Induction of Bystander T Cell Proliferation by Viruses and Type I Interferon in Vivo

David F. Tough, Persephone Borrow, Jonathan Sprent*

T cell proliferation in vivo is presumed to reflect a T cell receptor (TCR)–mediated polyclonal response directed to various environmental antigens. However, the massive proliferation of T cells seen in viral infections is suggestive of a bystander reaction driven by cytokines instead of the TCR. In mice, T cell proliferation in viral infections preferentially affected the CD44^{hi} subset of CD8$^+$ cells and was mimicked by injection of polyinosinic-polycytidylic acid [poly(I:C)], an inducer of type I interferon (IFN I), and also by purified IFN I; such proliferation was not associated with up-regulation of CD69 or CD25 expression, which implies that TCR signaling was not involved. IFN I [poly(I:C)]–stimulated CD8$^+$ cells survived for prolonged periods in vivo and displayed the same phenotype as did long-lived antigen-specific CD8$^+$ cells. IFN I also potentiated the clonal expansion and survival of CD8$^+$ cells responding to specific antigen. Production of IFN I may thus play an important role in the generation and maintenance of specific memory.

Viral infections generally induce vigorous immune responses that culminate in a long-lasting state of immune memory. The sharp transient increase in total T cell numbers found in certain viral infections and the activation of specific T cells by heterologous viruses suggest that a substantial component of the T cell proliferative response is not antigen-specific (*1*). T cell proliferation in viral infections has not been quantitated, although the number of cycling CD8$^+$ cells in mice with influenza infection greatly exceeds the number of virus-specific cells (*2*). To quantitate T cell proliferation, the DNA precursor bromodeoxyuridine (BrdU) was given to mice to label T cells during infection with lymphocytic choriomeningitis virus (LCMV).

Administration of BrdU in the drinking water to uninfected (control) adult thymectomized (ATx) C57BL/6 (B6) mice led to slow and progressive BrdU labeling of CD4$^+$ and CD8$^+$ cells in the spleen (*3*) and in pooled lymph nodes (LNs) (Fig. 1, A and B) (*4*). Labeling occurred more rapidly for "memory-phenotype" CD44^{hi} cells (20 to 25% of T cells) than for "naïve-phenotype" CD44^{lo} cells (Fig. 1, C through F). The rate and extent of BrdU labeling of T cells increased after infection with LCMV and was most conspicuous for the CD44^{hi} subset of CD8$^+$ cells, both in the spleen (Fig. 1G) and in LNs (Fig. 1C). BrdU labeling of CD44^{hi} CD8$^+$ cells was >90% by 1 week after infection (as compared with 25% in uninfected mice) and was associated with up-regulation of Ly-6C (Fig. 1H). Mice infected with two unrelated viruses, vaccinia virus (VV) or vesicular stomatitis virus (VSV), had similar responses (Fig. 1, G and H). The selective labeling of CD44^{hi} CD8$^+$ cells during viral infection was associated with a substantial (50 to 100%) increase in total numbers of CD8$^+$ cells in LNs and the spleen and a marked increase in the proportion of CD44^{hi} CD8$^+$ cells (60% at day 7 after LCMV infection, as compared with 20% in controls).

Because the frequency of antigen-specific T cells during viral infection is low (≤1% even at the peak of the immune response) (*2*, *5*), it seemed likely that the intense proliferation of T cells elicited by the above viruses was non–antigen-specific and reflected bystander stimulation by cytokines (*1*, *2*). In considering which cytokines might be involved, type I interferon (IFN I, which includes IFN-α and IFN-β) was a likely candidate because (i) IFN I causes rapid up-regulation of Ly-6C on CD8$^+$ cells (*6*), and (ii) LCMV and other viruses induce strong production of IFN I (*7*). To assess the role of IFN I, we tested whether T cell proliferation could be elicited by injection of poly(I:C) (synthetic double-stranded RNA), a powerful inducer of IFN I (*8*).

