whereas no further decrease was observed in WT mice over this same time period (Figs. 2D and 4B). Conversely, the number of IFN-γ^+ (virus-specific) CD4$^+$ T cells remained elevated 100 days after LCMV-Arm (fig. S7) and –Cl 13 infection in $Il21r^{-/-}$ mice (Fig. 4C). Thus, despite the increased presence of virus-specific CD4$^+$ T cells in $Il21r^{-/-}$ mice, CD8$^+$ T cell responses are not sustained and the infection is not resolved.

IL-21 signaling also affects antibody production by B cells and antigen-presenting cell responses (19), which in addition to CD8$^+$ T cell deletion could contribute to the failure to resolve infection. Compared with that in WT mice, antigen-presenting cell maturation was not differentially affected in $Il21r^{-/-}$ mice after LCMV-Arm or –Cl 13 infection and by some measurements was increased in the absence of IL-21 signaling (fig. S8). Furthermore, the total number of B cells was similar in WT and $Il21r^{-/-}$ mice during chronic infection (fig. S9). We observed a 1.9-fold decrease in LCMV-specific immunoglobulin G antibody titers in $Il21r^{-/-}$ mice, although substantial antibody titers were observed in both WT and $Il21r^{-/-}$ mice (fig. S9). LCMV-neutralizing antibody titers were undetectable in both WT and $Il21r^{-/-}$ mice at day 30 after LCMV-Cl 13 infection. Together, these data suggest that antigen-presenting cells impairment or impaired humoral immunity are unlikely to underlie the reduced control of chronic infection in $Il21r^{-/-}$ mice.

The requirement for CD4$^+$ T cell help to control chronic viral infection has long been established (5, 6); however, because CD4$^+$ T cells rapidly lose the ability to produce traditional helper cytokines such as IL-2, the specific mechanisms and factors that comprise CD4$^+$ T cell help have remained elusive. Our results suggest that CD4$^+$ T cells do not necessarily "exhaust" or "lose" function during chronic viral infection. Instead, we propose that CD4$^+$ T cell function is diverted toward the production of factors such as IL-21 that sustain effector activity to control infection. Multiple effector mechanisms probably contribute to the long-term development of CD8$^+$ T cell responses. In combination with (or in the absence of) other yet unidentified helper factors, IL-21 maintains the CD8$^+$ T cell effector activity required to control infection and thus provides a mechanism for CD4$^+$ T cell help in response to chronic viral infection. Failure of CD4$^+$ T cell help is associated with the inability to acutely clear HCV infection and with the progression to AIDS after HIV infection (8–12). Thus, loss of IL-21 as CD4$^+$ T cell responses decline may hinder control of these human chronic viral infections. Further identification of helper factors and how they regulate precise immune outcomes will provide valuable insight into the generation and maintenance of antiviral immunity to prevent and treat chronic viral infections.

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24. We thank M. Oldstone for his generous support (AI09484). Our work was supported by the UCLA Center for AIDS Research, the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research at UCLA, and NIH grants AI077012, AI082975 (to D.G.B.), and AI070845 (to K.S.).

A Vital Role for Interleukin-21 in the Control of a Chronic Viral Infection

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Understanding the factors that regulate the induction, quality, and longevity of antiviral T cell responses is essential for devising rational strategies to prevent or combat infections. In this study, we show that interleukin-21 (IL-21), likely produced by CD4$^+$ T cells, directly influences the generation of polyfunctional CD8$^+$ T cells and that the number of CD4$^+$ T cells that produce IL-21 differs markedly between acute and chronic infections. IL-21 regulates the development of CD8$^+$ T cell exhaustion and the ability to contain chronic lymphocytic choriomeningitis virus infection. Thus, IL-21 serves as a critical helper factor that shapes the functional quality of antiviral CD8$^+$ T cells and is required for viral control.

A hallmark of robust antiviral immunity is the induction of CD4$^+$ and CD8$^+$ T cell responses, which act cooperatively and in conjunction with other immune system components, to control infection. The consequences of an ineffective immune response can be catastrophic, favoring viral persistence or the erosion of long-lived immunological memory. During the initial phases of many chronic infections, including hepatitis C virus (HCV) and human immunodeficiency (HIV) infections, CD8$^+$ T cell responses are induced but can fail to attain or lose the ability to elaborate key effector functions (1–9).

