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Cutting Edge: Innate Immune Cells Contribute to the IFN-γ-Dependent Regulation of Antigen-Specific CD8+ T Cell Homeostasis1

Özen Sercan, Günter J. Hämmerling, Bernd Arnold, and Thomas Schüler2

IFN-γ has a dual function in the regulation of T cell homeostasis. It promotes the expansion of effector T cells and simultaneously programs their contraction. The cellular mechanisms leading to this functional dichotomy of IFN-γ have not been identified to date. In this study we show: 1) that expansion of wild-type CD8+ T cells is defective in IFN-γ-deficient mice but increased in IFN-γR-deficient mice; and 2) that contraction of the effector CD8+ T cell pool is impaired in both mouse strains. Furthermore, we show that CD11b+ cells responding to IFN-γ are sufficient to limit CD8+ T cell expansion and promote contraction. The data presented here reveal that IFN-γ directly promotes CD8+ T cell expansion and simultaneously induces suppressive functions in CD11b+ cells that counter-regulate CD8+ T cell expansion, promote contraction, and limit memory formation. Thus, innate immune cells contribute to the IFN-γ-dependent regulation of Ag-specific CD8+ T cell homeostasis. The Journal of Immunology, 2006; 176: 735–739.

Brief TCR signals are sufficient to activate naive CD8+ T cells and induce their expansion and subsequent differentiation into effector/memory cells (1–3). Effector cell numbers reach a maximum 7–8 days after primary Ag contact and then start to decline. Ninety to 99% of effector CD8+ T cells die during the contraction phase, and the few survivors differentiate into long-lived memory CD8+ T cells (4–6).

IFN-γ is essential for pathogen clearance and tumor rejection in many experimental systems (7, 8). In addition to its effector functions, IFN-γ contributes to the regulation of T cell homeostasis (9). On the one hand, IFN-γ promotes priming and expansion of naive CD4+ and CD8+ T cells (10–13). On the other hand, it induces the apoptosis of effector T cells (14–17). The functional dichotomy of IFN-γ is most evident in IFN-γ−/− mice, where effector CD8+ T cell expansion and elimination are impaired at the same time (9). This can lead to the accumulation of effector/memory T cells and subsequent immune pathology (16, 18, 19).

To date, IFN-γ produced by and acting on T cells has been the center of interest when T cell homeostasis was studied (10–19). However, T cells are not the only source of IFN-γ, and most if not all cells in the body express the IFN-γR (7, 8). We therefore analyzed whether cells other than Ag-specific CD8+ T cells are involved in the IFN-γ-dependent regulation of CD8+ T cell homeostasis. To study CD8+ T cell responses in the absence of host IFN-γ production and signaling, IFN-γ-deficient (IFN-γ−/−) and IFN-γ receptor 1-deficient (IFN-γR−/−) mice, respectively, were reconstituted with wild-type (wt)3 TCR transgenic CD8+ T cells to study the kinetics of their response in vivo. We show here that host-derived IFN-γ directly acts on CD8+ T cells to promote their expansion. At the same time, IFN-γ acts on host cells to limit expansion and induce contraction. As a consequence, the numbers of effector and memory CD8+ T cells are strikingly increased in IFN-γR−/− mice. NK cells are the major source of IFN-γ in our experimental system, and CD11b+ cells are sufficient to limit effector cell expansion and memory formation in an IFN-γR-dependent fashion. CD8+ T cell function is largely unaffected by host IFN-γ/IFN-γR, suggesting that host IFN-γ regulates quantitative rather than qualitative aspects of T cell homeostasis. Therefore, early IFN-γ production by innate immune cells not only contributes to the initiation of adaptive immune responses but also counter-regulates the generation of effector/memory CD8+ T cells to maintain immunological self-tolerance and memory diversity.

