

THE DIFFERENCE OF
BREAKING THROUGH BARRIERS
WITH VIBRANCE

ENABLING GREATER EXPERIMENTAL
INSIGHTS WITH THE POWER OF
BD HORIZON™ RED 718 REAGENTS



Achieve your resolution goals today >



An IFN- γ -Dependent Pathway Controls Stimulation of Memory Phenotype CD8⁺ T Cell Turnover In Vivo by IL-12, IL-18, and IFN- γ

This information is current as of April 12, 2021.

David F. Tough, Xiaohong Zhang and Jonathan Sprent

J Immunol 2001; 166:6007-6011; ;
doi: 10.4049/jimmunol.166.10.6007
<http://www.jimmunol.org/content/166/10/6007>

References This article **cites 31 articles**, 18 of which you can access for free at:
<http://www.jimmunol.org/content/166/10/6007.full#ref-list-1>

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at:
<http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at:
<http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at:
<http://jimmunol.org/alerts>

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852
Copyright © 2001 by The American Association of
Immunologists All rights reserved.
Print ISSN: 0022-1767 Online ISSN: 1550-6606.



An IFN- γ -Dependent Pathway Controls Stimulation of Memory Phenotype CD8⁺ T Cell Turnover In Vivo by IL-12, IL-18, and IFN- γ ¹

David F. Tough,^{2*} Xiaohong Zhang,[†] and Jonathan Sprent[†]

Unlike naive T cells, memory phenotype (CD44^{high}) T cells exhibit a high background rate of turnover in vivo. Previous studies showed that the turnover of memory phenotype CD8⁺ (but not CD4⁺) cells in vivo can be considerably enhanced by products of infectious agents such as LPS. Such stimulation is TCR independent and hinges on the release of type I IFNs (IFN-I) which leads to the production of an effector cytokine, probably IL-15. In this study, we describe a second pathway of CD44^{high} CD8⁺ stimulation in vivo. This pathway is IFN- γ rather than IFN-I dependent and is mediated by at least three cytokines, IL-12, IL-18, and IFN- γ . As for IFN-I, these three cytokines are nonstimulatory for purified T cells and under in vivo conditions probably act via production of IL-15. *The Journal of Immunology*, 2001, 166: 6007–6011.

It is well recognized that immunological memory is long-lived, with a single infection often inducing life-long immunity (1). How this longevity is achieved, however, remains poorly understood. Answering this question hinges on understanding the kinetic behavior of memory cells and the factors that regulate their life span. In this regard, studies in animals and humans have revealed that the majority of T cells with a memory phenotype (CD44^{high} in mice) exhibit a rapid rate of turnover (2–6). This was also shown to apply to memory T cells of known antigenic specificity using TCR-transgenic mice (7, 8). The rapid turnover of memory T cells contrasts with that of naive T cells, which rarely, if ever, divide in the absence of overt stimulation by specific Ag (3, 4, 9, 10). The implication is that the memory T cell pool is maintained at least in part by periodic cell division, raising the important question of what factors are driving the cell turnover.

The most straightforward explanation for the observed rapid turnover of memory T cells is that cell division is occurring in response to antigenic stimulation. However, it is clear that memory T cells can persist long term in the absence of specific Ag (11–16). Furthermore, it has been shown that memory CD8⁺ T cells divide after adoptive transfer into recipient mice in the absence of Ag (8, 17) and also after adoptive transfer into MHC class I-deficient mice (16, 17). The latter result strongly suggests that memory T cells can be driven to divide by signals delivered independently of TCR-MHC interactions. Consistent with this, recent studies have shown that certain cytokines can induce bystander proliferation of memory phenotype T cells in vivo (18, 19).

Cytokine-induced bystander proliferation is largely restricted to cells having a memory phenotype. Thus, injection of either type I IFN (IFN-I)³ or inducers of IFN-I into normal mice induced a marked increase in CD44^{high} CD8⁺ T cell proliferation, whereas little if any increase in proliferation was observed for naive phenotype CD8⁺ T cells (18); a recent study has shown that injection of poly(I:C), an inducer of IFN-I, also stimulates bystander proliferation of memory but not naive phenotype CD4⁺ T cells (20). Importantly, CD44^{high} CD8⁺ T cells divided in vivo in response to IFN-I after adoptive transfer to β_2 -microglobulin^{-/-} mice, indicating that TCR stimulation was likely not involved. Proliferation in response to IFN-I implies that bystander proliferation of CD8⁺ memory T cells might be a frequent occurrence, since rapid induction of IFN-I is a common response to infection. Indeed, marked proliferation of CD44^{high} CD8⁺ T cells occurs after injection of poly(I:C) (18), LPS (21), or CpG DNA (22, 23); these compounds are powerful inducers of IFN-I in vivo. However, experiments in mice lacking a functional IFN-I receptor (IFN-IR^{-/-}) suggested that IFN-I might not be unique in its ability to induce bystander proliferation. In these mice, memory phenotype CD8⁺ T cells failed to proliferate in response to a low dose of LPS, but did so after injection of a higher dose. An obvious possibility is that the higher dose of LPS induced additional cytokines that were also able to stimulate CD44^{high} CD8⁺ T cells. Subsequent work showed that IL-15 was one such cytokine, as injection of IL-15 into mice stimulated potent and selective proliferation of CD44^{high} CD8⁺ T cells (19). Furthermore, it has been reported that IFN-I-independent bystander proliferation of CD44^{high} CD8⁺ T cells also occurs after injection of the NKT cell stimulator α -galactosylceramide (20). In this study, injection of neutralizing anti-IFN- $\alpha\beta$ Ab failed to inhibit α -galactosylceramide-induced proliferation of CD44^{high} CD8⁺ T cells in the liver. There was, however, a partial reduction in the bystander response in IL-12-deficient mice also injected with anti-IFN- γ (but not in IL-12-deficient mice themselves), suggesting a possible role for IFN- γ .