Although the cellular and molecular cues that dictate the development of robust CD8$^+$ T cells are not fully elucidated, in the absence of CD4$^+$ T cell help, CD8$^+$ T cell responses are compromised (2, 5, 6, 10–18). CD4$^+$ T cells are the primary producers of interleukin-21 (IL-21), a member of the common-γ chain family of cytokines (19–21). Functions of IL-21 are wide-ranging and include promoting B cell and antibody responses and inducing the development of Th17 and follicular helper CD4$^+$ (Tfh) lineages (19–24). Given that CD4$^+$ T cells are necessary for optimal antiviral CD8$^+$ T cell responses, we investigated the role of IL-21 in CD8$^+$ T cell responses to viral infections.

A comparative analysis of lymphocytic choriomeningitis virus (LCMV)–specific CD4$^+$ T cells revealed marked differences in the induction of IL-21$^+$ CD4$^+$ T cells after acute LCMV-Armstrong (Arm) and chronic LCMV–clone 13 (Cl 13) infections of C57BL/6 (B6) mice (Fig. 1) (25). By 8 days, both infections elicited polyclonal virus-specific IL-21$^+$ CD4$^+$ T cells; however, this response was 7.8 times lower in the LCMV–Cl 13–infected cohort (Fig. 1B; $P < 0.001$). Because CD4$^+$ T cells are obligatory for the control of LCMV–Cl 13, these results suggest that the CD4$^+$ T cell response to this infection, albeit weak, is capable of providing some help (2, 5, 6, 10).

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The pronounced IL-21+ CD4+ T cell response after acute LCMV-Arm infection prompted us to analyze whether IL-21 influenced the generation of germinal center (GC) B cells, Tfh cells, and antibody responses (fig. S1). The percentage and number of GC B cells and Tfh (ICOS+, CXCR5+) T cells as well as LCMV-specific antibody titers were similar between 8 to 9 days after LCMV-Arm infection of *Il21*+/+ and −/− mice. Thus, acute LCMV infection can trigger the development of these responses even in the absence of IL-21. Moreover, all cohorts successfully controlled LCMV-Arm infection because serum viral titers were below the limits of detection [<50 plaque-forming units (PFU)/ml] by this time point. Although appreciable antibody levels were detectable between days 44 to 120 after infection, endpoint titers were three times lower in *Il21*−/− mice.

To further analyze the role of IL-21 in promoting antiviral immunity, we evaluated the responses of *Il21*+/+, +/−, and −/− mice to LCMV–Cl 13 infection. Mice infected with LCMV–Cl 13 typically exhibit high-grade viremia and progressive reductions in the functional capacity of antiviral CD8+ T cells, termed exhaustion (1, 2, 5, 6, 9, 26). Eight days after LCMV–Cl 13 infection, during the effector phase, the magnitudes of the antiviral CD4+ and CD8+ T cell responses and the frequency of interferon-γ (IFN-γ)–producing antiviral CD8+ T cells were similar among *Il21*+/+, +/−, and −/− cohorts (figs. S2 and S3, A and B); however, we observed differences in their functional quality (Fig. 2, A and B, and fig. S3B). Both the percentages and absolute numbers of polyfunctional, IL-2–producing CD8+ T cells were reduced in *Il21*−/− mice, with *Il21*+/− mice exhibiting an intermediate phenotype (Fig. 2, A and B, and fig. S3B). We observed similar trends for tumor necrosis factor–α (TNF-α) production (fig. S4A). Thus, IL-21 deficiency results in impaired polyfunctional effector CD8+ T cell responses during the initial phases of LCMV infection.

Given the altered cytokine responses by antiviral CD8+ T cells after LCMV–Cl 13 infec-

tion of *Il21*−/− mice, we next tracked viral loads over time to determine whether the absence of IL-21 compromised the containment of the infection (Fig. 2C and fig. S5). As expected, *Il21*+/+ hosts slowly controlled LCMV–Cl 13 infection (1, 2, 5, 9, 26). By contrast, viral titers in the serum, livers, and lungs of *Il21*−/− mice remained high, a phenotype similar to that observed in mice lacking CD4+ T cells (Fig. 2C and fig. S5). *Il21*+/− mice showed an intermediate pattern of clearance, with viral loads decreasing more slowly than in *Il21*+/+ mice and the infection persisting at high levels or breaking through in three of the five *Il21*+/− mice tested between days 136 and 148 after infection. These data suggest that IL-21 is critical for control of chronic viral infection.