Materials and Methods

Mice

The wt C57BL/6J mice (B6; Thy 1.2+), IFN-γ−/− (B6.129S7-Ifngrtm1Agt; Thy 1.2+), iNOS−/− (B6.129P2-Nos2−/−; Thy 1.2+), and congenic B6.PL-Thyl1a/Cy mice were purchased from Charles River Laboratories and bred in our animal facility. C57BL/6 OT-I mice express a transgenic TCR (Vα2Vβ8.1) specific for the H2-Kb-restricted peptide OVA257–264 (SIINFEKL) derived from chicken OVA and were provided by M. Zenke (Max Delbrück Center for Molecular Medicine, Berlin, Germany) with the kind permission of F. Carbone and W. Heath. OT-I mice were crossed to B6.PL-Thyl1a/Cy to generate congenic OT-I mice homozygous for Thy1.1. All mice were housed under specific-pathogen-free conditions.

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3 Abbreviations used in this paper: wt, wild type; DPBS, Dulbecco’s PBS; iNOS, inducible NO synthase.
Cell transfers
CD8+ T cells from spleens and lymph nodes of Thy1.1-congenic OT-I mice were purified using CD8α-specific microbeads and autoMACS (both from Miltenyi Biotec). At day −1, 1 × 10^6 to 2 × 10^6 CD8+ T cells were injected i.v. into the tail vein. At day 0, recipient mice were immunized i.v. with 50 μg of LPS (Sigma-Aldrich) and 250 μg of SIINFEKL peptide. Control animals were injected with DPBS instead. At the indicated time points, spleens were isolated and single cell suspensions were prepared. After erythrocyte lysis, spleen cells were counted. Additionally, spleen cells were stained with mAbs for CD8α (53-6.7) and Thy1.1 (OX-7) (BD Pharmingen). Cell separation was performed with autoMACS (Miltenyi Biotec). CD11b+ cells (1–1.3 × 10^6) were injected i.v. into the respective recipients.

Intracellular detection of IFN-γ
To stimulate IFN-γ production by OT-I cells, spleen cells from individual mice were cultured in 96-well tissue culture plates for 3 h at 37°C in RPMI 1640 with 10% FCS, penicillin/streptomycin, and 2-ME (50 μM) (complete RPMI 1640) with or without 10 μM peptide SIINFEKL in the presence of brefeldin A. For intracellular staining, the IFN-γ-specific mAb XMG1.2 (BD Pharmingen) and an intracellular cytokine staining kit (BD Pharmingen) were used according to the manufacturer’s recommendations.

To determine the source of host IFN-γ, the indicated mice were injected with 50 μg of LPS i.v. Three hours later, spleen cells were cultured for 3 h at 37°C in complete RPMI 1640 with brefeldin A. Spleen cells were then incubated with mAbs for DX5, CD3 (145-2C11; BD Pharmingen), CD8α, and IFN-γ using the intracellular cytokine staining kit.

Results and Discussion
To test whether IFN-γ and/or IFN-γR produced by host cells contribute to the regulation of CD8+ T cell responses, we studied the responses of wt TCR-transgenic CD8+ T cells in IFN-γ−/−, IFN-γR−/−, and wt mice. Thy1.1-congenic OT-I mice expressing an H-2Kb-restricted TCR specific for the chicken OVA-derived peptide SIINFEKL served as T cell donors. CD8+ Thy1.1+ OT-I cells were purified by MACS, labeled with CFSE, and injected i.v. into the different recipients. Because IFN-γ affects protein degradation by immunoproteasomes and subsequent MHC class I Ag presentation (20), recipient mice were not immunized with OVA protein. Instead, a mixture of LPS and SIINFEKL was used for vaccination. This mixture provided OT-I cells with the same amounts of TCR ligands in all experimental groups irrespective of differences in IFN-γ-dependent Ag presentation. Control wt mice were injected with DPBS instead (naive).

