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Cell-Intrinsic gp130 Signaling on CD4+ T Cells Shapes Long-Lasting Antiviral Immunity

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The IL-6 cytokine family utilizes the common signal transduction molecule gp130, which can mediate a diverse range of outcomes. To clarify the role of gp130 signaling in vivo during acute viral infection, we infected Cd4cre Il6stfl/fl mice, in which gp130 is conditionally ablated in T cells, with acute lymphocytic choriomeningitis virus. We found that by day 12, but not at day 8, after infection the number of virus-specific CD4+ T cells was reduced in the absence of gp130, and this was sustained for up to 2 mo postinfection. Additionally, gp130-deficient T follicular helper cells had lower expression of Maf, IL-21, and ICOS, and this was accompanied by a reduction in the proportion of germinal center B cells and plasmablasts. Remarkably, at 2 mo postinfection the proportion of IgG2a/c+ memory B cells and the systemic levels of lymphocytic choriomeningitis virus–specific IgG2 Abs were dramatically decreased, whereas there was a corresponding increase in IgG1+ memory B cells and virus-specific IgG1 Abs. In the same animals gp130-deficient virus-specific CD8+ T cells showed a reduced proportion of memory cells, which expressed lower levels of Tcf7, and displayed diminished recall responses on secondary infection. Mixed bone marrow chimeras revealed that the aforementioned gp130 effects on CD4+ T cells were cell intrinsic. Overall, our data show that gp130 signaling in T cells influences the quantity and quality of long-lasting CD4+ T cell responses as well as CD8+ T cell– and Ab-mediated immunity after acute viral infection.

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The IL-6 cytokine family utilizes the common signal transduction molecule gp130, which can mediate a diverse range of outcomes. To clarify the role of gp130 signaling in vivo during acute viral infection, we infected Cd4cre Il6stfl/fl mice, in which gp130 is conditionally ablated in T cells, with acute lymphocytic choriomeningitis virus. We found that by day 12, but not at day 8, after infection the number of virus-specific CD4+ T cells was reduced in the absence of gp130, and this was sustained for up to 2 mo postinfection. Additionally, gp130-deficient T follicular helper cells had lower expression of Maf, IL-21, and ICOS, and this was accompanied by a reduction in the proportion of germinal center B cells and plasmablasts. Remarkably, at 2 mo postinfection the proportion of IgG2a/c+ memory B cells and the systemic levels of lymphocytic choriomeningitis virus–specific IgG2 Abs were dramatically decreased, whereas there was a corresponding increase in IgG1+ memory B cells and virus-specific IgG1 Abs. In the same animals gp130-deficient virus-specific CD8+ T cells showed a reduced proportion of memory cells, which expressed lower levels of Tcf7, and displayed diminished recall responses on secondary infection. Mixed bone marrow chimeras revealed that the aforementioned gp130 effects on CD4+ T cells were cell intrinsic. Overall, our data show that gp130 signaling in T cells influences the quantity and quality of long-lasting CD4+ T cell responses as well as CD8+ T cell– and Ab-mediated immunity after acute viral infection.

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To clarify the role of signaling through gp130 on T cells, and to eliminate any redundancy within the IL-6 family of cytokines, we infected mice with conditional ablation of gp130 in T cells with the prototypic acute arenavirus LCMV ARM. It has previously been shown that T cell–specific deletion of gp130 during infection with gastrointestinal nematode Trichuris muris strongly polarizes the immune responses away from pathogenic Th1/Th17 responses toward protective Th2 responses (27). In the strongly Th1 environment of LCMV ARM infection, we found little evidence of increased Th2 cell differentiation in the absence of gp130. We did, however, find that the number of virus-specific CD4+ T cells was compromised at day 12 and long after infection. Additionally, gp130-deficient T cells had lower expression of Maf, IL-21, and ICOS. These CD4+ T cell defects were cell intrinsic and accompanied by reductions in the proportion of GC B cells and plasmablasts. A significant decrease in IgG2a/c memory B cells and virus-specific IgG2 levels was also observed in concert with increased IgG1 2 mo postinfection (p.i.). At the same time after infection, gp130-deficient virus-specific CD8+ T cells exhibited increased IgG1 2 mo postinfection (p.i.). At the same time after secondary infection. Overall, our data show that gp130 signaling in T cells is vital for optimal virus-specific CD8+ and CD4+ T cell responses long after acute infection and that disrupting this pathway has significant effects on lasting humoral immunity and recall responses.