Evaluation of antiviral CD8+ T cell responses at later stages after LCMV–Cl 13 infection revealed severe functional exhaustion in *Il21*−/− mice (Fig. 2D and figs. S3, C and D, and S4). By 136 days, *Il21*+/+ mice had brought the infection under control, and IFN-γ, IL-2 (Fig. 2D), and TNF-α (fig. S4B) production was detectable by antiviral, primarily glycoprotein (GP)–specific, CD8+ T cells. Moreover, these antiviral CD8+ T cells expressed intermediate levels of CD43 (CD43int) and low levels of programmed death–1 (PD-1low), a phenotype more similar to resting memory T cells (Fig. 2, E and F). In *Il21*+/− mice, the phenotypic and functional properties of the antiviral CD8+ T cells diverged depending on their virological status. CD8+ T cells from mice able to contain the infection resembled antiviral CD8+ T cells from *Il21*+/+; however, in mice with high viral titers, CD8+ T cell responses were more similar to those observed in *Il21*−/− hosts (fig. S4B). IL-2, IFN-γ, and TNF-α production by *Il21*−/− virus-specific CD8+ T cells were greatly reduced or absent (Fig. 2D and figs. S3D and S4B), but these T cells were CD43high, PD-1high (Fig. 2, E and F), a hallmark of the exhaustion that develops in chronically infected hosts (6, 9). Interestingly, the emergence of exhausted virus-specific CD8+ T cells in *Il21*−/− mice parallels what is observed after

LCMV–Cl 13 infection of CD4-deficient hosts (2, 5, 6, 10). Thus, the absence of IL-21 production results in a failure to contain the infection and a silencing of antiviral CD8+ T cell functions.

We next examined whether IL-21 was acting directly to promote and sustain CD8+ T cell responses. To directly compare, within the same host, the responses and fates of cells of the immune system that can and cannot perceive IL-21–derived signals, we generated bone-marrow chimeras with use of a mixture of allelically marked *Il21r*+/+ (CD45.1) and *Il21r*−/− (CD45.2) donor cells. As a control, we also generated mixed bone-marrow chimeric mice that were reconstituted with *Il21r*+/+ (CD45.1) bone marrow and *Il21r*+/+ (CD45.2) bone marrow prepared from the littermates of *Il21r*−/− mice. Before infection, the ratio of CD45.1:CD45.2 CD8+ T cells was 52:48 and 70:30 in the control and experimental cohorts, respectively. Nevertheless, both *Il21r*+/+ and *Il21r*−/− virus-specific CD8+ T cells were detectable in the circulation by 8 days after LCMV–Cl 13 infection (Fig. 3). Thus, the elaboration of primary virus-specific CD8+ T cell responses can occur independently of IL-21. Further tracking of virus-specific CD8+ T cells in the circulation at 16 days after infection revealed a preferential and rapid loss of *Il21r*−/− antiviral CD8+ T cells. This was confirmed by enumeration of splenic responses 3 weeks after infection, which revealed a 40 times fewer number of *Il21r*−/− virus-specific CD8+ T cells by comparison with *Il21r*+/+ counterparts in the same host (Fig. 3, C and D). These data illustrate the direct requirement of IL-21 for supporting and maintaining antiviral CD8+ T cells during chronic viral infections.

We next evaluated whether the addition of IL-21 enhanced antiviral CD8+ T cells because the absence of IL-21–dependent signaling impaired these responses. We investigated whether administration of IL-21 could improve responses and viral control in *Cd4*−/− mice because CD4+ T cells are a principal source of this cytokine. These "helpless" mice do not usually control

