-$ hosts.

The data from many published experiments by other laboratories are in accord with this interpretation: proliferation was seen in each