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Cutting Edge: Homeostatic Proliferation of Peripheral T Lymphocytes Is Regulated by Clonal Competition

Amy E. Troy and Hao Shen

Homeostatic proliferation functions to maintain peripheral T cell numbers and is regulated by cytokines. In this study, we provide evidence that T cell homeostasis is also regulated by clonal competition. Naive polyclonal T cells divided when transferred to TCR transgenic mice, as did monoclonal T cells when transferred to TCR transgenic hosts of differing clonotype. However, T cells did not divide in hosts of identical clonotype. Transgenic T cell proliferation was inhibited in irradiated hosts of the same clonotype, while cotransferred nontransgenic T cells proliferated extensively. These results show that clonal competition is a component of homeostasis that may contribute to selection of the peripheral T cell repertoire.

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All mammals maintain a roughly constant number of T cells throughout their lifetime despite Ag-driven expansion and T cell-depleting events. This ability to maintain a large and diverse body of T cells is not solely due to thymic output (1), continuous low-level division of T cells in the periphery, termed homeostatic proliferation (HP), also shapes the immune repertoire (2–4). This process is continuous in adults and is particularly important during ontogeny (5, 6), following T cell-depleting therapy (7), and in old age, when thymic output has decreased (8).

HP occurs following a decrease in T cell numbers and can be visualized experimentally by transfer of T cells to lymphopenic mice. The sensor that allows T cells to respond to a decrease in their numbers remains largely unknown. Space “sensing” in the form of decreased competition for cytokines or MHC/self peptide has been proposed. In support of the former hypothesis, IL-15 and IL-12 can contribute to HP of memory T cells, and IL-7 is necessary for homeostasis of both naive and memory T cells (9–13). Interactions between MHC and TCR complexes are also essential for survival and HP of naive T cells (14–20). A requirement for MHC/peptide implies that T cells of identical specificity could compete for these peptides on APCs. However, direct evidence for intraclonal competition during HP has not previously been reported. In contrast, existing evidence suggests that regulation of HP is independent of interaction with MHC/peptide complexes. Cotransfer of large numbers of CD8 T cells can inhibit CD4 as well as CD8 T cell proliferation in lymphopenic hosts (16, 21, 22). Inhibition of HP by cotransfer of T cells does not require that these competing cells interact with MHC (22). Finally, T cells with a low affinity for a particular peptide can inhibit the expansion of T cells with a higher affinity for the same peptide (22). Together these data have been interpreted as evidence that HP is not controlled by competition for MHC/peptides on APCs.

In this study, we show that CD4 and CD8 polyclonal T cells divided and expressed activation markers when transferred to naive TCR transgenic hosts. Proliferation did not occur when T cells were transferred to hosts with a diverse repertoire of T cells. Further transfer experiments revealed that clonal T cell populations inhibited the division of T cells of their own clonotype but not T cells of differing peptide specificity. Our data thus reveal competition between clonal T cells to be a novel component of homeostatic regulation.

Materials and Methods

Mice

All mice were maintained under specific pathogen-free conditions in the University Lab Animal Resources of the University of Pennsylvania Medical School (Philadelphia, PA). Male and female 6- to 8-wk-old BALB/c and C57BL/6 mice were purchased from the National Cancer Institute Animal Production Program (Frederick, MD). P14 and OT-I mice have been described previously (23, 24). TCR transgenic mice which had been backcrossed greater than nine times to C57BL/6 (B6.OT-I Thy 1.1 and Thy 1.2; B6.P14 Thy 1.1 and Thy 1.2) were maintained as breeding colonies.