Fig. 1. Diminished IL-21+ CD4+ T cell responses during the initial phase of LCMV–Cl 13 infection. IL-21 and IFN-γ production by LCMV GP$_{61-80}$ CD4+ T cells was determined 8 days after LCMV-Arm or –Cl 13 infections of B6 mice. (**A**) Flow cytometric analysis of intracellular staining for IL-21 and IFN-γ in splenocytes from LCMV-infected *Il21*−/− and *Il21*+/+ mice after stimulation with GP$_{61-80}$ peptide. Gated total CD4+ T cells are shown. (**B**) Enumeration of IL-21–producing CD4+ T cells at 8 days after LCMV-Arm or –Cl 13 infection. Graphs represent mean ± SD; ***P < 0.001. Representative results are shown from two independent experiments (n = 8 to 9 for *Il21*+/+ cohorts and n = 2 for *Il21*−/− mice).
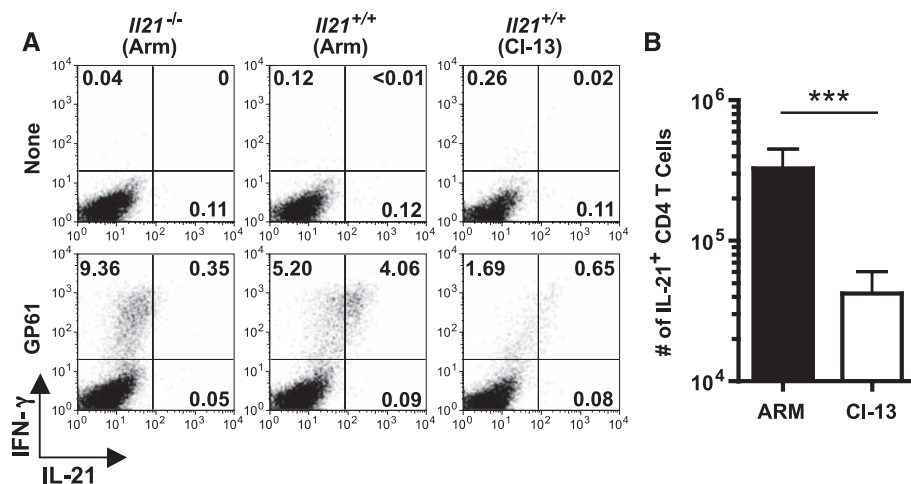
LCMV–Cl 13 infection and develop severe CD8+ T cell exhaustion (2, 5, 6, 10). Daily injections of recombinant IL-21 to LCMV–Cl 13–infected $Cd4^{-/-}$ mice enhanced the functional quality of virus-specific CD8+ T cells, particularly the nucleoprotein (NP)$_{396}$ epitope-specific population, which usually rapidly succumbs to exhaustion (Fig. 4). Importantly, IL-21 treatment resulted in lower viral titers (Fig. 4C). The delicate balance between the quality and size of the antiviral immune response and the hosts' viral burden be-

came apparent as 70% of the treated mice became moribund, reaching experimental endpoints requiring euthanasia. Illness and death after LCMV infection is classically associated with immunopathology (27). Thus, although IL-21 administration can improve antiviral CD8+ T cell responses and viral clearance and has been safely used in the context of tumor immunotherapy in mice and humans, care will need to be taken before applying this treatment strategy to chronic viral infections (21, 28, 29).

Collectively, our findings provide insights into the determinants of the functional quality of CD8+ T cell responses and the role of IL-21 in ensuring the successful control of infection. The results implicate IL-21 as a critical helper factor that couples the requirement for CD4+ T cells, which produce this cytokine, to the elaboration and maintenance of polyfunctional CD8+ T cells capable of clearing virus-infected cells. We observed that induction of IL-21–producing CD4+ T cells is markedly reduced during the ini-