Thus, although it has been demonstrated that memory phenotype CD8⁺ T cells are sensitive to bystander stimulation in vivo, the

*Edward Jenner Institute for Vaccine Research, Compton, Newbury, Berkshire, United Kingdom; and [†]Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Received for publication November 29, 2000. Accepted for publication March 6, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ This work was supported by Grants CA38355, CA25803, AI21487, and AG01743 from the U.S. Public Health Service and by a collaborative grant from the Edward Jenner Institute for Vaccine Research. This is Publication No. 23 from the Edward Jenner Institute for Vaccine Research.

² Address correspondence and reprint requests to Dr. David F. Tough, Edward Jenner Institute for Vaccine Research, Compton, Newbury, Berkshire RG20 7NN, U.K. E-mail address: david.tough@jenner.ac.uk

³ Abbreviations used in this paper: IFN-I, type I IFN; IFN-IR, IFN-I receptor; BrdU, bromodeoxyuridine; LN, lymph node.

range of infection-induced cytokines capable of inducing proliferation is not known. In this paper, we report that three additional cytokines, IL-12, IL-18, and IFN- γ , share the capacity of IFN-I to induce selective proliferation of CD44^{high} CD8⁺ T cells in vivo but not in vitro. In contrast to IFN-I, however, these three cytokines induce T cell proliferation in vivo by an IFN- γ -dependent pathway.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from either the rodent breeding colony at The Scripps Research Institute, the specific pathogen-free unit at the Institute for Animal Health (Compton, U.K.), or The Jackson Laboratory (Bar Harbor, ME). 129/SvEvTacfBR (129) mice were purchased from Taconic Farms (Germantown, NY). 129 background mice defective in IFN-IR function (IFN-IR^{-/-}) (24) were originally purchased from B&K Universal (North Humberston, U.K.) and were maintained and bred in the animal facility at The Scripps Research Institute. IFN- γ -deficient (IFN- γ ^{-/-}) mice were purchased from The Jackson Laboratory or the specific pathogen-free unit at the Institute for Animal Health. C3H/HeJ and C3H/HeOuj mice were purchased from The Jackson Laboratory.

Cytokines and injection

Recombinant murine IL-12 used was either that provided as a gift from Genetics Institute (Cambridge, MA) or that purchased from R&D Systems (Minneapolis, MN). Recombinant mouse IFN- γ and recombinant human IL-15 were purchased from R&D Systems. IL-18 was purchased from BioSource International (Camarillo, CA). The specified doses of cytokines were injected into mice i.v. as indicated.

Treatment of mice with bromodeoxyuridine (BrdU)

Mice were given BrdU (Sigma, St. Louis, MO) in their drinking water at a concentration of 0.8 mg/ml. BrdU administration was started immediately after injections and mice were sacrificed 3 days later. BrdU was dissolved in sterile water and changed daily.

In vitro culture of T cells

CD8⁺ T cells were purified by staining total LN or spleen cells with anti-CD8-PE (Life Technologies, Grand Island, NY) followed by cell sorting on a MoFlo flow cytometer (Cytomation, Fort Collins, CO). Sorted cells were resuspended in complete medium (RPMI 1640 + GlutaMAX I (Life Technologies) supplemented with 10% FCS, 5% NCTC-135 (Life Technologies), 5 \times 10⁻⁸ M 2-ME (Sigma), 250 μ g/ml gentamicin (Life Technologies), and 50 U/ml penicillin/streptomycin (Life Technologies)), and plated at 1 \times 10⁶ cells/ml (1 ml/well) in 24-well plates. Cells were cultured in medium alone, or medium supplemented with recombinant murine IL-12, recombinant murine IL-18, or recombinant human IL-15 as indicated. BrdU was added to wells at 12.5 μ g/ml from the beginning of culture, and cells were assayed 40 or 72 h later as indicated. Harvested cells were

depleted of dead cells by centrifugation over Histopaque-1083 (Sigma) before staining.