Adoptive transfer

Lymphocytes from spleen and/or lymph nodes were purified, when indicated, by negative selection with B220 and MHC class II Ab-coupled magnetic beads or by positive selection with CD4 and/or CD8 beads per the manufacturer’s protocol (Miltenyi Biotec, Auburn, CA). Highly purified naive T cells were prepared by sorting Thy 1.1+ and CD44+CD62L low cells on a FACSVantage sorter (BD Biosciences, San Jose, CA). Highly purified naive T cells were prepared by sorting Thy 1.1+ and CD44+CD62L low cells on a FACSVantage sorter (BD Biosciences, San Jose, CA), and sorted cells were >99% pure. Cells were labeled with 5 μM CFSE (Molecular Probes, Eugene, OR) and suspended in PBS for injection. Purified T cells (0.5–3 million) or 10–50 million splenocytes were injected into the lateral tail vein of each recipient mouse. In some experiments, recipient mice were gamma-irradiated with 450 or 650 cGy in a Cs 137 irradiator 24 h before transfer.

Ab staining and analysis of cell surface markers

Cells from recipient spleen and lymph nodes were labeled at 4°C with one or more of the following Abs, all purchased from BD PharMingen (San Diego, CA): CD4, CD8, CD44, CD62L, CD69, CD127, and MHC class II. Labeled cells were stained with PI for dead cell discrimination. Data were acquired on a FACSCalibur with CellQuest software (BD Biosciences, San Jose, CA) and analyzed with FlowJo (Tree Star, Ashland, OR).

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2 Address correspondence and reprint requests to Dr. Hao Shen, Department of Microbiology, School of Medicine, University of Pennsylvania, 3610 Hamilton Walk, Philadelphia, PA 19104-6076. E-mail address: hshen@mail.med.upenn.edu

3 Abbreviations used in this paper: HP, homeostatic proliferation; CD62L, CD62 ligand.
CA): PE-CD90.1 and PerCP-CD90.1 (Thy1.1; clone OX-7), allophycocyanin-CD90.2 (Thy 1.2; clone 53-2-1.1), PE-CD4 (clone IM7), APC-CD62L (clone MEL-14), PE-β75.1, 5.2 (clone MR9-4), PE-Vβ 8.1, 8.2 (clone MR5-2), PE-Vα2, and biotin-Vα2 (clone B20.1). After washing in PBS/BSA, cells were fixed with 2% paraformaldehyde in PBS. Cell suspensions were analyzed by FACS on a FACSCalibur machine (BD Biosciences) using the CellQuest program (BD Biosciences). Acquired data was analyzed using FlowJo software (Tree Star, San Carlos, CA).

**Results**

Initially, we attempted to design an adoptive transfer system to visualize the division of polyclonal T cells in response to antigenic stimulation during infection. We wanted to minimize host T cell responses that obscure the response of transferred cells, but HP precluded the use of T cell-deficient mice as recipients. Thus, we selected TCR transgenic mice as recipients, reasoning that they would be unable to mount a broad response due to a limited T cell repertoire and would not support HP of donor T cells. However, to our surprise, we observed that polyclonal T cells proliferated when transferred to TCR transgenic hosts in our uninfected control groups.

To characterize this initial observation, we adoptively transferred CFSE-labeled BALB/c (Thy1.2) spleenocytes to naive DO11.10 (Thy1.1) TCR transgenic or BALB/c (Thy1.1) mice. Thirteen days after transfer, recipient mice were sacrificed and splenocyte suspensions were stained with fluorescent Abs to Thy1.1, CD4, and CD8. Analysis by FACS showed that Thy1.1+ donor T cells divided greater than seven times to become CFSE-negative in DO11.10, but not in BALB/c, recipients (Fig. 1A). CFSE-negative donor T cells were CD44high and CD62Llow, indicating that they were activated as a result of their proliferation. Kinetic analysis showed that proliferating donor T cells became detectable in the spleen by day 5, persisted for at least 1 mo in transgenic hosts, and remained CD44high and CD62Llow (data not shown). The proliferation kinetics and activation phenotype of donor cells are similar to those previously observed for T cells that have undergone HP (25-28).

Dividing donor cells included those of both CD4 and CD8 lineage (Fig. 1B). The ability of CD8 donor cells to divide in DO11.10 mice might be the result of greatly reduced numbers of host CD8 T cells, because peripheral T cells in DO11.10 mice consist largely of a single I-Aγ-restricted CD4+ T cell clone specific to an OVA peptide (29). However, donor CD4 T cells also proliferated despite the presence of a full CD4+ T cell compartment in DO11.10 mice, indicating that low cell numbers may not be the sole reason for the proliferation.