The abundance of OT-I cells in the spleen (Fig. 1A) and their frequency within the CD8+ T cell pool (Fig. 1B) were determined for the expansion, contraction, and memory phase at days 7, 14, and 30 after immunization, respectively. At day 7, only very few CD8+ Thy1.1+ OT-I cells were detectable in naive control mice (Fig. 1, A and B). In immunized wt mice, OT-I cells had expanded and represented 2.4% of cells in the spleen (Fig. 1A). In contrast, 5-fold fewer OT-I cells were found in IFN-γ−/− mice, but 2-fold more OT-I cells were found in IFN-γR−/− mice (Fig. 1, A and B). This result demonstrates: 1) that host-derived IFN-γ is required for efficient CD8+ T cell expansion; and 2) that its actions on host cells are not required for expansion but rather limit it. Irrespective of the host, OT-I cells were negative for CFSE at day 7 (data not shown), indicating that host IFN-γ/IFN-γR did not affect cell cycle progression.

At day 14, OT-I cell numbers were reduced in all recipients, demonstrating that the effector OT-I pool had contracted irrespective of host IFN-γ/IFN-γR expression (Fig. 1, A and B). However, the numbers of OT-I cells were increased in IFN-γR−/− mice as compared with wt and IFN-γ−/− mice, both of which contained similar numbers (Fig. 1, A and B). At day 30, OT-I numbers had further decreased in all experimental groups (Fig. 1A, and B). Nevertheless, IFN-γR−/− mice contained 4–5 times more memory OT-I cells than wt and IFN-γ−/− mice (Fig. 1, A and B).

Memory CD8+ T cells are derived from effector cells that survive the contraction phase. Therefore, the number of memory CD8+ T cells is determined by both the size of the effector cell pool and the degree of its contraction (4–6). To analyze whether OT-I memory cells accumulated in IFN-γR−/− mice due to impaired effector cell contraction, OT-I responses were normalized. For this purpose, OT-I effector cell numbers at day...
7 were defined as 100%, and the values at days 14 and 30 were calculated in relation to the numbers at day 7. This allowed the comparison of all experimental groups irrespective of the differences in total OT-I cell numbers (Fig. 1B). At day 30, 41% of OT-I cells were still present in IFN-γR-/- mice, but only 10% were present in wt mice (Fig. 1C). Effector cell survival was similarly improved in IFN-γR-/- mice, where 54% of OT-I cells survived until day 30 (Fig. 1C). Therefore, the data shown in Fig. 1C demonstrate: 1) that IFN-γR signaling in host cells contributes to effector CD8+ T cell contraction; and 2) that host but not CD8+ T cells are the source of the “regulatory” IFN-γ.

At day 7, the percentages of annexin V+ OT-I cells were comparable in all experimental groups (data not shown), indicating that differences in apoptotic cell death were not responsible for the different contraction rates. Whether the host IFN-γ/IFN-γR supports a caspase-independent pathway of CD8+ T cell contraction (21) remains to be shown.

Next, we aimed to identify the source of host IFN-γ. Because SIINFEKL or DPBS alone did not induce IFN-γ production in spleens of wt mice (data not shown), the effect of LPS was tested. To mimic vaccination, LPS was injected i.v. into untreated wt, IFN-γ-/-, and IFN-γR-/- mice, and intracellular cytokine staining for IFN-γ was performed. As shown in Fig. 2A, 1.9% and 1.6% of wt and IFN-γR-/- spleen cells, respectively, produced IFN-γ 6 h after LPS injection. DX5+CD3+CD8+ NK cells were the major source of IFN-γ, which was not detectable in IFN-γ-/- mice (Fig. 2A and B). It was shown only recently that NK-derived IFN-γ promotes CD4+ T cell priming (10). A similar scenario can be anticipated for CD8+ T cells, because host-derived IFN-γ is required for CD8+ T cell expansion (Fig. 1A and B) and NK cells are the major source of IFN-γ in our system (Fig. 2A and B). Because NK cells produce IFN-γ only after 24 h after LPS injection (22) and CD8+ T cells lose their IFN-γ sensitivity within 12 h after primary TCR triggering (23), we conclude that IFN-γ exerts its growth-promoting effect in the very early priming phase of the CD8+ T cell response. However, because NK cells are not the only source of early IFN-γ (Fig. 2A and B), we cannot exclude a contribution of other cells to the IFN-γ-dependent regulation of CD8+ T cell homeostasis.