Poly(I:C) mimicked the effects of viruses by causing marked proliferation of CD44^{hi} cells, especially CD44^{hi} CD8$^+$ cells (Fig. 2A), and up-regulation of Ly-6C (Fig. 2C); proliferation of CD4$^+$ cells was minimal (*9*). The proliferative response of CD44^{hi} CD8$^+$ cells was transient and declined abruptly after day 3 (Fig. 2A). LNs were enlarged at day 1 but returned to near normal size by day 2 to 3; at this stage, there was a small (20 to 30%) increase in the total number of CD8$^+$ cells and in the proportion of CD44^{hi} CD8$^+$ cells.

Poly(I:C) also caused T cell stimulation in mice transgenic for the 2C T cell receptor (TCR) (*10*, *11*); the clonotype-positive T cells in this strain were detected by monoclonal antibody (mAb) 1B2. In 2C mice, poly(I:C) caused selective stimulation of clonotype-negative (1B2$^-$) CD44^{hi} CD8$^+$ cells (Fig. 2B, left). Most (>95%) of the clonotype-positive (1B2$^+$) 2C cells had a naïve CD44^{lo} phenotype and proliferation of these cells was minimal. However, poly(I:C) caused substantial proliferation of the minor (<5%) subset of CD44^{hi} 1B2$^+$ cells (Fig. 2B, middle), which implies that proliferation was independent of antigen specificity, did not require TCR triggering, and depended only on the expression of a memory (CD44^{hi}) phenotype (*12*). In support of

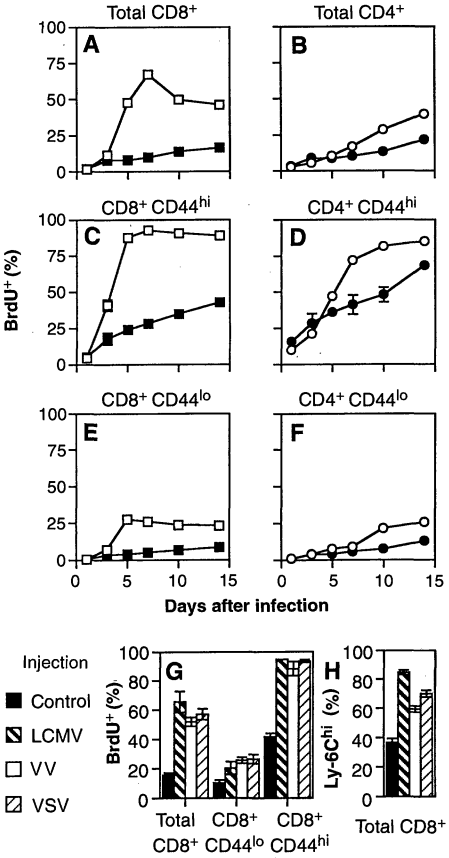

Fig. 1. Proliferation of T cells after viral infection (*34*). (**A** through **F**) Kinetics of BrdU labeling of CD8$^+$ [(A), (C), and (E)] and CD4$^+$ [(B), (D), and (F)] LN T cells in ATx B6 mice after LCMV infection (open symbols) or in uninfected controls (solid symbols). Proliferation kinetics are shown for total CD8$^+$ (A), total CD4$^+$ (B), CD8$^+$ CD44^{hi} (C), CD4$^+$ CD44^{hi} (D), CD8$^+$ CD44^{lo} (E), and CD4$^+$ CD44^{lo} (F) cells. (**G**) BrdU-labeling and (**H**) Ly-6C expression of CD8$^+$ spleen T cells from B6 mice measured 7 days after injection with LCMV, VV, or VSV compared with uninfected controls. All mice were given BrdU continuously in their drinking water from the time of viral infection. The data represent mean values from two or three mice per group (±SD).

D. F. Tough and J. Sprent, Department of Immunology, IMM4, Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, USA.
P. Borrow, Department of Neuropharmacology, IMM6, Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037, USA.
*To whom correspondence should be addressed.

this possibility, proliferation of CD44^{hi} CD8$^+$ cells induced by poly(I:C) was not associated with up-regulation of markers found on TCR-activated T cells, that is, CD69 or CD25 IL-2 receptor α-chain cells (Fig. 2C); and occurred in the absence of major histocompatability complex (MHC) class I expression, that is, when class I$^-$ [β_2-microglobulin$^-$ (β_2M$^-$)] CD8$^+$ cells (generated in chimeras) were exposed to poly(I:C) after transfer to class I$^-$ hosts (Fig. 2B, right). Collectively, these findings indicate that poly(I:C)-induced proliferation correlates closely with high CD44 expression and appears to be independent of TCR specificity (*13*).

