MHC Class I-Positive Dendritic Cells (DC) Control CD8 T Cell Homeostasis In Vivo: T Cell Lymphopenia as a Prerequisite for DC-Mediated Homeostatic Proliferation of Naive CD8 T Cells

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The sizes of peripheral T cell pools are regulated by competition for environmental signals within a given ecological T cell niche. Cytokines and MHC molecules have been identified as resources for which naive T cells compete to proliferate homeostatically in lymphopenic hosts to fill up their respective compartments. However, it still remains unclear to what extent CD4 and CD8 T cells intercompete for these resources and which role dendritic cells (DC) play in this scenario. Using transgenic mice in which only DC express MHC class I, we demonstrate that this type of APC is sufficient to trigger complete homeostatic proliferation of CD8 T cells in vivo. However, normal numbers of endogenous naive CD4 T cells, but not CD25+CD4+ T regulatory cells, efficiently suppress this expansion in vivo. These findings identify DC as a major resource and a possible target for homeostatic competition between naive CD4 and CD8 T cells. The Journal of Immunology, 2005, 175: 201–206.

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MHC Class I-Positive Dendritic Cells (DC) Control CD8 T Cell Homeostasis In Vivo: T Cell Lymphopenia as a Prerequisite for DC-Mediated Homeostatic Proliferation of Naive CD8 T Cells

The peripheral T cell pool size is tightly regulated by endogenous stimuli, which are indispensable both for survival and homeostatic proliferation (HP) (1, 2). Although proliferative expansion of naive T cells is rare under steady state conditions, it occurs in lymphopenic hosts, where space in T cell niches is abundant (1). Exploitation competition seems to be operational as a major regulatory mechanism controlling HP (3); different naive T cell populations have a common need for limiting resources such as self-peptide/MHC-complexes (4–8), IL-7 (9, 10), or thymic stromal lymphopoietin (TSLP) (11). Also during immune responses, CTLs compete for access to APC (12–14), a phenomenon that was termed interference competition. From this struggle for contact with APC, it has been extrapolated that homeostatic competition of naive T cells for physical APC access could also regulate peripheral T cell pool sizes (15). In fact, naive CD4+ (16) as well as CD8+ (17) T cells do compete among each other, and clones with the highest TCR avidity and homeostatic expansion capacity have growth advantages (18). However, in this scenario several questions remain unanswered. First, the type of APC responsible and sufficient to induce homeostatic expansion is still elusive. Although some in vitro coculture experiments indicated that in mice, dendritic cells (DC) are essential for HP of peripheral T cells (19, 20), a direct in vivo demonstration of this hypothesis is still lacking. Second, it is unclear whether CD4 and CD8 T cells intercompete for shared resources or if they have strictly defined subset preferences for their respective definitions of space. Because cotransfer experiments showing partially reciprocal CD4-CD8 inhibition were performed in irradiated hosts (5, 21), irradiation side effects, such as excess cytokine production (22, 23), could have masked the physiological in vivo situation. Third, it remains unclear whether the competition takes place at the level of MHC-mediated TCR signals, cytokine signals, or both.

In this study we investigated whether DC are the APC in charge of providing MHC class I (MHC1) for HP of CD8 T cells and analyze competition between CD4 and CD8 T cells for homeostatic expansion in nonirradiated recipients. Using B2-microglobulin (B2m)-deficient mice that are tolerant to MHC1 by transgenic B2m expression on DC (24), we demonstrate that 1) MHC1 expression on DC is sufficient to induce homeostatic CD8 T cell proliferation; 2) despite an empty CD8 compartment, this proliferation is completely suppressed by normal numbers of endogenous CD4 T cells; and 3) partial or complete depletion of CD4+ T cells, but not depletion of CD25+CD4+ regulatory T cells, allows DC to instruct naive CD8 T cells to proliferate homeostatically in an MHC-dependent fashion.

Materials and Methods

Mouse strains

All mice were bred and maintained at the animal facility of the Institute for Immunology (Ludwig Maximilians University, Munich, Germany) in accordance with established guidelines. CD11c-MHC1-transgenic (CD11c-MHC1) and K14-B2m x CD11c-MHC1-transgenic mice have been described previously (24, 25). All mice were backcrossed >12 times to the B2m-deficient (26) C57BL/6 background. C57BL/6 Thy1.1 mice were provided by J. Kirberg (MPI, Freiburg, Germany).