Fig. 2. Severe CD8+ T cell exhaustion and viral persistence in the absence of IL-21. Splenic CD8+ T cell responses and viral titers were evaluated after LCMV–Cl 13 infection of $Il21^{+/+}$, $^{+/-}$, and $^{-/-}$ mice. (**A**) Flow cytometric analysis of intracellular cytokine staining for IFN-γ and IL-2 production by CD8+ T cells at 8 days after infection after restimulation without or with the indicated peptide epitopes. Gated CD8+ T cells are shown, and the percentages of CD8+, IFN-γ+ cells that co-produce IL-2 are reported in parentheses. (**B**) Percentages of epitope-specific CD8+, IFN-γ+ cells that co-produce IL-2 at 8 days after infection. Error bars are SEM; *$P <$ 0.05 by comparison with $Il21^{+/+}$ group. (**C**) Serum viral titers over time after LCMV–Cl 13 infection of $Il21^{+/+}$, $^{+/-}$, $^{-/-}$, and $Cd4^{-/-}$ mice. Results from individual mice are shown; the dotted line represents the limit of detection. (**D**) IFN-γ and IL-2 production by LCMV-specific CD8+ T cells at 136 days after infection. Gated CD8+ T cells are shown. (**E** and **F**) CD43 and PD-1 expression by GP33 tetramer+ CD8+ T cells from $Il21^{+/+}$ (shaded), $^{+/-}$ (dashed line), and $^{-/-}$ (bold line) mice at 8 (E) and 136 (F) days postinfection. The $Il21^{+/-}$ data shown in (D) and (F) are from mice that were aviremic at the time of analysis. Representative or composite data are shown from two independent experiments ($n = 3$ to 6).
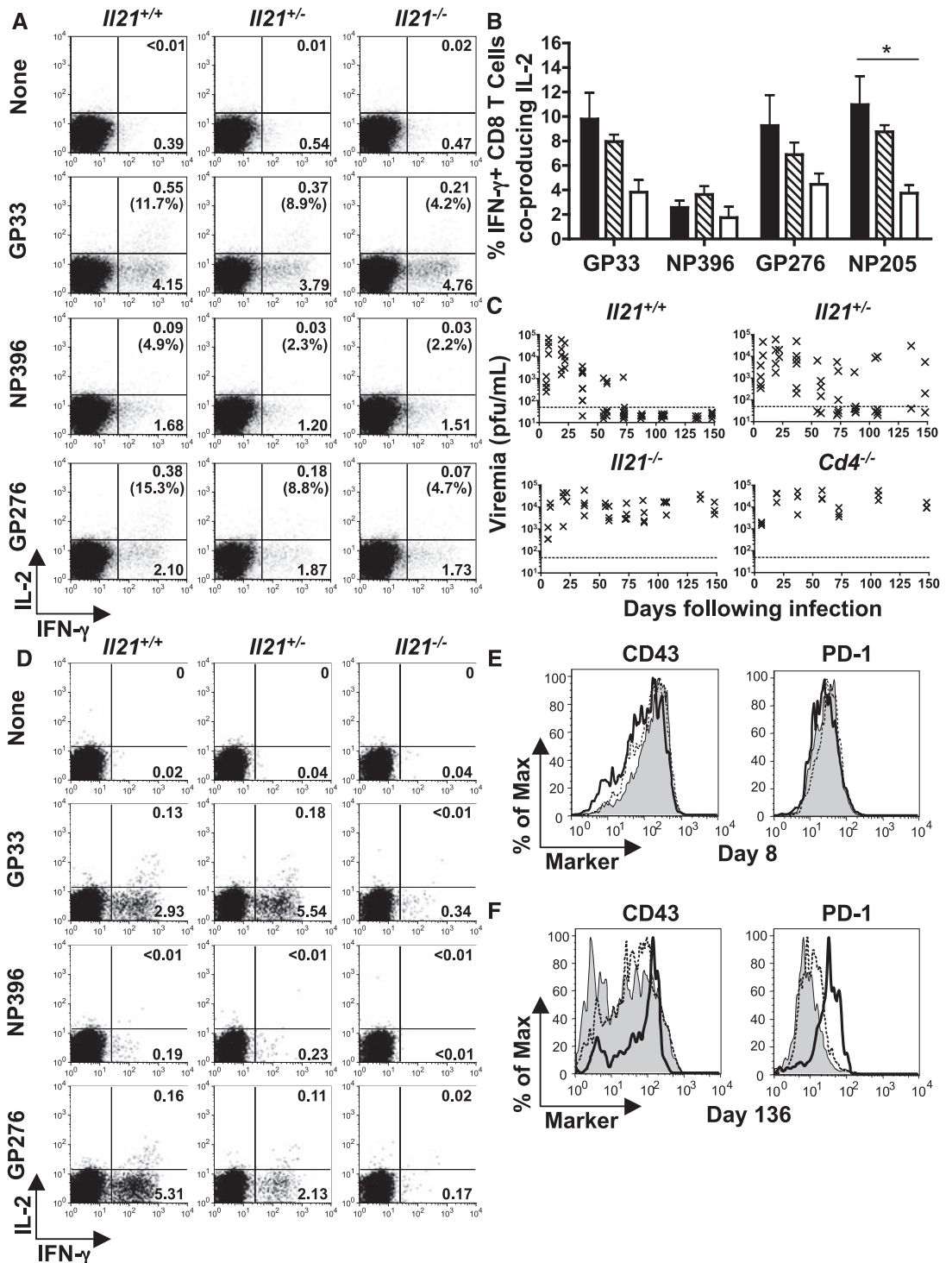