To determine whether this phenomenon was restricted to the DO11.10 mouse, we performed additional transfers using CD8 TCR transgenics on a C57BL/6 (B6) background as hosts. Polyclonal T cells from B6 mice were labeled with CFSE and transferred to Thy-congeneic B6, B6.P14, or B6.OT-I mice. P14 and OT-I mice express transgenic TCR specific to an H-2Db-restricted epitope from lymphocytic choriomeningitis virus and an H-2Kb-restricted epitope from OVA, respectively. FACS analysis of transgenic host spleenocytes 8 days posttransfer showed that a population of B6 donor cells had divided in P14 and OT-I hosts while minimal cell division was observed in B6 hosts (Fig. 2). Both CD4 and CD8 donor T cells divided in P14 and OT-I hosts, despite the presence of mainly CD8 T cells in these transgenic mice. Thus, polyclonal T cells divided when transferred to CD4 or CD8 TCR transgenic mice, and this division was not simply due to decreased numbers of CD8 or CD4 T cells in these respective hosts.

Polyclonal T cells from normal mice contain a small population of cells that have a memory phenotype (CD44high).

**FIGURE 1.** Polyclonal T cells from BALB/c mice divide and express activation markers upon transfer to DO11.10 TCR transgenic mice. Splenocytes from naive BALB/c (Thy 1.1) mice were labeled with CFSE and transferred to BALB/c (Thy 1.2) and DO11.10 (Thy1.2) mice. At day 13 posttransfer, splenocytes were stained with fluorescent Abs to surface markers (Thy1.1, CD4 or CD8, and CD44 or CD62L). Donor (Thy1.1+) populations were analyzed for CFSE fluorescence and CD44 or CD62L expression (A). Donor T cells were further gated on CD4+ or CD8+ cells and analyzed for CFSE fluorescence (B). Numbers in histograms indicate the percentage of gated donor CD4 or CD8 cells that have divided two or more times. Plots for one representative mouse from a group of three are shown. The experiment was repeated twice with similar results.

**FIGURE 2.** Polyclonal T cells from B6 mice undergo proliferation upon transfer to P14 or OT-I TCR transgenic mice. Splenocytes from B6 were labeled with CFSE and transferred to Thy-congeneic B6, P14, and OT-I mice. Eight days posttransfer, splenocytes were stained with fluorescent Abs to Thy 1.1 or Thy 1.2, CD4, and CD8. Proliferation of donor CD4+ or CD8+ cells was visualized by analyzing their CFSE fluorescence. Numbers in histograms indicate the percentage of gated donor CD4 or CD8 cells that have divided two or more times. Plots for one representative mouse from a group of three are shown. Each experiment was repeated at least twice with similar results.
It was possible that dividing cells came exclusively from the memory subset of polyclonal donor T cells due to a reduced memory cell pool in TCR transgenic mice. In addition, naive and memory T cells have different requirements for survival and HP. In particular, HP of naive T cells is dependent on interactions with MHC/peptide while HP of memory T cells is not (16, 18, 30). Furthermore, memory T cells are known to undergo slow continuous turnover in intact hosts (30). Thus, we transferred naive (CD62Llow) polyclonal T cells enriched by MACS to transgenic hosts and observed that donor cells proliferated to an extent similar to that of unpurified donor T cells (data not shown). To obtain highly purified naive T cells, we sorted B6 lymphocytes for Thy1.1+/CD44low/CD62Lhigh cells (sorted cells >99% pure) on the flow cytometer and transferred them to Thy-congenic B6 and OT-I recipients (Fig. 3). Transferred T cells retained their naive phenotype and CFSE fluorescence in the B6 host. In contrast, both CD4 and CD8 T cells divided in the transgenic host. Dividing cells expressed activation markers (CD44high/CD62Llow) while undivided donor cells remained CD44low and CD62Lhigh. These results conclusively demonstrate that naive T cells divide when transferred into intact transgenic recipients.