Abs and flow cytometry

The following fluorochrome-conjugated mAbs were used in this study: FITC-anti-CD44 (IM7), PE-anti-CD3e (145-2c11), PerCP-anti-CD4 (RM4-5), PerCP-anti-CD8a (53-6.7), allophycocyanin-anti-CD62L (MEL-14; BD Biosciences), PE-anti-CD44 (IM7 8.1), and allophycocyanin-anti-CD6a (CT-CD8a; Caltag Laboratories). For flow cytometry, organs were prepared as single cell suspensions according to standard protocols. After data acquisition on a FACSCalibur (BD Biosciences), analysis was performed using FloJo software (Tree Star).
CFSE labeling and adoptive cell transfer

Naive CD8<sup>+</sup>CD44<sup>+</sup> T cells were isolated from pooled spleen and LN by combined negative and positive selections using the MACS CD8<sup>+</sup> T cell isolation kit, FITC-anti-CD44, and anti-FITC MicroBeads (Miltenyi Biotec). The purity of CD8<sup>+</sup>CD44<sup>+</sup> cells was ≥95%. CFSE labeling (Molecular Probes) was performed as described previously (24, 25). Cells (2 × 10<sup>5</sup>) were injected into the lateral tail veins of age- and sex-matched recipient mice. The transferred CD44<sup>+</sup>CD8<sup>+</sup> T cell fraction did not contain fast-proliferating cells. Therefore, under the conditions used in this study, transferred CD8<sup>+</sup> T cells divided a maximum of five to seven times without complete dilution of their CFSE dye and remained clearly distinguishable from endogenous T cells. Nevertheless, in some experiments Thy1.1<sup>+</sup> or Ly5.1<sup>+</sup> CD8<sup>+</sup> T cells were used, and the transferred CD8<sup>+</sup> T cells were subsequently identified using anti-Thy1.1 or anti-Ly5.1 mAb (data not shown).

In vivo induction of lymphopenia

Recipient mice were either sublethally irradiated (600 rad; gammacell 40; Atomic Energy of Canada) 1 day before T cell transfer or treated with an opsonizing mAb to deplete specifically CD4<sup>+</sup> T cells by i.p. injection of 500 μg of GK 1.5 on days 3 and 1 before and days 2, 5, 7, and 11 after T cell transfer. For partial CD4<sup>+</sup> T cell depletion, 300 μg was used at each time point. For T cell depletion, 750 μg of opsonizing anti-Thy1.2-Ab 30-H12 (American Type Culture Collection) was injected i.p. under the same regimen.

Results

An empty CD8<sup>+</sup> T cell compartment is not sufficient to allow homeostatic proliferation of naive CD8<sup>+</sup> T cells in mice with MHCI<sup>+</sup> DC

To investigate whether DC are sufficient to trigger HP of naive CD8<sup>+</sup> T cells, we took advantage of CD11c-MHCI-transgenic mice (24, 25). In these otherwise MHCI-negative (β<sub>2m</sub>-deficient) mice, CD11c promoter-driven transgenic β<sub>2m</sub> expression reconstitutes wild-type levels of MHCI selectively on DC, but not on other cell types (24, 25). Moreover, MHCI<sup>+</sup> DC do not induce positive thymic selection of CD8<sup>+</sup> T cells, but mediate central tolerance toward MHCI (25). As a result, these mice 1) are tolerant to MHCI and 2) have an empty peripheral CD8<sup>+</sup> T cell compartment (Fig. 1A), but 3) possess normal numbers of CD4<sup>+</sup> T cells in their periphery (25) (Fig. 1A): 40% (total CD4 number, 25.5 × 10<sup>6</sup>) in CD11c-MHCI mice and 38% (total CD4 number, 26.6 × 10<sup>6</sup>) in C57BL/6 controls. To analyze the behavior of CD8<sup>+</sup> T cells in CD11c-MHCI mice, we purified naive (CD44<sup>+</sup>CD62L<sup>high</sup>; data not shown) CD8<sup>+</sup> T cells from C57BL/6 donors, labeled them with CFSE, and transferred them into either C57BL/6 or CD11c-MHCI recipients. Surprisingly, despite an empty CD8<sup>+</sup> T cell compartment in CD11c-MHCI recipients (Fig. 1A), naive CD8<sup>+</sup> T cells did not show any cell division 7 or 14 days after transfer (Fig. 1A). They behaved similarly to the cells transferred into C57BL/6 hosts with full CD8<sup>+</sup> T cell compartments. In addition, T cell surface activation markers, CD44 and CD62L, were not modulated differentially in either type of hosts (Fig. 1A), nor were the recovery rates of transferred T cells significantly different (Fig. 1A). These data indicate the absence of T cell rejection, activation, or expansion (Fig. 1A) and could be interpreted as an insufficiency of MHCI<sup>+</sup> DC in CD11c-MHCI mice that triggers homeostatic CD8<sup>+</sup> T cell proliferation.