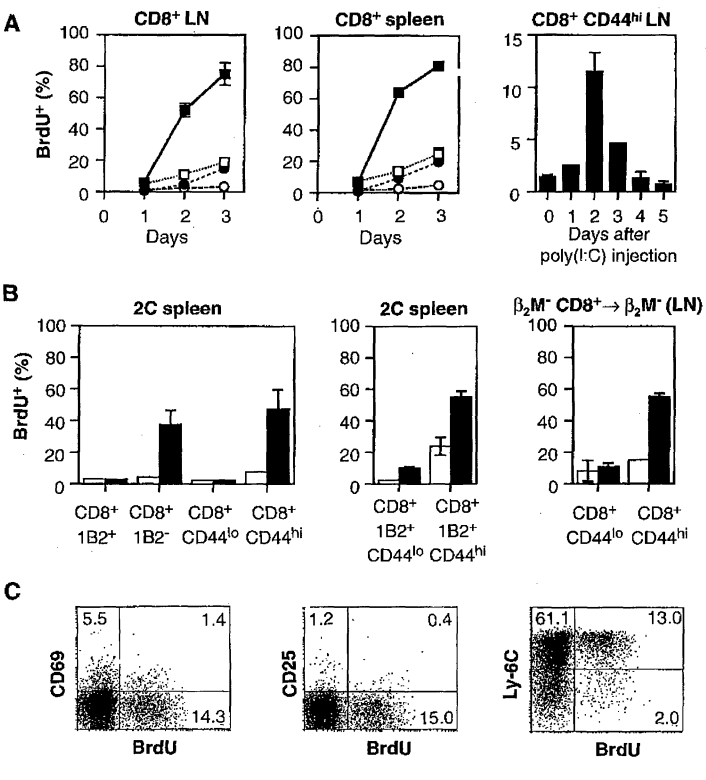

Fig. 2. Proliferation of T cells after poly(I:C) injection (*35*). (**A**) BrdU labeling of CD8$^+$ T cells in control (open symbols) and poly(I:C)-injected (solid symbols) ATx B6 mice. Left and middle panels show BrdU labeling of CD8$^+$ CD44^{hi} (squares) versus CD8$^+$ CD44^{lo} (circles) T cells in LNs and spleens of ATx B6 mice given BrdU drinking water continuously for 1, 2, or 3 days after poly(I:C) injection. The right panel shows the percentage of BrdU labeling of CD8$^+$ CD44^{hi} LN T cells in mice pulsed with BrdU for 4 hours at different times after poly(I:C) injection. (**B**) Effect of poly(I:C) injection on the turnover of CD8$^+$ cells in 2C TCR transgenic mice (left and middle panels) and in an MHC class I$^-$ environment (right panel). Left panel shows BrdU labeling of CD8$^+$ 1B2$^+$, CD8$^+$ 1B2$^-$, CD8$^+$ CD44^{lo}, and CD8$^+$ CD44^{hi} splenic T cells in 2C TCR transgenic bone marrow chimeras that were either uninjected (open bars) or injected with poly(I:C) 3 days previously (solid bars). The middle panel shows BrdU labeling of CD44^{lo} and CD44^{hi} CD8$^+$ 1B2$^+$ spleen cells in ATx 2C mice injected with poly(I:C) (solid bars) or PBS (open bars) 3 days previously. The right panel shows BrdU labeling of CD8$^+$ LN cells transferred from (β_2M$^-$ → B6) bone marrow chimeras into β_2M$^-$ mice 3 days after injection of recipient mice with PBS (open bars) versus poly(I:C) (solid bars); the data show staining of cells recovered from LNs. (**C**) Phenotype of CD8$^+$ T cells proliferating after poly(I:C) injection. Dot plots show CD69 (left), CD25 (middle), and Ly-6C (right) staining versus BrdU incorporation for CD8$^+$ LN T cells from normal B6 mice injected with poly(I:C) 3 days previously. Except for the 4-hour labeling in (A) (right panel), the mice in each experiment were placed on BrdU water from the time of poly(I:C) injection. The data represent mean values from two or three mice per group (±SD).

