Is IL-7 from dendritic cells essential for the homeostasis of CD4$^+$ T cells?

To the Editor:

In an article published in the February 2009 issue of *Nature Immunology*[1], Guimond *et al.* offered an interesting explanation for the slower interleukin 7 (IL-7)-driven homeostatic proliferation of naive CD4$^+$ T cells than of CD8$^+$ T cells[1,2]. Guimond *et al.* concluded that CD4$^+$ T cells can respond efficiently only to IL-7 expressed by dendritic cells but not to IL-7 expressed by stromal cells, which are the main IL-7 producers[3,4]. As direct evidence of their hypothesis, Guimond *et al.* showed that polyclonal CD4$^+$ T cells and CD4$^+$ T cells transgenic for the Marilyn T cell antigen receptor (TCR) proliferated abundantly in bone marrow chimeras in which IL-7 was expressed only by bone marrow–derived cells (IL-7-deficient (*Il7*$^{-/-}$) hosts given bone marrow from mice deficient in recombination-activating gene 1 (*Rag1*$^{-/-}$)).

We were intrigued by the rate of CD4$^+$ T cell population expansion in those chimeras, which was 50- to 100-fold higher than the typical IL-7-driven homeostatic proliferation in wild-type lymphopenic hosts. In the case of polyclonal T cells, the caveat is that a fraction of these cells respond strongly to environmental and commensal microflora antigens in lymphopenic hosts[5,6]. This concern does not apply to Marilyn cells and most other CD4$^+$ TCR-transgenic lines, which undergo minimal proliferation in lymphopenic hosts[2,7]. Therefore, we assessed the proliferation of SMARTA-transgenic CD4$^+$ cells (which express a TCR specific for lymphocytic choriomeningitis virus glycoprotein). In contrast to Marilyn cells, SMARTA cells failed to undergo any detectable proliferation in *Il7*$^{-/-}$ chimeras given *Rag1*$^{-/-}$ bone marrow (**Supplementary Fig. 1**).

Unlike SMARTA CD4$^+$ T cells, Marilyn cells are specific for the mouse male HY antigen and undergo intense proliferation in male hosts but not in female hosts (**Fig. 1a**). We tested whether female bone marrow chimeras would still support the considerable proliferation of Marilyn cells and found that the proliferation of Marilyn cells in female *Il7*$^{-/-}$ chimeras given bone marrow from female *Rag1*$^{-/-}$ mice was almost completely undetectable (**Fig. 1a**). In contrast, as expected, female *Il7*$^{-/-}$ chimeras given bone marrow from male *Rag1*$^{-/-}$ mice supported massive proliferation of donor Marilyn cells (**Fig. 1a**). These results suggest that the prominent population expansion of Marilyn cells reported by Guimond *et al.* was not due to IL-7 expressed by bone marrow–derived cells but instead reflected an immune response to contaminating male cells in their chimeras.

In a related experiment, Guimond *et al.* showed that Marilyn cells undergo faster homeostatic proliferation in female lymphopenic IL-7 receptor-α-deficient (*Il7r*$^{-/-}$) hosts than in *Rag1*$^{-/-}$ hosts, a finding we have confirmed (**Fig. 1b**). Although Guimond *et al.* interpreted this as an indication that signaling through the IL-7 receptor on antigen-presenting cells inhibits the homeostasis of CD4$^+$ T cells, further analysis suggests otherwise. Notably, we found that *Il7r*$^{-/-}$ hosts supported enhanced homeostatic proliferation not only of CD4$^+$ T cells but also of polyclonal and TCR-transgenic CD8$^+$ T cells (**Fig. 1b**). Therefore, the simplest explanation for these findings is that the considerable homeostatic proliferation of T cells in *Il7r*$^{-/-}$ mice merely reflects a higher concentration of IL-7 in these mice than in normal lymphopenic hosts.