DC are sufficient to induce homeostatic proliferation of CD8<sup>+</sup> T cells in irradiated hosts

Next, we induced lymphopenia by irradiation of the hosts to induce maximal depletion of endogenous lymphocytes. This treatment reduced the total number of lymphocytes to 10%, but also reduced

![FIGURE 1. In irradiated lymphopenic hosts, MHCI<sup>+</sup> DC are sufficient to induce homeostatic proliferation. Naive (CD44<sup>+</sup>CD62L<sup>high</sup>) polyclonal CD8<sup>+</sup> T cells (1 × 10<sup>6</sup>) from C57BL/6 donor mice were CFSE labeled and injected i.v. into untreated (A) or sublethally irradiated (B) recipients. A, The flow cytometric analysis of peripheral T cell compartments in untreated C57BL/6- or CD11c-MHCI mice is shown as dot plots. Numbers above the gates indicate the percentage of CD8<sup>+</sup> T cells within each gate as well as the total cell numbers (in parentheses). Data are from pooled lymph nodes and spleen (average of five mice). Histograms (middle panel) show CFSE-labeled CD8<sup>+</sup> T cells 7 or 14 days after transfer. Right panel histograms, CD44 or CD62L expression of these cells before and 14 days after transfer. B, CFSE profiles of CD8<sup>+</sup> T cells transferred into the indicated sublethally irradiated hosts. Graphs (right panel) show the percentage of CD44<sup>+</sup> or CD62L<sup>low</sup> cells within each peak of CFSE-labeled cells corresponding to one cell division. For CD44/CD62L analysis, gates were set as shown in the respective histograms in A. rec, Recovered total numbers (×10<sup>6</sup>) of transferred CD8<sup>+</sup> T cells from pooled lymph nodes and spleens (average from three mice per group ± SEM).](http://www.jimmunol.org/)

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DC number to 10–20% by day 7 after irradiation (data not shown). Despite this loss of DC as the only cell type expressing MHCI in CD11c-MHCI recipients, the remaining DC were sufficient to induce similar proliferation rates of transferred CFSE⁺ CD8 T cells compared with a C57BL/6 wild-type environment (Fig. 1B). As reported previously, in β₂m⁻/⁻ hosts, HP occurred, an effect that has been attributed to residual MHCI expression in β₂m⁻/⁻ mice (5) or cytokine-driven homeostasis of CD8 T cells (27). Although in β₂m⁻/⁻ hosts the transferred CD8 T cells divided only once in 7 days, in irradiated C57BL/6 and CD11c-MHCI mice they showed up to five divisions and similar recovery rates (Fig. 1B). In addition, concomitant weak up-regulation of CD44 (Fig. 1B) was observed on dividing CD8 T cells in all hosts. However, all transferred CD8 T cells modulated CD62L, even those that did not divide (Fig. 1B). This indicates that, in contrast to transferred CD8 T cells in untreated hosts that showed no or modest modulation of CD62L (Fig. 1A), environmental conditions such as lymphopenia and/or irradiation-induced cytokine storm and abundant cell death might have a strong impact on naive T cells. Nevertheless, these data indicate that MHCI⁺ DC can provide signals via MHCI, which are not present in β₂m-deficient mice, and allow homeostatic expansion of naive CD8 T cells with the efficiency of a normal MHCI⁺ C57BL/6 environment (Fig. 1B).