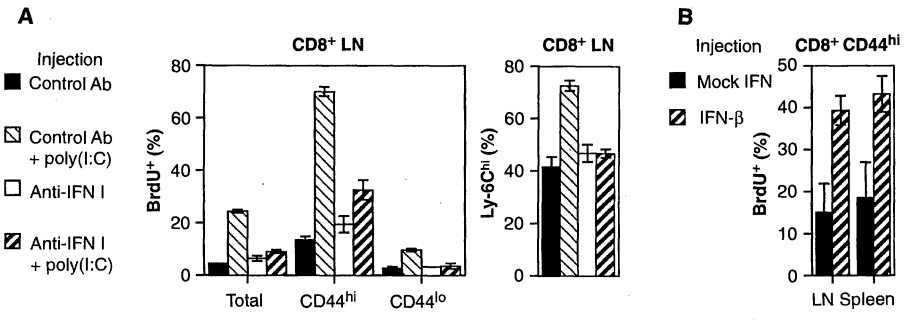

Fig. 3. Role of IFN I in CD8$^+$ T cell proliferation (*36*). (**A**) Inhibition of poly(I:C)-induced proliferation by antibody to IFN α/β. Data show BrdU labeling (left) and Ly-6C expression (right) of CD8$^+$ LN cells in ATx B6 mice injected with control sheep serum plus either PBS or poly(I:C), or with sheep antibody to IFN α/β plus either PBS or poly(I:C). (**B**) Effect of IFN-β injection on CD8$^+$ T cell proliferation. Data show BrdU labeling of CD8$^+$ CD44^{hi} LN and spleen cells 3 days after injection of purified murine IFN-β or mock IFN α/β. The data represent the mean values from two to four mice per group (±SD).

Although the entire range of cytokines elicited by poly(I:C) injection is unknown, the proliferation of CD44^{hi} cells induced by poly(I:C) was substantially reduced by coinjection of antibody to IFN I (anti–IFN I) (Fig. 3A, left); this applied to both CD4$^+$ and CD8$^+$ CD44^{hi} cells (Fig. 3) and was seen in both LNs and spleens (*9*). Anti–IFN I also prevented poly(I:C)-induced up-regulation of Ly-6C (Fig. 3A, right). These findings suggest that the effects of poly(I:C) are mediated largely through production of IFN I. If so, injection of mice with purified IFN I would be expected to reproduce the effects of poly(I:C). This was indeed the case. Thus, like poly(I:C), injection of mice with a large dose of purified IFN I (IFN-β; 4.4×10^5 U per mouse) induced marked proliferation of CD44^{hi} CD8$^+$ cells (Fig. 3B).

The above findings regarding poly(I:C) and purified IFN I provide the first direct evidence that T cell proliferation in vivo can reflect a bystander (non–antigen specific) response driven by cytokines. Thus, most of the CD44^{hi} CD8$^+$ cells proliferating in viral infections could be bystander cells stimulated by locally produced cytokines, especially IFN I. Whether these cytokines act directly on CD8$^+$ cells or through the production of other mediators is unknown. In preliminary experiments, CD44^{hi} cells also proliferated after injection of recombinant IFN-γ (*9*); other cytokines have yet to be tested. Hence, cytokine-induced proliferation of T cells in viral infections may not be restricted to IFN I. The capacity of cytokines, notably IFN I, to cause bystander stimulation of T cells in vivo may explain the "homeostatic" expansion of CD8$^+$ cells seen in patients with acquired immunodeficiency syndrome (*14*) and in nude mice and rats given small numbers of T cells (*15*, *16*). Thus, rather than reflecting homeostasis, the expansion of CD8$^+$ cells could simply reflect a proliferative response driven by the cytokines released during opportunistic viral infections (*17*). The capacity of cytokines to cause selective stimulation of CD44^{hi} cells could also explain why T cell proliferation in nude mice injected with TCR transgenic CD8$^+$ cells is marked for the clonotype-negative cells (which are predominantly CD44^{hi}) but minimal for the clonotype-positive cells (mostly CD44^{lo}) (*18*). Because the proliferation of T cells in nude rodent hosts ceases when the pool size reaches a certain threshold (*15*, *19*), the homeostasis in this situation could simply reflect restoration of immunocompetence: Pathogens are eliminated and cytokine concentrations return to normal.