Fig. 3. IL-21 acts directly to sustain virus-specific CD8+ T cells during an ongoing infection. Cohorts of control $Il21r^{+/+}/Il21r^{+/+}$ (CD45.1/CD45.2) and experimental $IL21r^{+/+}/IL21r^{-/-}$ (CD45.1/CD45.2) mixed bone-marrow chimeras were infected with LCMV-Cl 13 and CD8+ T cell responses evaluated over time. (**A**) Peripheral blood mononuclear cells were evaluated by flow cytometry to check reconstitution of CD8+ T cells in $Il21r^{+/+}/Il21r^{+/+}$ or $Il21r^{+/+}/Il21r^{-/-}$ mixed bone-marrow chimeras before infection. Gated CD8+ T cells are shown. (**B**) Flow cytometric analysis of GP$_{33}$- and GP$_{276}$-specific CD8+ T cell responses in the circulation at days 8 and 16 after infection. Gated tetramer+ CD8+ T cells are shown. (**C**) Flow cytometric analysis of splenic CD8+ T cells and GP$_{33}$- and GP$_{276}$-specific responses at 3 weeks after infection. Gated CD8+ (left) or CD8+ tetramer+ (right) cells are shown. (**D**) Absolute numbers of GP$_{33}$- and GP$_{276}$-specific CD8+ T cells in mixed bone-marrow chimeras 3 weeks after infection. Graphs represent average + SD of $Il21r^{+/+}$ CD45.1 CD8+ T cells (black), $Il21r^{+/+}$ CD45.2 CD8+ T cells (gray), and $Il21r^{-/-}$ CD45.2 CD8 T cells (white). **$P < 0.01$, ***$P < 0.001$. Representative results are shown from one of two similar experiments ($n = 7$ and 8 for $Il21r^{+/+}/Il21r^{+/+}$ and $IL21r^{+/+}/Il21r^{-/-}$ cohorts, respectively).
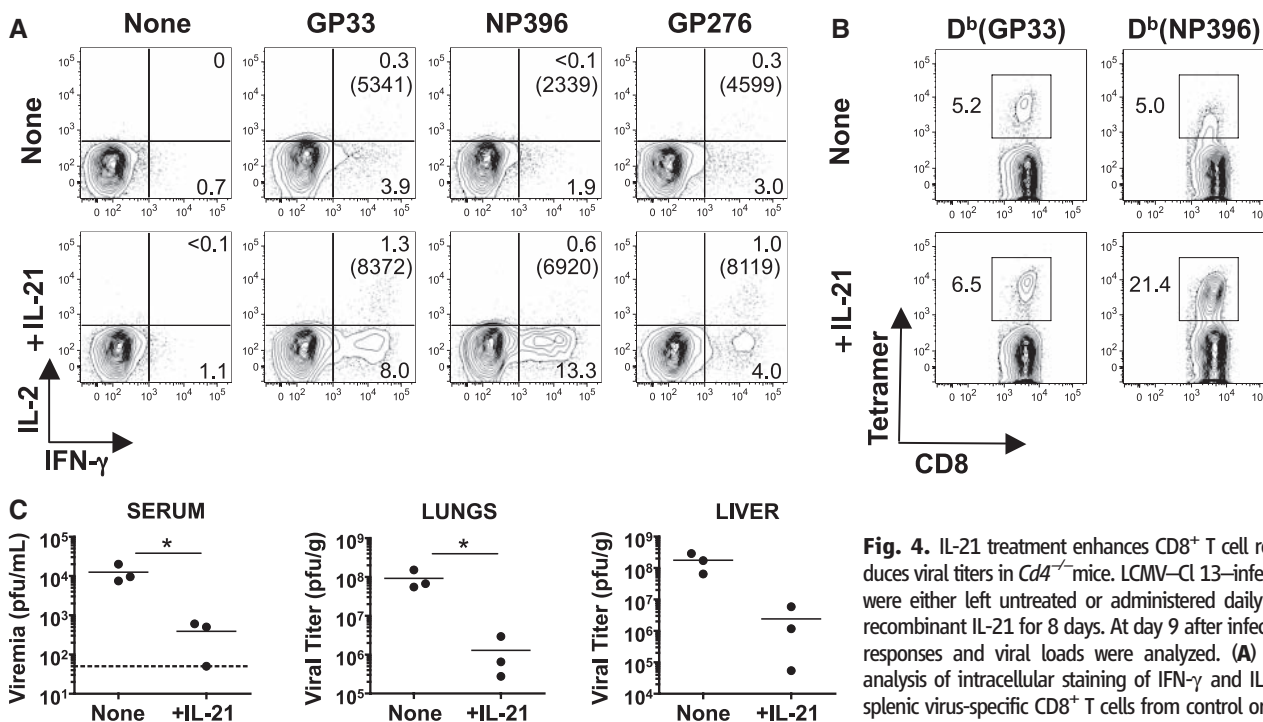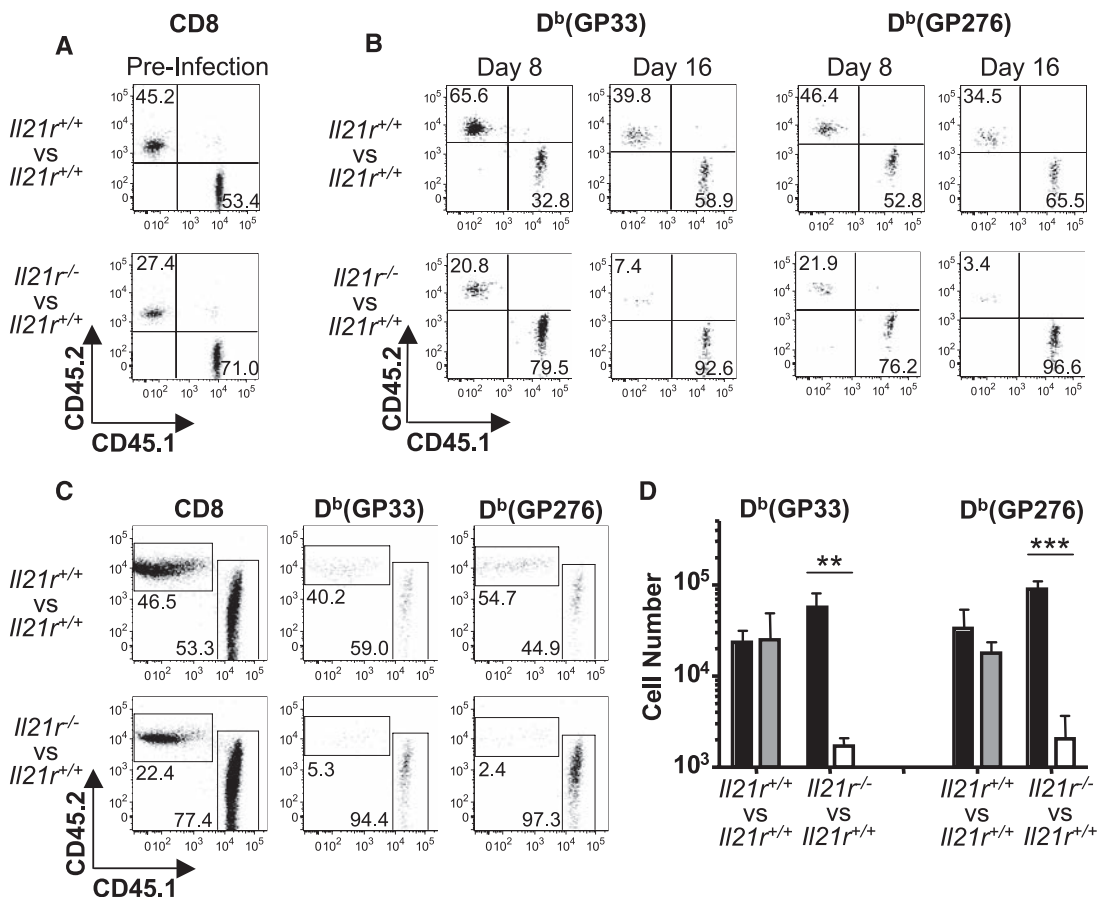