Our finding of polyclonal T cell proliferation in TCR transgenic hosts was unexpected. It is possible that proliferation was driven by a lymphopenic environment in transgenic mice, although TCR transgenic mice and age-matched background strains did not have significantly different total T cell numbers (data not shown). Alternatively, because TCR transgenic and normal hosts differ in the diversity of their T cell repertoire, proliferation might be driven by homeostatic mechanisms dependent on this property of the host environment. To distinguish between these two possibilities, we performed transfers of transgenic T cell populations to different TCR transgenic mice. Splenocytes from P14 or OT-I mice were enriched for T cells using MACS and labeled with CFSE before transfer to Thy-congenic B6, P14, or OT-I recipients. Eight days after transfer, proliferation of donor cells was analyzed. P14 and OT-I transgenic T cells did not divide when transferred to B6 hosts (data not shown) or hosts of the same transgenic TCR specificity (Fig. 4). In contrast, both P14 and OT-I donor cells divided more than seven times in hosts transgenic for a different TCR. Thus, transgenic T cells did not divide appreciably when transferred to a host of the same clonotype, yet divided many times in hosts of a different clonotype. This indicates that T cell division was inhibited by the presence of clonal T cells but not by T cells of another clonotype. Therefore, these results support the regulation of T cell homeostasis by competition among cells with identical TCR specificity.

We next examined whether the phenomenon of clonal competition could be visualized in irradiated mice, which are commonly used as hosts to study HP. We exposed B6 (Thy1.1) and OT-I (Thy1.1) mice to sublethal doses of radiation and 24 h later adoptively transferred purified CD8 T cells from OT-I (Thy1.2) mice. Most T cells from OT-I Rag+/+ mice express the transgenic (Vα2/Vβ5) TCR but a small percentage do not.

![Figure 3](http://www.jimmunol.org/)

**Figure 3.** Purified naive T cells from B6 mice proliferate in OT-I, but not in Thy-congenic B6 hosts. Splenocytes from B6 were stained for Thy1.1, CD44, and CD62L and sorted on the FACSVantage flow cytometer for Thy1.1+/CD44low/CD62Lhigh cells. These cells were labeled with CFSE and transferred to Thy-congenic B6 and OT-I mice. Eight days after transfer, donor (Thy1.1+) populations in the spleen of recipient mice were analyzed for CFSE fluorescence and CD44 or CD62L expression (A). Donor T cells were further gated on CD4+/CD8- cells and analyzed for CFSE fluorescence (B). Numbers in histograms indicate the percentage of gated donor CD4+ or CD8+ cells that have divided two or more times. Plots for one representative mouse from a group of three are shown.

![Figure 4](http://www.jimmunol.org/)

**Figure 4.** Transgenic T cells divide when transferred to TCR transgenic hosts of a different clonotype. CFSE-labeled splenocytes from P14 or OT-I transgenic mice were enriched for CD8 T cells by positive selection (CD8+ MicroBeads; Miltenyi Biotec) and transferred to Thy-congenic B6, P14, and OT-I mice. Recipients that received OT-I cells were sacrificed 8 days posttransfer, recipients that received P14 cells were sacrificed 14 days posttransfer, and their splenocytes were stained with fluorescent Abs to cell surface markers (Thy1.1 or Thy1.2, CD8, and Vβ5 or Vβ8). Proliferation of donor P14 (Thy1.2+/CD8+Vβ8+) and OT-I (Thy1.2+/CD8+Vβ5+) cells was analyzed by visualizing their CFSE fluorescence. Numbers in histograms indicate the percentage of gated donor cells that have divided two or more times. Plots for one representative mouse from a group of three are shown. Experiments were repeated with similar results.
We hypothesized that this small population of nontransgenic cells would divide more than transgenic cells in irradiated OT-I hosts because of greater clonal competition against donor transgenic cells. Eight days after transfer, we examined proliferation of transgenic (Vβ5+)) and nontransgenic (Vβ5−) populations of donor T cells (Fig. 5). In irradiated B6 hosts, the Vβ5+ and Vβ5− donor cell populations proliferated to a similar extent, and the majority of them divided two to four times. In contrast, nontransgenic (Vβ5−) cells divided more than transgenic (Vβ5+) cells in irradiated OT-I mice. Most transgenic donor T cells divided three or four times, while a population of nontransgenic (Vβ5−) donor cells divided more than seven times. Donor T cells divided more extensively in hosts that received a greater dose of radiation (650 cGy), yet nontransgenic cells still divided more than transgenic cells. This differential proliferation was not due to inherent differences in the proliferative capacity of transgenic and nontransgenic cells, as they divided similarly in irradiated B6 (Fig. 5) and in Rag−/− mice (data not shown). Together, these results indicate that proliferation of transgenic donor cells is inhibited by clonal competition from host transgenic cells.