Monoclonal Abs and flow cytometry

mAbs used for cell surface staining were the following: anti-CD8-PE, anti-CD4-PE (Life Technologies), anti-CD44-biotin (IM7.8.1), anti-Ly-6C-biotin (PharMingen, San Diego, CA), anti-CD4-Cy-5 (GK1.5), and anti-CD8-Cy-5. Anti-CD4-Cy-5 was prepared using a kit from Amersham Life Science (Arlington Heights, IL). Anti-CD8-Cy-5 was either purchased from Caltag (Burlingame, CA) or prepared as for anti-CD4-Cy-5. Biotinylated Abs were detected with streptavidin-Red670 (Life Technologies).

After surface staining, BrdU labeling was assessed with anti-BrdU-FITC (Becton Dickinson, Mountain View, CA) as described elsewhere (4). Stained cells were analyzed on either FACScan or FACSCalibur flow cytometers (Becton Dickinson).

Results and Discussion

Effect of IL-12 on T cell turnover

To determine the effect of IL-12 on T cell turnover, graded doses of recombinant murine IL-12 were injected i.v. into B6 mice and the recipients were given BrdU in the drinking water for 3 days (Fig. 1, A–D). Injection of IL-12 stimulated a marked proliferation of memory phenotype CD44^{high} CD8⁺ T cells, with the percentage of BrdU-labeled cells increasing in a dose-dependent manner; this was true in both LN and spleen (Fig. 1, A and B). In contrast to CD44^{high} CD8⁺ T cells, IL-12 had no effect on the BrdU labeling of naive phenotype CD44^{low} CD8⁺ T cells. Similarly, injection of IL-12 induced minimal proliferation among LN CD4⁺ T cells, although a small increase in BrdU labeling was observed at the highest dose of IL-12 injected for CD44^{high} cells only (Fig. 1C). Of note, significant proliferation of splenic CD44^{high} CD4⁺ cells was observed after injection of IL-12, but such stimulation was largely restricted to a subset of NK1.1⁺ cells (data not shown). How these cells are stimulated by IL-12 will be the subject of another publication. The remainder of this paper will focus on the effects of IL-12 on memory phenotype CD8⁺ T cells.

Corroborating previous data on LN cells (19), IL-12 was non-stimulatory for purified CD8⁺ cells in vitro. Thus, when increasing concentrations of IL-12 were added to T cells purified from either LN (Fig. 1E) or spleen (data not shown), BrdU incorporation in vitro was virtually undetectable, both for CD44^{high} and CD44^{low} CD8⁺ cells. By contrast, strong stimulation of CD44^{high} (but not CD44^{low}) CD8⁺ cells occurred following addition of IL-15. These results therefore suggest that the in vivo induction of proliferation of CD44^{high} CD8⁺ cells by IL-12 was mediated by indirect mechanisms rather than direct stimulation of the T cells.

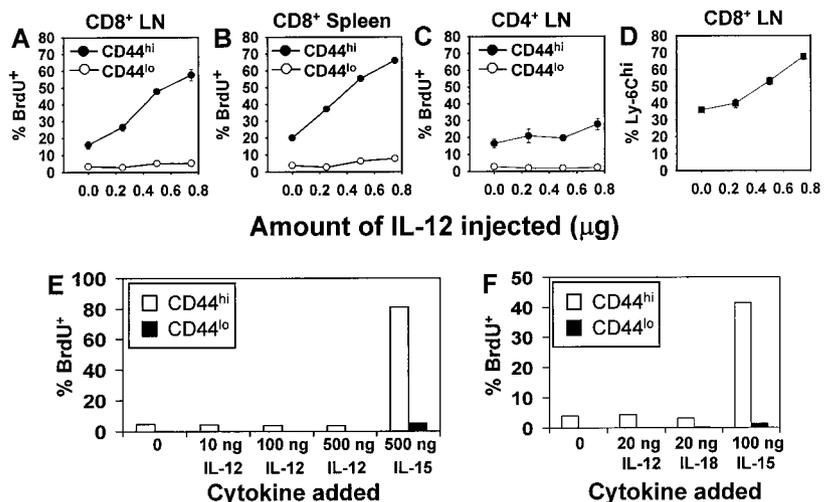


FIGURE 1. IL-12 induction of T cell proliferation in vivo but not in vitro. A–D, B6 mice were injected with the indicated amount of IL-12 i.v. and given BrdU in their drinking water for 3 days. The percentage of CD44^{low} (○) and CD44^{high} (●) CD8⁺ (A and B) or CD4⁺ (C) T cells labeled with BrdU is shown for T cells isolated from LN (A and C) or spleen (B). Shown in D is the percentage of LN CD8⁺ T cells expressing high levels of Ly-6C. Data represent the mean \pm SD for two mice per point. E and F, CD8⁺ T cells were purified from pooled LN of normal B6 mice by cell sorting and placed in culture for 40 h (E) or 72 h (F) in the presence of BrdU and the indicated cytokine. Data show percent BrdU⁺ cells among CD44^{high} (□) or CD44^{low} (■) CD8⁺ T cells.