Absence of MHCI-independent homeostatic proliferation in nonirradiated, T cell-depleted, β₂m-deficient hosts can be restored by MHCI⁺ DC

To eliminate irradiation effects and to examine the MHCI-independent proliferation observed in irradiated, β₂m-deficient recipients (5, 27) (Fig. 1B), we used the CD11c-MHCI × K14-β₂m mice described previously (25). In these otherwise β₂m-deficient animals, a β₂m transgene under control of the K14 promoter reconstituted MHCI expression on thymic cortical epithelium, inducing positive selection of CD8 T cells in the absence of negative selection (28). Crossing these mice to CD11c-MHCI mice introduced MHCI tolerance by negative selection via thymic MHCI⁺ DC (25), leading to normal numbers of peripheral MHCI-deficient CD8 T cells (25). When these MHCI⁻ CD8 T cells were transferred to K14-β₂m × CD11c-MHCI (Fig. 2A; full CD4 and CD8 compartments, MHCI⁺ DC), to CD11c-MHCI (full CD4, empty CD8 compartment, MHCI⁺ DC), or β₂m-deficient hosts (full CD4, empty CD8 compartment, no MHCI), they did not proliferate in any of the hosts (Fig. 2A, upper panel). To create space in the T cell compartments of these mice, we Ab-depleted CD4 T cells from the various recipients. The depletion efficiency was 90–95% in all mice and resulted in T cell lymphopenic β₂m⁻/⁻ and CD11c-MHCI mice, but did not alter the endogenous CD8 compartment in K14-β₂m × CD11c-MHCI (data not shown). The adoptively transferred CD8 T cells proliferated only in CD11c-MHCI recipients, divided two or three times in 7 days (Fig. 2A, lower panel), doubled in total numbers (Fig. 2A), and modulated activation markers CD44 and CD62L (data not shown). However, the same T cells did not divide in either β₂m⁻/⁻ or in K14-β₂m × CD11c-MHCI mice (Fig. 2A). Typically the recoveries of naive CD8 T cells in all experiments were between 5 and 10% of input cell numbers, and we detected no differences in the efficiencies of recovery that correlated with either donor or recipient mouse genotypes. These data demonstrate that MHCI⁺ DC in nonirradiated, T cell lymphopenic mice are sufficient to induce CD8 T cell expansion (Fig. 2A; CD11c-MHCI, CD4 depleted), which is efficiently suppressed by endogenous CD8 T cells in the absence of CD4 T cells (Fig. 2A; K14-β₂m × CD11-MHCI, CD4 depleted). Furthermore, MHCI-dependent expansion, as observed in irradiated, β₂m⁻/⁻ hosts (5, 27) (Fig. 1B), seems to be an irradiation artifact, because in CD4-depleted, β₂m⁻/⁻ mice, CD8 T cells did not divide (Fig. 2A).

DC are sufficient to induce complete homeostatic proliferation of CD8 T cells in T cell-depleted hosts

Next, we wanted to investigate whether the HP induced by DC would be quantitatively comparable to the proliferation induced by the totality of MHCI⁺ APC plus nonprofessional, nonlymphoid MHCI⁺ cells in C57BL/6 mice. The latter have previously been speculated to drive homeostatic proliferation of CD8 T cells (1, 29). Therefore, we depleted endogenous T cells by repeated injections of opsonizing anti-Thy1.2-specific mAb in the various hosts, as described previously (5). Subsequently, we transferred naive
Thy1-congenic (Thy1.1\(^+\)), CFSE-labeled CD8 T cells into the various recipients. The depletion efficiency in both types of recipients at the point of CD8 T cell transfer until the termination of the experiments was >95% for CD4 and CD8 T cells (data not shown). MHCI\(^+\) DC were sufficient to trigger CD8 T cell expansion in T cell-depleted, CD11c-MHCI mice (Fig. 2B), and the resulting proliferation was similar to that observed in C57BL/6 mice (Fig. 2B), with up to four cell divisions during 7 days. Also, the modulation of activation markers, CD44 and CD62L (Fig. 2B, lower panel), as well as the recovery rates of CD8 T cells (Fig. 2A) in both hosts were comparable. These data indicate that MHCI\(^+\) DC are sufficient to induce maximal HP of CD8 T cells in hosts with T cell lymphopenia; however, the presence of nonprofessional, nonlymphoid MHCI\(^+\) APC (29) in T-depleted C57BL/6 recipients did not increase T cell expansion.