Many of the antigen-specific T cells proliferating in viral infections are rapidly eliminated (*1*), but some of the responding cells survive and differentiate into long-lived resting memory cells (*5*). The factors controlling the survival of long-lived memory cells are controversial. For $CD8^+$ cells, there is evidence that memory to LCMV (*20*) and the HY antigen (*21*) requires persistent contact with residual antigen. However, more recent studies suggest that $CD8^+$ cell memory to viruses (*5, 22*) and the HY antigen (*23*) is remarkably long-lived in the absence of specific antigen. It has been suggested that the maintenance of long-term memory to viruses could reflect repeated TCR stimulation by cross-reactive environmental antigens (*24*). Because memory cells remain $CD44^{hi}$, an alternative possibility is that memory is not maintained through TCR stimulation but through intermittent contact with cytokines, such as IFN I, released during infection with other viruses. This hypothesis has the underlying assumption that stimulating T cells with IFN I provides a protective signal that promotes long-term survival of the cells rather than rapid elimination.

To address this question, we used a BrdU pulse-chase approach. Mice were injected with poly(I:C), placed immediately on BrdU water for 3 days (pulse), and then switched to normal water (chase) (Fig. 4, A and B). Placing the mice on normal water for 1 to 2 months after the BrdU pulse caused little change either in the numbers of BrdU-labeled $CD8^+$ cells or in the surface phenotype of these cells; in particular, the BrdU-labeled $CD8^+$ cells remained $CD44^{hi}$ Ly-$6C^{hi}$. Like virus-specific memory cells tested late after priming (*25*), the "rested" BrdU-labeled $CD8^+$ cells were predominantly $CD45RB^{hi}$ $CD62L^{hi}$ (indicative of a partial reversion to a naïve phenotype) (*9*). Thus, exposure to IFN I caused $CD44^{hi}$ $CD8^+$ cells to proliferate for a short period ($\leq$3 days) (see also Fig. 2A, right) and then differentiate into long-lived resting memory cells; these cells displayed a phenotype identical to that of typical late-stage memory $CD8^+$ cells specific for viral antigens.

In addition to promoting the survival of preexisting memory cells, IFN I could play a role in initial memory cell generation, perhaps by delivering survival signals to unprimed precursors responding to specific antigen during viral infection. We addressed this question with 2C TCR transgenic mice. Small numbers of 2C cells were adoptively transferred to B6 mice together with specific antigen (B10.D2 spleen cells), and the mice were placed on BrdU water for 7 days (Fig. 4C). Injection of poly(I:C) 1 to 2 days after immunization enhanced the production of $BrdU^+$ $1B2^+$ $CD8^+$ cells, both during initial priming (day 7) and after a 4-week rest period on normal water (day 35); most of the $BrdU^+$ $1B2^+$ cells expressed intermediate to high levels of CD44 (*9*). These findings imply that exposure to IFN I during a primary immune response promotes both the generation and survival (memory) of antigen-specific $CD8^+$ cells.

The results presented here suggest that release of IFN I during viral infections could play two different roles in promoting long-term memory carried by $CD8^+$ cells. First, the survival of preexisting memory cells may not require continuous TCR contact with specific antigen or cross-reactive environmental antigens but simply intermittent contact with IFN I released during intercurrent viral infections; stimulation of memory cells by IFN I could require minimal TCR ligation or be totally TCR-independent. Second, during the primary response to viruses, local production of IFN I may act as an adjuvant and augment both the intensity of the response and the survival of early memory cells. An adjuvant effect of IFN I could explain why most live viruses elicit strong immune responses, whereas viral peptides are poorly immunogenic or tolerogenic unless supplemented with exogenous adjuvant (*26*).