Fig. 4. IL-21 treatment enhances CD8+ T cell responses and reduces viral titers in $Cd4^{-/-}$ mice. LCMV–Cl 13–infected $Cd4^{-/-}$ mice were either left untreated or administered daily doses of 10-μg recombinant IL-21 for 8 days. At day 9 after infection, CD8+ T cell responses and viral loads were analyzed. (**A**) Flow cytometric analysis of intracellular staining of IFN-γ and IL-2 production in splenic virus-specific CD8+ T cells from control or treated cohorts. Gated CD8+ T cells are shown. The mean fluorescence intensity (MFI) of IFN-γ–producing CD8+ T cells is reported in parentheses. (**B**) Flow cytometric analysis of GP$_{33}$ and NP$_{396}$ tetramer+ CD8+ T cells. Plots show gated CD8+ T cells. (**C**) Viral titers were assessed in the serum, lungs, and liver of control and IL-21–treated mice. Dotted line indicates the limit of detection (50 PFU/ml) for serum samples. *$P < 0.05$. Representative results from one of two independent experiments are shown ($n = 7$ and 6 for control and treated groups, respectively).

tial phase of chronic viral infection, suggesting that this could be a general feature of infections that elicit nonprotective adaptive immune responses, such as hepatitis B virus, HCV, and HIV ([8], [30]). This poor induction of IL-21 may result from the failure to initiate robust CD4^{+} T cell responses as well as the exhaustion or the viral-induced depletion of these cells that may occur during certain chronic infections. Therefore, we anticipate that the cautious development of approaches to modulate the levels of IL-21, or regulate the induction of cellular subsets that generate IL-21, will provide new therapeutic opportunities to improve immunity to diseases that require CD8^{+} T cell responses to be controlled, such as chronic viral infection and tumors.