Role of IFN-I in IL-12-induced turnover

In addition to inducing T cell proliferation, *in vivo* injection of IL-12 induced CD8⁺ T cells to up-regulate Ly-6C (Fig. 1D). Since Ly-6C was reported to be selectively up-regulated by IFN-I (25), this result suggested that IL-12 induced synthesis of IFN-I. Hence, bearing in mind that IFN-I elicits strong proliferation of CD44^{high} CD8⁺ T cell turnover *in vivo* (18), stimulation of these cells by IL-12 could be mediated via IFN-I. To test this possibility, IL-12 was injected into mice deficient for the IFN-IR (IFN-IR^{-/-}) and the response was compared with that in control mice. As shown in Fig. 2, A–D, CD44^{high} CD8⁺ T cells showed similar increases in BrdU labeling in IFN-IR^{-/-} and IFN-IR^{+/+} mice. Therefore, IL-12 induction of T cell turnover was independent of IFN-I.

Ly-6C expression was also studied in IFN-IR^{-/-} and control mice after IL-12 injection. Notably, baseline Ly-6C expression was much lower in IFN-IR^{-/-} mice, indicating an important role for IFN-I in determining Ly-6C expression under “resting” conditions (Fig. 2, E and F). Nevertheless, this was not an absolute requirement, since some Ly-6C^{high} CD8⁺ T cells were present in the IFN-IR^{-/-} mice. Injection of IL-12 resulted in increased numbers of Ly-6C^{high} CD8⁺ T cells in both control and IFN-IR^{-/-} mice. The implication is that the up-regulation of Ly-6C observed in normal mice after IL-12 injection is partly IFN-I independent.

Role of IFN- γ in the induction of memory phenotype T cell turnover

Since IL-12 is a strong inducer of IFN- γ expression (26), IL-12 might stimulate CD44^{high} CD8⁺ cells via production of IFN- γ . To

examine this possibility, we compared the response to IL-12 injection in mice deficient for IFN- γ with that in control mice. Strikingly, CD8⁺ T cells showed little if any increase in BrdU labeling after IL-12 injection into IFN- γ ^{-/-} mice (Fig. 3, A–D). Also, unlike in control mice, the percentage of CD8⁺ T cells expressing high levels of Ly-6C was not increased after IL-12 injection (Fig. 3, E and F). Therefore, the ability of IL-12 to induce both bystander proliferation of memory phenotype CD8⁺ T cells and up-regulation of Ly-6C was highly dependent on IFN- γ .

Based on the finding that IL-12-induced CD8⁺ T cell proliferation was dependent on IFN- γ , it would be predicted that other cytokines capable of inducing IFN- γ should also be able to stimulate the turnover of CD44^{high} CD8⁺ T cells *in vivo*. To test this hypothesis, we examined the effect on T cell turnover of IL-18, another strong inducer of IFN- γ (27). In fact, the activity of IL-18 was very similar to that of IL-12. Thus, although addition of IL-18 to purified CD8⁺ T cells failed to cause T cell division *in vitro* (Fig. 1F), injection of IL-18 stimulated a strong increase in the BrdU labeling of memory phenotype CD8⁺ T cells *in vivo* (Fig. 4, A and B). Also like IL-12, IL-18 had only a small effect on the turnover of CD44^{high} CD4⁺ T cells (Fig. 4C) and did not stimulate increased BrdU labeling of naive T cells. Finally, although small increases in BrdU labeling were detectable among CD44^{high} CD8⁺ T cells after IL-18 injection into IFN- γ ^{-/-} mice, proliferation was markedly reduced compared with that in control mice (Fig. 4, D and E). Thus, as for IL-12, induction of bystander CD8⁺ T cell proliferation by IL-18 is largely IFN- γ dependent.

These data are consistent with the idea that cytokines capable of inducing IFN- γ promote the turnover of memory phenotype CD8⁺