CD11b+ macrophage-like cells produce NO in response to IFN-γ (24, 25). NO interferes with IL-2 signaling in T cells, blocks the subsequent expansion (25), and induces apoptosis (15). To test whether CD11b+ myeloid cells suppress CD8+ T cell responses in an IFN-γR-dependent fashion, IFN-γR-/- mice were reconstituted with purified CD11b+ cells from wt (Fig. 2, C and D) or IFN-γR-/- (Fig. 2, E and F) mice. Simultaneously, these mice received CD8+ Thy1.1+ OT-I cells. Groups of IFN-γR-/- mice were reconstituted with CD8+ OT-I cells only and served as controls (Fig. 2, C–F). As expected, CD8+ OT-I cells were most abundant in control IFN-γR-/- mice at days 7 and 30 after immunization (Fig. 2, C–F). However, in IFN-γR-/- mice reconstituted with wt CD11b+ cells the frequency of CD8+ OT-I cells was reduced at day 7 (Fig. 2C), and the differences between both experimental groups were even more pronounced at day 30 (Fig. 2D). In contrast, the numbers of CD8+ OT-I cells did not differ between control IFN-γR-/- mice and those reconstituted with IFN-γR-/- CD11b+ cells (Fig. 2, E and F). Therefore, the data shown in Fig. 2, C–F, demonstrate that IFN-γR signaling in CD11b+ cells is sufficient to reduce CD8+ T cell expansion (day 7), promote contraction, and limit memory CD8+ T cell formation (day 30).

To analyze whether the IFN-γR-dependent counter-regulation of CD8+ T cell responses was mediated by NO, mice deficient for iNOS, the enzyme that is required for NO production in response to IFN-γ (26), were reconstituted with CD8+ OT-I cells, wt mice served as controls. As shown in Fig. 2, G and H, OT-I cell numbers were nearly identical in iNOS-/- and wt mice at days 7 and 30 after immunization. Because NO was not required to counter-regulate CD8+ T cell responses in our system, the molecular mechanisms by which CD11b+ cells
exert their IFN-γR-dependent regulatory function remain to be defined. The wt effector OT-I cells primed in vitro do not accumulate in IFN-γR−/− mice (27), suggesting that CD11b+ cells do not exert their regulatory function in the late phase of a CD8+ T cell response. Because our vaccination protocol induces the production of early IFN-γ (Fig. 2, A and B), which is known to program CD8+ T cell contraction (28), it seems likely that CD11b+ cells exert their IFN-γR-dependent regulatory functions in the priming phase. However, the contribution of IFN-γ to the regulation of T cell homeostasis will vary with the experimental system, because the induction of early IFN-γ is strongly pathogen dependent (29).

The question of whether host IFN-γ/IFN-γR affected the function of CD8+ T cells was analyzed next. The wt, IFN-γ−/−, and IFN-γR−/− mice were reconstituted with CD8+ Thy1.1+ OT-I cells and vaccinated as before. On days 7, 14, and 30 after vaccination, recipient spleen cells were cultured for 3 h with SIINFEKL in the presence of brefeldin A. Subsequently, spleen cells were stained for CD8, Thy1.1, and intracellular IFN-γ. The percentages of IFN-γ+ OT-I cells were determined after gating on as CD8+ Thy1.1+ OT-I cells. Shown are mean values with SD for one representative experiment. Two independent experiments with three mice per group and time point were performed.

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

**FIGURE 3.** Host IFN-γ/IFN-γR expression does not affect CD8+ T cell function. CD8+ Thy1.1+ OT-I cells were transferred, and recipients were immunized as before. At days 7, 14, and 30 after immunization, recipient spleen cells were cultured for 3 h with SIINFEKL in the presence of brefeldin A. Subsequently, spleen cells were stained for CD8, Thy1.1, and intracellular IFN-γ. The percentages of IFN-γ+ OT-I cells were determined after gating on as CD8+ Thy1.1+ OT-I cells. Shown are mean values with SD for one representative experiment. Two independent experiments with three mice per group and time point were performed.