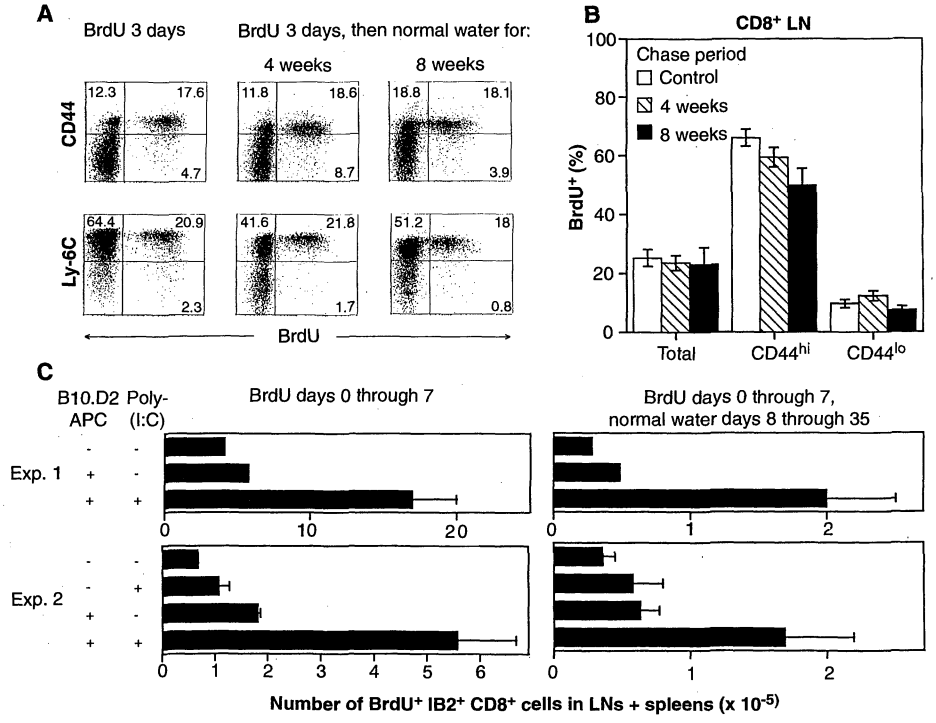

Fig. 4. Persistence of BrdU-labeled $CD8^+$ T cells after poly(I:C)-induced proliferation (*37*). (**A**) Dot plots showing CD44 (upper) and Ly-6C (lower) staining versus BrdU labeling of $CD8^+$ LN T cells in ATx B6 mice injected with poly(I:C), then given BrdU water for 3 days (left), followed by normal drinking water for 4 weeks (middle) or 8 weeks (right). (**B**) Percent of BrdU-labeled $CD8^+$ $CD44^{lo}$ and $CD44^{hi}$ cells in ATx B6 mice injected with poly(I:C), then given BrdU water for 3 days (open bars), followed by normal drinking water for 4 weeks (striped bars) or 8 weeks (solid bars). (**C**) Total number of $BrdU^+$ $1B2^+$ $CD8^+$ T cells found in pooled LNs plus spleens after adoptive transfer of 2C LN cells plus or minus B10.D2 T-depleted spleen cells as antigen-presenting cells (APCs) to B6 recipients with or without poly(I:C) injection. Mice were given BrdU water for the first 7 days after injection of cells (left), then transferred to normal drinking water for a further 4 weeks (right). Where indicated, recipients were also injected with poly(I:C) 1 or 2 days after injection of cells. Data points lacking error bars were derived from one mouse, whereas the remainder represent the mean values of two or three mice per group (±SD).

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11. 2C mice on a B6 ($H\text{-}2^b$) background were used. The 2C TCR has specificity for $H\text{-}2^d$ alloantigens and has