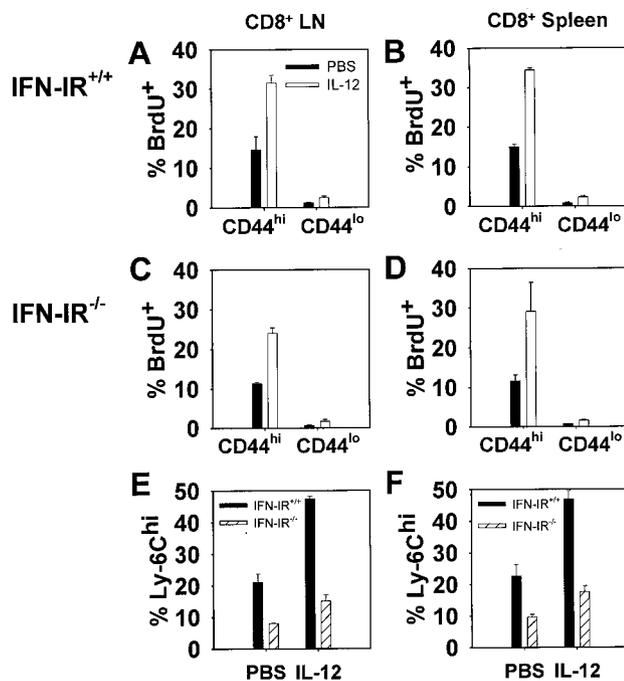


FIGURE 2. Induction of CD8⁺ T cell proliferation by IL-12 in mice lacking a functional IFN-IR. Control (129 background, IFN-IR^{+/+}) or IFN-IR^{-/-} mice were injected *i.v.* with PBS or with 0.75 μ g of IL-12 and given BrdU for 3 days. A and B, Percent BrdU⁺ of CD44^{high} and CD44^{low} CD8⁺ T cells in LN (A) and spleen (B) of control mice injected with PBS (■) or IL-12 (□). C and D, Percent BrdU⁺ of CD44^{high} and CD44^{low} CD8⁺ T cells in LN (C) and spleen (D) of IFN-IR^{-/-} mice injected with PBS (■) or IL-12 (□). E and F, Percent Ly-6C^{high} of total CD8⁺ T cells in LN (E) and spleen (F) of control (■) and IFN-IR^{-/-} (▨) mice injected with PBS or IL-12. Data represent the mean \pm SD for two mice injected with PBS and two mice injected with IL-12.

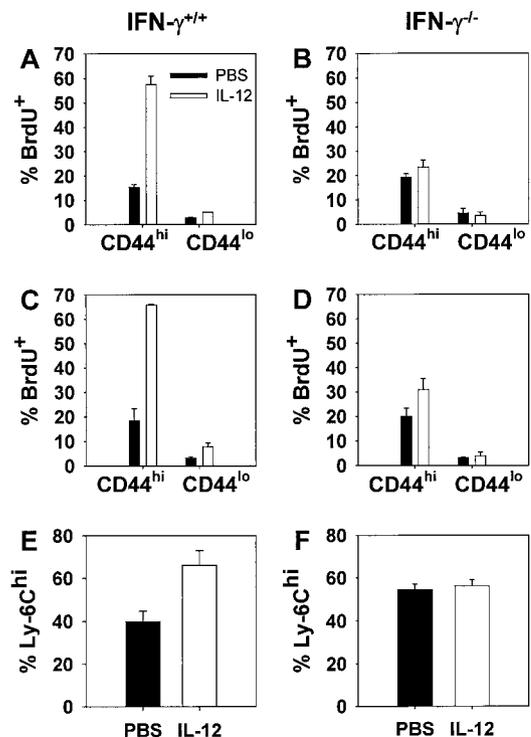


FIGURE 3. Dependence on IFN- γ of IL-12 induction of T cell proliferation. Control or IFN- γ ^{-/-} mice were injected with PBS or IL-12 (0.75 μ g) *i.v.* and given BrdU for 3 days. A–D, Percent BrdU⁺ cells among CD44^{high} and CD44^{low} CD8⁺ T cells in LN (A and B) or spleen (C and D) of control (A and C) and IFN- γ ^{-/-} (B and D) mice injected with PBS (■) or IL-12 (□). E and F, Percent of CD8⁺ spleen T cells expressing high levels of Ly-6C in control (E) and IFN- γ ^{-/-} (F) mice injected with PBS (■) or IL-12 (□). Data represent the mean \pm SD for two control and two IFN- γ ^{-/-} mice injected with PBS or IL-12.

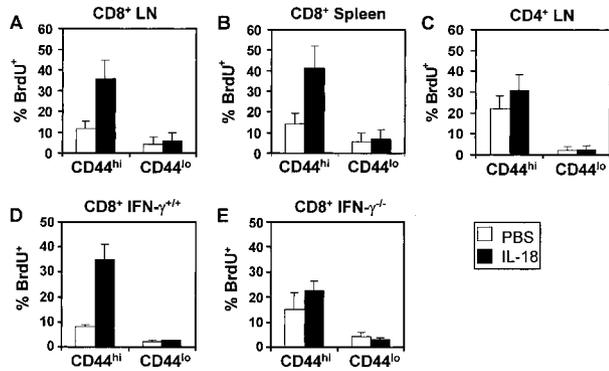


FIGURE 4. Stimulation of CD44^{high} CD8⁺ T cell proliferation in vivo by IL-18 and role of IFN- γ . A–C, PBS or IL-18 (5 μ g) was injected i.v. into B6 mice and recipients were given BrdU for 3 days. Shown are percent BrdU⁺ among CD44^{high} and CD44^{low} CD8⁺ T cells in LN (A) or spleen (B), or among CD44^{high} and CD44^{low} CD4⁺ T cells in LN (C) of mice injected with PBS (\square) or IL-18 (\blacksquare). Data represent the mean \pm SD for five mice injected with PBS and six mice injected with IL-18. D and E, PBS or IL-18 (5 μ g) was injected i.v. into control or IFN- γ ^{-/-} mice and recipients were given BrdU for 3 days. Shown are percent BrdU⁺ cells among CD44^{high} and CD44^{low} CD8⁺ T cells in spleen of control (D) and IFN- γ ^{-/-} (E) mice injected with PBS (\square) or IL-18 (\blacksquare). Data represent the mean \pm SD for three control and three IFN- γ ^{-/-} mice injected with PBS or IL-18.