**CD4 T cells, but not CD25\(^+\) T cells, suppress homeostatic expansion of CD8 T cells**

To determine to what extent CD4 T cells intercompete with naive CD8 T cells for common resources during HP, we selectively depleted CD4 T cells in the various hosts (Fig. 3A). Although treatment with a high dose of anti-CD4 Ab eliminated >90% of endogenous CD4 T cells (Fig. 3A), the remaining endogenous CD8 T cells efficiently inhibited the proliferation of wild-type CD8 T cells in C57BL/6 mice with a wild-type MHC expression pattern (Fig. 3A) as seen previously for MHCI-deficient CD8 T cells in K14-\(\beta_2m\) \times CD11c-MHCI recipients (see above; Fig. 2A). In contrast, CD4 depletion in CD11c-MHCI hosts allowed the transferred CD8 T cells to proliferate and divide more than twice (Fig. 3A). As a result, the recovery rates of CD8 T cells were twice as high as those found in C57BL/6 mice 7 days after transfer (Fig. 3A). Together with the data from Fig. 2A, these results indicate that CD4 T cells can efficiently compete with CD8 T cells for resources. To test whether this competition by CD4 T cells was an all or nothing course of action or if partial reductions of CD4 numbers were sufficient to allow CD8 T cells to expand, we lowered the dose of anti-CD4 Ab for in vivo treatment (Fig. 3B). This treatment induced a partial (Fig. 3B; 40–50%) depletion of CD4 T cells during the 14-day observation period. Although as expected from the results presented in Fig. 3A, partial CD4 T cell depletion did not allow transferred CD8 T cells to proliferate homeostatically in C57BL/6 controls after 7 or 14 days, it was sufficient to induce some expansion of CD8 T cells in CD11c-MHCI mice, leading to increased recovery rates of the transferred T cells (Fig. 3B). However, the division rates (approximately one division; Fig. 3B) after 7 days were clearly lower compared with those in the same hosts with complete CD4 T cell depletion (Fig. 3A). Only after 14 days did the division rate (about two or three divisions; Fig. 3B) in partially depleted hosts reach approximately the level observed in fully CD4-depleted hosts at 7 days (Fig. 3A). To investigate whether CD25\(^+\) CD4\(^+\) T regulatory cells would be responsible for controlling HP of CD8 T cells as shown previously for CD4 T cells (30, 31), we depleted recipient mice with an anti-CD25 mAb as described previously (32). The depletion efficiency was between 80 and 90% (data not shown). However, in neither C57BL/6 nor CD11c-MHCI mice was this sufficient to allow homeostatic expansion of CD8 T cells (Fig. 3C). Because the expanding CD8 T cells themselves were CD25-negative during their proliferation (data not shown), we can exclude the possibility that the anti-CD25 treatment depleted the proliferating CD8 T cells. These data indicate a tight coregulation of CD8 and CD4 T cell compartments, demonstrated by the fact that partial liberation of resources by CD4 T cell removal allows homeostatic expansion of CD8 T cells, and full liberation of resources allows a proportionally stronger CD8 T cell expansion.

**Discussion**

To date the identity of an HP-triggering APC has been unclear, although DC have been candidates due to several indirect lines of evidence (1). The fact that HP of CD8 T cells was reduced in lymphotixin \(\alpha\)-deficient mice (21), which have, among other defects, diminished numbers of DC in lymphoid tissues (33), was a sign that DC could be involved. In addition, entering T zones of the follicles was a precondition for HP (21), and there the density of DC is elevated compared with other regions. In contrast, several arguments existed against an involvement of DC. 1) CD8 T cells proliferated normally in splenectomized mice without lymph nodes (alvitaly mice) (29); however, this animal model did not exclude T cell-DC encounters in other tissues. 2) In mixed radiation chimeras, MHCI\(^+\) radioreistant, non-BM-derived stromal...
cells seemed to trigger HP of CD8 T cells via MHC expression and IL-7 production (10). Our results clearly identify DC as the APC with the capacity to induce HP. However, we cannot formally exclude that other cell types are also able to induce HP, yet the presence of other nucleated MHCI - cells in T-depleted C57BL/6 mice did not increase the proliferative rate, recovery, or activation marker modulation (Fig. 2B). Therefore, DC are sufficient to trigger maximal CD8 T cell HP. These findings are in line with our previous demonstration that MHCI DC are sufficient to support long term survival of naive CD4 T cells (34), another DC function where presentation of MHC/self-peptide ligands for naive CD8 T cells. Therefore, MHC signals provided by DC and the availability of sufficient IL-7 are prerequisites for CD8 T cell proliferation in lymphopenic hosts. CD4 T cell inhibition of this process is most likely mediated by consumption of soluble factors such as IL-7. However, additional work is needed to determine whether competition for soluble factors also occurs during DC-T cell interaction, whether DC present/produce these factors, or whether TCR/MHC-self-peptide interaction is pre-conditioning CD8 T cells for the subsequent reception of cytokine signals.

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Disclosures
The authors have no financial conflict of interest.

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