**Discussion**

We have observed that T cell division occurs following transfer of naive polyclonal T cells to nonirradiated TCR transgenic hosts. Previous studies have shown that T cell clones from old mice can divide in young, nonirradiated syngeneic mice (31), but donor cells in our experiments were from young (6- to 16-wk-old) mice and did not divide in Thy-congenic BALB/c or B6 hosts. The proliferation we observed could be due instead to either an allogeneic response or HP. An allogeneic response is highly unlikely for several reasons. First, our TCR transgenic mice were extensively bred to donor strains (greater than nine generations). Second, CD8 T cells from transgenic mice do not divide in B6 mice, thus arguing against possible allogeneic responses between B6 and P14 or OT-I mice. Lastly, allogeneic responses typically begin within 24 h, with multiple divisions of transferred T cells occurring by 72 h (32, 33), while we did not observe any significant division before day 5. These slow kinetics are more consistent with HP (17, 28). These data suggest that transgenic mice with a limited TCR repertoire might allow naive donor T cells to undergo HP, even in the absence of overt lymphopenia. Further analysis shows that monoclonal P14 and OT-I cells divide upon transfer to TCR transgenic hosts of a differing clonotype but not hosts of identical clonotype. Results similar to these have been observed when T cells are transferred between OT-I and 2C TCR transgenic mice (C. Surh, personal communication). Furthermore, we have shown that nontransgenic T cells divide many times more than OT-I transgenic T cells when transferred to irradiated OT-I hosts. Together, these results indicate that HP is inhibited by competition among naive T cells with the same TCR.

Our results do not contradict the previous finding that HP can be inhibited by large numbers of nonspecific T cells. Inhibition by nonspecific T cells occurs independent of MHC interaction (16, 21, 22) and is likely due to competition for common resources, such as IL-7 (9, 11–13). This does not preclude the existence of another level of regulation, such as the clonal competition that is demonstrated by our results. Clonal inhibition likely results from reduced interaction of these T cells with cognate MHC/peptide complexes. This was suggested by previous work which clearly demonstrated that HP is critically dependent on TCR interaction with MHC (14–16, 18, 19) and in several cases, on the same peptides mediating positive selection in the thymus (15, 17, 18). HP occurs mainly in the T cell compartment of secondary lymphoid organs and requires interaction with professional APC (14, 22). These requirements suggest that T cells with the same specificity are likely to compete for cognate MHC/self-peptide complexes during HP. Indeed, the capacity of transgenic T cells to undergo HP has been shown to be dependent on the amount of available self-peptides and TCR affinity for these peptides (20). In addition, T cell competition has been demonstrated during T cell responses to viral infection (34). Our results show that clonal competition for MHC/peptide complexes also occurs during HP. Thus, in addition to their established role in thymic selection, self-peptides may play an important role in maintaining the diversity of the peripheral T cell repertoire by regulating HP.

In conclusion, our results show that HP is regulated by competition between clonal naive T cells as well as by total cell numbers and cytokines. Clonal competition during HP may play an important role in the development and maintenance of a diverse peripheral T cell repertoire.

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**References**