no detectable cross-reactivity for H-2^b (*10*).
12. The origin of the small number of CD44^{hi} 1B2$^+$ cells in 2C mice is unknown. These cells may be recent thymic emigrants because about 5% of thymic emigrants in normal mice are CD44^{hi} (*4*).
13. The possibility that poly(I:C)-induced proliferation of T cells adoptively transferred to β_2M^- mice reflected TCR contact with residual class I molecules is unlikely for two reasons. First, the injected β_2M^- CD8$^+$ cells were thoroughly depleted of non–T cells. Second, the low concentration of cell-surface class I molecules in β_2M^- mice causes only minimal positive selection of CD8$^+$ cells in the thymus and is essentially nonimmunogenic for normal CD8$^+$ cells.
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34. B6 mice were thymectomized at 5 to 6 weeks of age and left for 4 weeks before viral infection. Viruses used were the Armstrong 53b strain of LCMV (*27*); the Mudd-Summers strain of the Indiana serotype of VSV; and a thymidine kinase–negative VV recombinant, VVSC11, which expresses β-galactosidase (*28*). Viral titers were determined by plaque assay on Vero (LCMV and VSV) or 143TK$^-$ (VV) cells (*27*). All virus stocks were free of mycoplasma contamination. Mice were intravenously (iv) injected with LCMV [2×10^6 plaque-forming units (PFU)] or VSV (5×10^5 PFU), or were intraperitoneally (ip) injected with VV (2×10^6 PFU), and then immediately given BrdU (Sigma) drinking water (0.8 mg/ml) (*4*). BrdU water was made fresh and changed daily. LN cells were stained for flow cytometry with mAbs to CD8-PE (Gibco-BRL, Gaithersburg, MD), to CD4-PE (Collaborative Biomedical Products, Bedford, MA), to CD44-biotin (IM7.8.1), or to Ly-6C–biotin (Pharmingen, San Diego, CA); with streptavidin-RED613 (Gibco-BRL); and, after fixation, with mAb to BrdU-FITC (Becton Dickinson, Mountainview, CA) (*4*). Stained cells were analyzed on a FACScan flow cytometer (Becton Dickinson).
35. For poly(I:C) experiments, mice were injected ip with poly(I:C) (150 μg) (Sigma) in 150 μl of phosphate-buffered saline (PBS). For 4-hour BrdU labeling, mice were injected ip with 1 mg of BrdU in 200 μl of PBS and then given BrdU drinking water for 4 hours before being killed. Bone marrow chimeras were constructed by injection of T-depleted bone marrow into B6 mice irradiated with 10Gy (*29*). Donors were either β_2M^- (*30*) or B6 2C (*10*) mice. Six weeks after construction of ($\beta_2M^- \rightarrow$ B6) chimeras, purified CD8$^+$ LN cells were prepared by depletion of CD4$^+$, MHC class I$^+$ and class II$^+$ cells with antibody plus complement, followed by positive panning on plates coated with antibody to CD8 (*31*). Class I$^-$ CD8$^+$ cells (8×10^5) were injected iv into β_2M^- recipients. Six days later, these mice were injected ip with poly(I:C) or PBS. Three-color staining of cells for flow cytometric analysis was carried out as in Fig. 1, with the use of additional mAbs to CD25-biotin (7D4) and CD69-biotin (Pharmingen). For analysis of CD8$^+$ 1B2$^+$ CD44^{lo} and CD44^{hi} cells in ATx 2C mice, cells were stained with mAb to CD8-CyChrome (Pharmingen), 1B2-biotin (*32*), Texas Red–streptavidin (Gibco-BRL), and mAb to CD44-PE (Pharmingen) before being stained with mAb to BrdU-FITC. Four-color analysis was done with a FACStar Plus flow cytometer (Becton Dickinson).
36. To test the ability of anti–IFN I to inhibit poly(I:C)-induced proliferation, ATx B6 mice were injected iv three times with either adsorbed neutralizing antiserum to murine IFN α/β (neutralizing titer: 5.3×10^{-5} against 8 IU of murine IFN α/β) (*33*) or normal adsorbed sheep serum. Mice were injected with 0.2 ml of sera 2 hours before and 24 and 48 hours after being injected ip with either 50 μg of poly(I:C) in 200 μl of PBS or with 200 μl of PBS alone. BrdU water was given to the mice from the time of PBS or poly(I:C) injection. To test the effect of murine IFN I in vivo, ATx B6 mice received a single iv injection of either 4.4×10^5 U of purified murine IFN-β (Lee Biomolecular Laboratory, San Diego, CA) or an equivalent volume (0.45 ml) of mock IFN α/β (Lee Biomolecular Laboratory) and were immediately started on BrdU drinking water. Staining of LN and spleen cells for flow cytometry was carried out as described in Fig. 1.
37. For adoptive transfer experiments (shown in Fig. 4C), B6 2C LN cells (5.5×10^6) were injected iv plus or minus T-depleted (*31*) B10.D2 (H-2^d) spleen cells (2.6×10^7) into normal B6 mice. BrdU water was given for 7 days from the time of immunization; where indicated, mice were injected ip with a single dose of 100 μg of poly(I:C) 1 or 2 days later; the effects of poly(I:C) given 1 or 2 days after immunization were quite similar, and pooled data for these time points are shown. Mice were either killed 7 days after injection of cells or transferred to normal water for a further 4 weeks. The number of BrdU$^+$ 1B2$^+$ CD8$^+$ cells was calculated from total cell counts of pooled LNs and spleens. Experiments 1 and 2 represent independent experiments.
38. We thank M. B. A. Oldstone for advice on viral experiments, D. Y. Loh for 2C mice, I. Gresser for providing antiserum to IFN, T. Knapp for assistance with four-color analysis, and B. Marchand for typing the manuscript. Supported by grants CA38355, CA25803, AI21487, and AI32068 from the USPHS and by a Centennial Fellowship from the Medical Research Council of Canada (to D.F.T.). This is publication 9662-IMM from the Scripps Research Institute.