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IL-21R on T Cells Is Critical for Sustained Functionality and Control of Chronic Viral Infection

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Chronic viral infection is often associated with the dysfunction of virus-specific T cells. Our studies using *Il21r*-deficient (*Il21r*^{−/−}) mice now suggest that interleukin-21 (IL-21) is critical for the long-term maintenance and functionality of CD8^{+} T cells and the control of chronic lymphocytic choriomeningitis virus infection in mice. Cell-autonomous IL-21 receptor (IL-21R)–dependent signaling by CD8^{+} T cells was required for sustained cell proliferation and cytokine production during chronic infection. *Il21r*^{−/−} mice showed normal CD8^{+} T cell expansion, effector function, memory homeostasis, and recall responses during acute and after resolved infection with several other nonpersistent viruses. These data suggest that IL-21R signaling is required for the maintenance of polyfunctional T cells during chronic viral infections and have implications for understanding the immune response to other persisting antigens, such as tumors.

Chronic viral infections, including HIV and hepatitis B and C viruses (HBV and HCV), afflict >0.5 billion people worldwide. Although the reasons for ineffective antiviral immunity remain poorly defined, studies of chronic infection with lymphocytic choriomeningitis virus (LCMV) in mice and HIV, HCV, and HBV in humans show that the loss of T cell functionality, termed exhaustion, is a hallmark of chronic infection ([1]). Long-term maintenance of potent virus-specific CD8^{+} T cell responses requires help from CD4^{+} T cells and cytokines produced by T and non-T cells ([2], [3]). Specifically, members of a cytokine subfamily with receptors sharing the common gamma (γ$_c$) chain such as interleukin-2 (IL-2), IL-7, and IL-15 have distinct activities on the development and maintenance of antiviral effector and memory T cells ([4]–[8]). IL-21, an additional family member, has pleiotropic activities on CD4^{+} T cells ([9]), although its role in antiviral CD8^{+} T cell responses is unknown.

LCMV can cause acute or persistent infection, depending on the viral isolate and the dose of infection. High-dose infection with the fast-replicating strain LCMV-Docile (or strain Clone 13) results in virus persistence and exhausted virus-specific CD8^{+} T cells. In contrast, low-dose infection is efficiently cleared in immunocompetent mice.

To study the role of IL-21 in acute and chronic viral infection, we infected control and *Il21r*^{−/−} mice with low- [200 plaque-forming units (PFU)], intermediate- (2000 PFU), or high-dose (2 × 10^{6} PFU) LCMV-Docile. Comparable expansion, cytokine production, and killing of virus-infected targets by viral epitope gp$_{33-41}$–specific CD8^{+} T cells were observed 8 days after infection, indicating that the IL-21 receptor (IL-21R) was not required for priming and differentiation of virus-specific CD8^{+} T cells during the acute response (Fig. 1, A to C, and fig. S1, A to G). As expected, control mice infected with low or intermediate doses mounted efficient long-term antiviral CD8^{+} T cell responses and controlled the infection (Fig. 1, A to F), whereas infection with high-dose LCMV-Docile resulted in reduced frequencies of and interferon-γ (IFN-γ) production by virus-specific T cells (Fig. 1, A to C), in addition to viral persistence (Fig. 1F). We detected exhausted *Il21r*^{−/−} LCMV-specific CD8^{+} T cells in response to low and intermediate virus doses starting at day 15 after infection (Fig. 1, A to D). After 5 weeks, we observed reduced frequencies and total numbers of gp$_{33-41}$-specific *Il21r*^{−/−} CD8^{+} T cells in the blood and spleen. Moreover, the remaining gp$_{33-41}$-specific CD8^{+} T cells failed to produce IFN-γ, tumor necrosis factor–α, and IL-2 and did not proliferate upon stimulation, all of which are characteristics of exhausted T cells (Fig. 1C and fig. S1, H and I). Consequently, *Il21r*^{−/−} mice developed chronic viremia after exposure to low or intermediate doses of virus (Fig. 1E and fig. S1K). Even at high-dose exposure, when both control and *Il21r*^{−/−} mice developed a chronic infection, viral titers were increased in the latter (Fig. 1F). The fre-

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