T cells. To test directly the ability of IFN- γ to stimulate T cell turnover, rIFN- γ was injected i.v. into B6 mice and recipients were given BrdU for 3 days. As shown in Fig. 5, IFN- γ induced marked proliferation of CD44^{high} CD8⁺ T cells but failed to stimulate CD44^{high} CD4⁺ T cells. Like IL-12 and IL-18, injection of IFN- γ did not affect the turnover of naive phenotype T cells. Thus, IFN- γ may indeed serve as a common mediator linking IL-12 and IL-18 to the stimulation of CD44^{high} CD8⁺ T cell turnover. This cytokine pathway appears to be distinct from one that we previously described in which IFN-I, through the induction of an effector cytokine (see below), stimulates the turnover of memory phenotype CD8⁺ T cells.

It is unlikely that IFN- γ stimulates CD8⁺ T cell division directly. In fact, addition of IFN- γ to purified T cells in vitro does not induce CD8⁺ T cells to proliferate (19). However, a clue to the mechanism of action of this cytokine may come from the fact that injection of IFN- γ results in selective proliferation of CD122^{high} CD8⁺ T cells (Fig. 6, A and B). Like IL-12 (data not shown), IFN- γ stimulated an equivalent increase in BrdU labeling of these cells in C3H/HeJ and C3H/HeOuJ mice, discounting the possibility of contamination with endotoxin. Similarly, IL-18 also induced proliferation of CD122^{high} but not CD122^{low} CD8⁺ T cells (Fig. 6, C and D). The significance of this finding is that CD122^{high} CD8⁺

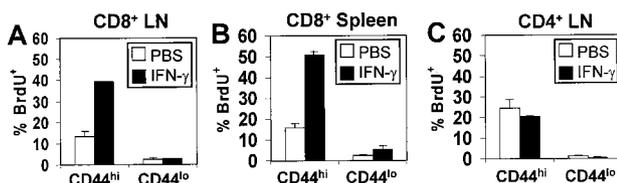


FIGURE 5. Stimulation of CD44^{high} CD8⁺ T cell proliferation in vivo by IFN- γ . A–C, PBS or IFN- γ (38 μ g) was injected i.v. into B6 mice and recipients were given BrdU for 3 days. Data show percent BrdU⁺ among CD44^{high} and CD44^{low} CD8⁺ T cells in LN (A) or spleen (B), or among CD44^{high} and CD44^{low} CD4⁺ T cells in LN (C) of mice injected with PBS (\square) or IFN- γ (\blacksquare). Data represent the mean \pm SD for two mice injected with PBS and two mice injected with IFN- γ .

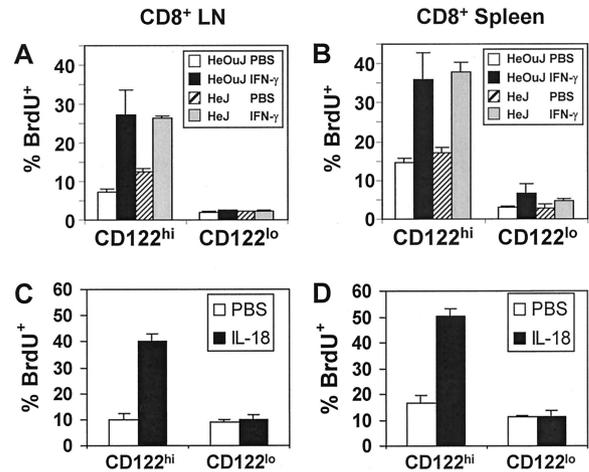


FIGURE 6. Selective stimulation of CD122^{high} CD8⁺ T cells by IFN- γ or IL-18. A and B, C3H mice that are able (HeOuJ) or unable (HeJ) to respond to LPS were injected with either PBS or IFN- γ (50 μ g) and given BrdU in their drinking water for 3 days. Graphs show percent BrdU⁺ among CD122^{high} or CD122^{low} CD8⁺ T cells in LN (A) or spleen (B). \square , C3H/HeOuJ mice injected with PBS; \blacksquare , C3H/HeOuJ mice injected with IFN- γ ; \square (hatched), C3H/HeJ mice injected with PBS; \square (grey), C3H/HeJ mice injected with IFN- γ . Data represent the mean \pm SD for two mice of each type injected with PBS and two mice of each type injected with IFN- γ . C and D, PBS or IL-18 (5 μ g) was injected i.v. into B6 mice and recipients were given BrdU for 3 days. Graphs show percent BrdU⁺ among CD122^{high} and CD122^{low} CD8⁺ T cells in LN (C) or spleen (D) of mice injected with PBS (\square) or IL-18 (\blacksquare). Data represent the mean \pm SD for two mice injected with PBS and three mice injected with IL-18.