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Nonselective and $G_{\beta\gamma}$-Insensitive *weaver* K^+ Channels

Betsy Navarro, Matthew E. Kennedy, Bratislav Velimirović, Deepti Bhat, Andrew S. Peterson, David E. Clapham*

Homozygous *weaver* mice are profoundly ataxic because of the loss of granule cell neurons during cerebellar development. This granule cell loss appears to be caused by a genetic defect in the pore region (Gly$^{156} \rightarrow$ Ser) of the heterotrimeric guanine nucleotide–binding protein (G protein)–gated inwardly rectifying potassium (K^+) channel subunit (GIRK2). A related subunit, GIRK1, associates with GIRK2 to constitute a neuronal G protein–gated inward rectifier K^+ channel. The *weaver* allele of the GIRK2 subunit (*wv*GIRK2) caused loss of K^+ selectivity when expressed either as *wv*GIRK2 homomultimers or as GIRK1-*wv*GIRK2 heteromultimers. The mutation also led to loss of sensitivity to G protein βγ dimers. Expression of *wv*GIRK2 subunits led to increased cell death, presumably as a result of basal nonselective channel opening.

A point mutation in the pore region of the GIRK2 K^+ channel subunit appears to cause cerebellar granule cell loss in *weaver* mice (*1*). G protein–gated inward rectifier K^+ channels are heteromultimers of GIRK1 and CIR [cardiac inward rectifier or GIRK4 (*2*, *3*)] or GIRK1 and GIRK2 subunits (*4*, *5*) that are directly bound and gated by $G_{\beta\gamma}$ subunits (*6*). When expressed alone, GIRK2 or CIR subunits display unusual kinetics [fast with variable amplitude (*3*, *5*)] that presumably represent homomultimeric channels. GIRK1 does not appear to form detectable homomultimeric K^+ channels in transfected Chinese hamster ovary (CHO) cells or human embryonic kidney (HEK; A293) cells (*2*) and forms a K^+ channel in *Xenopus* oocytes only by interacting with an intrinsic *Xenopus* CIR homolog, XIR (*7*). Coexpression of GIRK1 and GIRK2 subunits results in channels with conductance, kinetics, and regulation similar to those of GIRK1-CIR channels, which form the acetylcholine (ACh)–gated inward rectifier K^+ channel (I_{KACh}) in native heart tissue (*2*, *3*).

GIRK1 and GIRK2 mRNA are colocalized in the early postnatal mouse cerebellum, when *weaver* mice granule cells are lost (*8*). In situ hybridizations identified GIRK1 ex-

B. Navarro, M. E. Kennedy, B. Velimirović, D. E. Clapham, Department of Pharmacology, Mayo Foundation, Rochester, MN 55905, USA.
D. Bhat and A. S. Peterson, Departments of Genetics and Neurobiology, Duke University Medical Center, Durham, NC 27710, USA.
*To whom correspondence should be addressed.