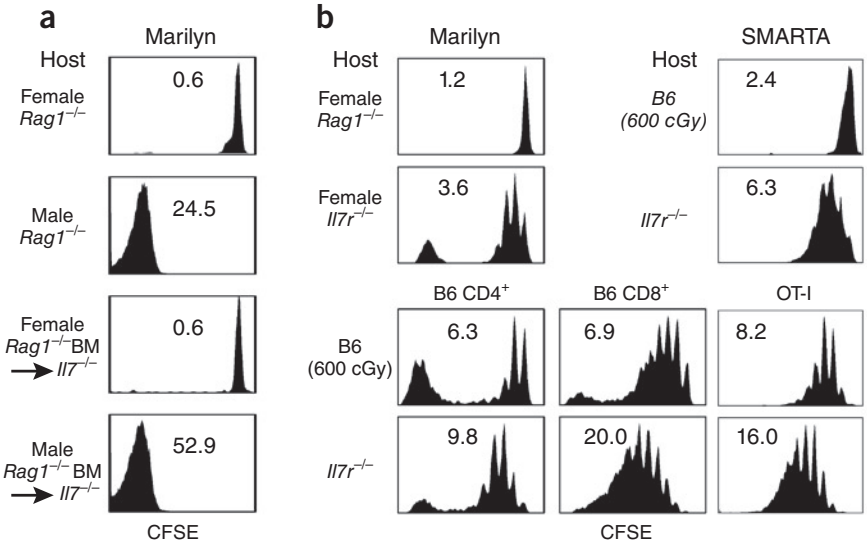

Figure 1 Homeostatic proliferation of naive CD4$^+$ T cells in lymphopenic hosts. (**a**) Proliferation of *Rag2*$^{-/-}$ Marilyn TCR-transgenic CD4$^+$ T cells (labeled with the cytosolic dye CFSE) in various hosts (left margin), analyzed 1 week after adoptive transfer into various chimeras (**Supplementary Fig. 1**). BM, bone marrow; →, transfer. Numbers in plots indicate donor cells recovered from host lymph node and/or spleen (mean × 10^5). (**b**) Proliferation of CFSE-labeled TCR-transgenic and polyclonal C57BL/6 (B6) Thy-1.1 T cells in various hosts (left margin) analyzed 1 week (Marilyn, SMARTA and B6) or 6 d (OT-I; ovalbumin-specific TCR) after adoptive transfer. The small population of CFSE$^-$ Marilyn cells in *Il7r*$^{-/-}$ hosts is due to host cells positive for the β-chain variable region 6 that could not be excluded because a donor-specific congenic marker was not available. Numbers in plots indicate donor cells recovered from host spleen (mean × 10^5). Data are representative of two experiments with two to three mice per group analyzed individually.

In summary, our findings obtained with sex-matched bone marrow chimeras, SMARTA cells and *Il7r*$^{-/-}$ hosts question the central conclusions reached by Guimond *et al.*

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Mackall and Guimond respond:
In our article[1], we concluded that interleukin 7 (IL-7) production is regulated by a simple feedback loop and that IL-7 signaling on antigen-presenting cells controls CD4$^+$ T cell homeostatic expansion. Marilyn HY-reactive T cells proliferated more in IL-7-deficient (*Il7*$^{-/-}$) recipients of bone marrow from female mice deficient in recombination-activating gene 1 (*Rag1*$^{-/-}$) than in *Rag1*$^{-/-}$ recipients of such bone marrow (Fig. 3d in ref. 1). Surh *et al.*[2] were surprised by the high degree of proliferation, could not reproduce our findings and assumed that contamination by bone marrow from male mice occurred in our experiments. However, we controlled these experiments by administering the same pool of marrow cells to *Rag1*$^{-/-}$ recipients, which did not support proliferation, thus ruling out the possibility of contamination by bone marrow from male mice (**Supplementary Fig. 1a**). Furthermore, Marilyn cells administered to male hosts underwent greater population expansion and dilution of the cytosolic dye CFSE (**Supplementary Fig. 1a**) than did those given to *Il7*$^{-/-}$ recipients. Our original manuscript contained additional data that supported the central model. Marilyn cells proliferated after being transferred into *Rag1*$^{-/-}$ recipients that received bone marrow from female mice deficient in the common γ-chain but not after being transferred into *Rag1*$^{-/-}$ recipients that received bone marrow from female *Rag1*$^{-/-}$ mice (**Supplementary Fig. 1b**). If contamination by bone marrow from male mice were to explain this, the bone marrow deficient in the common γ-chain (but not the *Rag1*$^{-/-}$ bone marrow) would have been consistently contaminated across three independent experiments, which seems implausible. Furthermore, Marilyn cells proliferated in female mice deficient in the IL-7 receptor α-chain (*Il7r*$^{-/-}$; **Supplementary Fig. 1c**), which were not chimeric, and we obtained similar results with chimeras generated by the transfer of bone marrow from female *Il7r*$^{-/-}$ mice into female *Il7r*$^{-/-}$ recipient mice (**Supplementary Fig. 1d**). In *Il7r*$^{-/-}$ mice and female *Il7r*$^{-/-}$ chimeras, Marilyn cells show a homeostatic pattern of proliferation, which differed from the rapid proliferation observed in the *Il7*$^{-/-}$ mouse (**Supplementary Fig. 1a**). We do not yet understand why the patterns differ, but we observed similar differences with polyclonal T cells (**Supplementary Fig. 1e**). Together, the multiple models demonstrating enhanced Marilyn cell proliferation when dendritic cells lack IL-7 signaling, the absence of Marilyn cell proliferation in *Rag1*$^{-/-}$ recipients and the homeostatic proliferation pattern in *Il7r*$^{-/-}$ recipients refute the notion that contamination by bone marrow from male mice confounded our conclusions[1].

Furthermore, we respectfully disagree with the suggestion that results obtained with polyclonal T cells are to be discounted. Polyclonal T cell proliferation in this system was IL-7 dependent (**Supplementary Fig. 1f**), and most polyclonal T cells, not just the fraction responsive to environmental antigens, proliferated in *Il7*$^{-/-}$ hosts (**Supplementary Fig. 1e**). Given that the goal of mouse experiments is to model the human condition, important insights are gleaned from studies of natural T cells. Depleted mouse and human immune systems reconstitute repertoires by responding to the wide array of antigens presented during lymphopenia, including self antigens, cognate antigens and cross-reactive antigens. We clearly demonstrated greater homeostatic expansion of naturally occurring CD4$^+$ T cells when stroma-derived IL-7 was absent[1].

We cannot explain why Surh *et al.* cannot replicate our data. Possibilities include differences in the number of bone marrow cells transferred, which perhaps limited reconstitution of the plasmacytoid dendritic cell niche that supports CD4$^+$ T cell population expansion; larger numbers of transferred Marilyn cells, which can compete for cross-reactive antigen; or variation in the T cell antigen receptor (TCR)-transgenic strains, as our Marilyn mice do not express Thy-1.1. However, potential explanations for discordant results in one small set of experiments do not undermine the global conclusions we presented[1]. Together, our data shed light on an entirely new axis of CD4$^+$ T cell regulation, were the first to our knowledge to explain why the regeneration of CD4$^+$ T cells in humans is exquisitely thymus dependent and opened new possibilities for the therapeutic manipulation of the homeostatic expansion of CD4$^+$ T cells in humans.

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