T cells proliferate in direct response to IL-15, which is inducible by IFN- γ (19). Thus, turnover of memory phenotype CD8⁺ T cells in response to IL-12 injection may be the end result of a cascade of cytokine production.

We showed previously that the turnover of memory phenotype CD8⁺ cells in vivo increases considerably for 2–3 days after injection of IFN-I or IFN-I-inducing agents such as LPS and poly(I:C) (18, 21). In this paper, we describe a second pathway in which selective stimulation of CD44^{high} CD8⁺ cells in vivo is controlled by IFN- γ rather than IFN-I. This pathway operates following injection of at least three cytokines, IFN- γ , IL-12, and IL-18. In the case of IL-12 and IL-18, these cytokines presumably function by inducing the production of IFN- γ .

It is notable that, despite their strong stimulatory effects in vivo, IFN- γ , IL-12, and IL-18 were all unable to induce proliferation of purified T cells in vitro. Hence, it is likely that the in vivo function of these cytokines reflects the subsequent production of an effector cytokine. For several reasons we suspect that IL-15 is the effector cytokine. First, both IFN-I and IFN- γ induce the production of IL-15 mRNA by macrophages in vitro (19). Second, CD122 (IL-2R β), an important component of the receptor for IL-15 (and IL-2), is expressed at a much higher level on memory phenotype CD44^{high} CD8⁺ cells than on CD44^{high} CD4⁺ cells (19, 28). Third, virtually all of the T cells responding to IFN- γ and IFN-I in vivo are CD122^{high}. Fourth, IL-15 induces selective stimulation of memory phenotype CD8⁺ cells not only in vivo but also in vitro (19). Fifth, at least for poly(I:C) injection, stimulation of memory phenotype CD8⁺ cells by cytokines in vivo does not apply to CD122^{-/-} mice (unpublished data of X. Zhang and J. Sprent). Sixth, preliminary results suggest that memory-phenotype CD8⁺ T cells do not proliferate in response to Poly I:C injection after adoptive transfer to IL-15^{-/-} mice (A. Gulbranson-Judge and J. Sprent, unpublished data). Based on these findings, our prediction is that

both the IFN-I-dependent and IFN- γ -dependent pathways of in vivo T cell proliferation will not operate in IL-15^{-/-} mice (29). We are currently in the process of testing this prediction.

The notion that IL-15 is the final effector cytokine for in vivo T cell proliferation elicited by IFN-I and IFN- γ is in agreement with recent studies showing a profound deficiency of memory phenotype T cells in IL-15R α ^{-/-} (30) and IL-15^{-/-} (29) mice. Significantly, the paucity of memory phenotype T cells in these two lines is largely restricted to CD8⁺ cells rather than CD4⁺ cells. Similarly, selective disappearance of memory phenotype CD8⁺ occurs following repeated injection of anti-CD122 mAb (28). The implication therefore is that the survival of memory phenotype CD8⁺ cells depends crucially upon continuous stimulation via background levels of IL-15. A corollary of this notion is that in vivo exposure to agents that lead to increased synthesis of IL-15 would augment the proliferation of CD44^{high} CD8⁺ cells. Our data on the effects of injecting mice with IL-15-inducing agents such as IFN-I and IFN- γ are consistent with this prediction. It is also notable that IL-15-transgenic mice show a marked overrepresentation of memory phenotype CD8⁺ (but not CD4⁺) cells (31).

Finally, it should be pointed out that the background rate of turnover of CD44^{high} CD8⁺ T cells in IFN- γ ^{-/-} mice was not reduced compared with control mice (see Figs. 3 and 5). Furthermore, normal numbers of memory phenotype CD8⁺ T cells were found in IFN- γ ^{-/-} mice (data not shown). At face value, these observations indicate that normal CD8⁺ T cell homeostasis is not dramatically altered in the absence of IFN- γ under resting conditions. This is not surprising, given that induction of IL-15 production by other means, including the IFN-I-dependent pathway, will still occur in these mice. Here, an important question is whether the turnover of CD44^{high} CD8⁺ T cells is reduced in the combined absence of both IFN- γ and IFN-I. We are currently breeding IFN-IR^{-/-} \times IFN- γ ^{-/-} mice to assess this possibility.

Acknowledgments

We thank Drew Worth for cell sorting and Genetics Institute for supplying some of the IL-12.

References

- Ahmed, R., and D. Gray. 1996. Immunological memory and protective immunity: understanding their relation. *Science* 272:54.
- Mackay, C. R., W. L. Marston, and L. Dudler. 1990. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J. Exp. Med.* 171:801.
- Michie, C. A., A. McLean, A. Alcock, and P. C. L. Beverley. 1992. Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature* 360:264.
- Tough, D. F., and J. Sprent. 1994. Turnover of naive- and memory-phenotype T cells. *J. Exp. Med.* 179:1127.
- Mohri, H., S. Bonhoeffer, S. Monard, A. S. Perelson, and D. D. Ho. 1998. Rapid turnover of T lymphocytes in SIV-infected rhesus macaques. *Science* 279:1223.
- McCune, J. M., M. B. Hanley, D. Cesar, R. Halvorsen, R. Hoh, D. Schmidt, E. Eieder, S. Deeks, S. Siler, and R. Neese. 2000. Factors influencing T-cell turnover in HIV-1-seropositive patients. *J. Clin. Invest.* 105:R1.
- Zimmerman, C., K. Brduscha-Riem, C. Blaser, R. M. Zinkernagel, and H. Pircher. 1996. Visualization, characterization, and turnover of CD8⁺ memory T cells in virus-infected hosts. *J. Exp. Med.* 183:1367.
- Bruno, L., H. von Boehmer, and J. Kirberg. 1996. Cell division in the compartment of naive and memory T lymphocytes. *Eur. J. Immunol.* 26:3179.
- von Boehmer, H., and K. Hafen. 1993. The life span of naive α/β T cells in secondary lymphoid organs. *J. Exp. Med.* 177:891.
- McLean, A., and C. A. Michie. 1995. In vivo estimates of division and death rates of human T lymphocytes. *Proc. Natl. Acad. Sci. USA* 92:3707.
- Hou, S., L. Hyland, K. W. Ryan, A. Portner, and P. C. Doherty. 1994. Virus-specific CD8⁺ T cell memory determined by clonal burst size. *Nature* 369:652.
- Lau, L. L., B. D. Jamieson, R. Somasundaram, and R. Ahmed. 1994. Cytotoxic T cell memory without antigen. *Nature* 369:648.
- Mullbacher, A. 1994. The long-term maintenance of cytotoxic T cell memory does not require persistence of antigen. *J. Exp. Med.* 179:317.
- Swain, S. L. 1994. Generation and in vivo persistence of polarized Th1 and Th2 memory cells. *Immunity* 1:543.
- Swain, S. L., H. Hu, and G. Huston. 1999. Class II-independent generation of CD4 memory T cells from effectors. *Science* 286:1381.
- Murali-Krishna, K., L. L. Lau, S. Sambhara, F. Lemonnier, J. Altman, and R. Ahmed. 1999. Persistence of memory CD8 T cells in MHC class I-deficient mice. *Science* 286:1377.
- Tanchot, C., F. A. Lemonnier, B. Perarnau, A. A. Freitas, and B. Rocha. 1997. Differential requirements for survival and proliferation of CD8 naive or memory T cells. *Science* 276:2057.
- Tough, D. F., P. Borrow, and J. Sprent. 1996. Induction of bystander T cell proliferation by viruses and type I interferon in vivo. *Science* 272:1947.
- Zhang, X., S. Sun, I. Hwang, D. F. Tough, and J. Sprent. 1998. Potent and selective stimulation of memory-phenotype CD8⁺ T cells in vivo by IL-15. *Immunity* 8:591.
- Eberl, G., P. Brawand, and H. R. MacDonald. 2000. Selective bystander proliferation of memory CD4⁺ and CD8⁺ T cells upon NK T or T cell activation. *J. Immunol.* 165:4305.
- Tough, D. F., S. Sun, and J. Sprent. 1997. T cell stimulation in vivo by lipopolysaccharide (LPS). *J. Exp. Med.* 185:2089.
- Sprent, J., D. F. Tough, and S. Sun. 1997. Factors controlling the turnover of T memory cells. *Immunol. Rev.* 156:79.
- Tough, D. F., S. Sun, X. Zhang, and J. Sprent. 1999. Stimulation of naive and memory T cells by cytokines. *Immunol. Rev.* 170:39.
- Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science* 264:1918.
- Dumont, F. J., and L. Z. Coker. 1986. Interferon- α/β enhances the expression of Ly-6 antigens on T cells in vivo and in vitro. *Eur. J. Immunol.* 16:735.
- Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251.
- Okamura, H., H. Tsutsui, T. Komatsu, M. Yutsudo, A. Hakura, T. Tanimoto, K. Torigoe, T. Okura, Y. Nukada, K. Hattori, et al. 1995. Cloning of a new cytokine that induces IFN- γ production by T cells. *Nature* 378:88.
- Ku, C. C., M. Murakami, A. Sakamoto, J. Kappler, and P. Marrack. 2000. Control of homeostasis of CD8⁺ memory T cells by opposing cytokines. *Science* 288:675.
- Kennedy, M. K., M. Glaccum, S. N. Brown, E. A. Butz, J. L. Viney, M. Embers, N. Matsuki, K. Charrier, L. Sedger, C. R. Willis, et al. 2000. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.* 191:771.
- Lodolce, J. P., D. L. Boone, S. Chai, R. E. Swain, T. Dassopoulos, S. Trettin, and A. Ma. 1998. IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity* 9:669.
- Nishimura, H., T. Yajima, Y. Naiki, H. Tsunobuchi, M. Umemura, K. Itano, T. Matsuguchi, M. Suzuki, P. Ohashi, and Y. Yoshikai. 2000. Differential roles of interleukin 15 mRNA isoforms generated by alternative splicing in immune responses in vivo. *J. Exp. Med.* 191:157.