Materials and Methods

**Mice and viral stocks**

Cd4-cre Il6srΔ9Δ10 mice (on a C57BL/6 background) were provided by Dr. Werner Mueller (University of Manchester, Manchester, U.K.). Cd45.1+ (B6.SJL-Plpcre<sup>Prpc</sup>/BoyJ) and inbred C57BL/6 mice were obtained from The Jackson Laboratory. All mice were bred and maintained in a closed breeding facility, and mouse handling conformed to the requirements of the National Institutes of Health and the Institutional Animal Care and Use Guidelines of the University of California San Diego. Unless otherwise stated, 6- to 8-wk old mice were infected i.v. with 2 × 10<sup>6</sup> PFU of LCMV ARM or with 2 × 10<sup>5</sup> LCMV C113. Viruses were grown, identified, and quantitated as described previously (28, 29). Viral quantification was carried out by six-well plate plaque assays on vero cells (American Type Culture Collection). For mixed bone marrow chimeras, recipient Cd45.1+ mice were exposed to 1000 rad. The following day they were injected with 5 × 10<sup>6</sup> bone marrow cells i.v. (an equal mix of CD45.1+ or Cd4-cd Il6sr<sup>Δ9Δ10</sup> donor cells). Mice were maintained on oral antibiotics for 2 wk, and reconstitution was continued for an additional 6 wk prior to infection (18).

**LCMV-specific Ab ELISAs**

LCMV-specific ELISAs were done as we and others have previously described using Ag prepared by purifying LCMV on a Renografin gradient (18, 30).

**Flow cytometry**

Flow cytometry was done as previously described (31). The following fluorochrome-labeled Abs purchased from BioLegend, eBioscience, or BD Biosciences were used to stain blood or spleen cells: anti–CD8-Pacific, anti–CD127-PerCP-Cy5.5, -CD45.1-PE, –IgM-allophycocyanin-Cy7, –CD19-PE, –B220-PE-CF594, –CD38-Alexa Fluor 700, –CD38-Cy7-FITC, –GL7-Fluor 660, –CD138-PE, –IgG-allophycocyanin-Cy7, –IgDb-PE, –CD1-PeCy7, –CD1-BV605, –IgCs-PE, –IgCs-Cy5.5-PE, –CD11a-FITC, –CD49d-PerCP-Cy5.5, –KLRLG1-FITC, –CD127-PerCP-Cy5.5, –CD8-eFluor 450, –CD45.1-PE-CF594, –CD45.2-BV605, –IgG1-FITC, and –IgG2a-biotin followed by anti–streptavidin-BV650, –IFN-γ-allophycocyanin, –TNF-α-FITC, –IL-2-PE, and –CXC5R5-BV421. Two-step CXC5R5 (BD Biosciences), Foxp3 (eBioscience), Tbet (Santa Cruz Biotechnology, Dallas, TX), and Bcl6 (BD Biosciences, K112-91) staining were done as previously described (18). Biotinylated D<sup>5</sup>GP<sub>53-41</sub> and D<sup>5</sup>NF<sub>396-406</sub> monomers along with allophycocyanin–L<sup>α</sup>A (IgG1) and streptavidin–allophycocyanin (Molecular Probes/Life Technologies). Class I tetramers were stained at 1:100 for 1 h 15 min on ice, and class II tetramers were stained 1:100 for 3 h at 37°C, followed by normal Ab staining. Cells were acquired using the digital LSR II flow cytometer (Becton Dickinson, San Jose, CA). Staining for fluorescence-assisted cell sorting was conducted in an identical fashion and cells were isolated on a FACSaria (Becton Dickinson, San Jose, CA) to >95% purity. Flow cytometric data were analyzed with FlowJo software (FlowJo LLC, Ashland, OR).

**Ex vivo T cell stimulation**

For MHC class I-restricted GP<sub>33-41</sub> peptide (2 µg/ml) or MHC class II-restricted Pgo<sub>7-27</sub> peptide (5 µg/ml), stimulation and staining were carried out as we have previously described (31). For polyclonal stimulation we used PMA (10 ng/ml) and ionomycin (0.5 µg/ml) in place of peptide. For intracellular IL-21 staining, cells were permeabilized with saponin and incubated with a 1:25 dilution of mouse IL-21R-human Fc (R&D Systems) for 30 min at 4°C, washed twice, and stained with a 1:200 anti-human FC-PE (BD Pharmingen).

**Real-time RT-PCR**

Total RNA was extracted from splenocytes using RNeasy kits (Qiagen) and reverse transcribed into cDNA using SuperScript III reverse transcriptase (Invitrogen). CDNA quantification was performed using SYBR Green PCR kits (Applied Biosystems) and a real-time PCR detection system (Applied Biosystems). Primers for the genes assessed are described in Harker et al. (18), as well as Tcf7 forward, 5'-CACTGCTGCTAGCCTACCC-3'; Tcf7 reverse, 5'-CTGCTCTGCTGATGATTGC-3'; Maf forward, 5'-AAA-TACAGAAGCTGTTGAGCA-3'; and Maf reverse, 5'-CGGGAGA-GGAAGGTTGCTC-3'.

**Statistical analysis**

Nonparametric Mann–Whitney tests or ANOVA tests were performed using the InStat 3.0 software (GraphPad Software, San Diego, CA). A p value of <0.05 was considered statistically significant.

**Results**

**gp130 signaling sustains virus-specific CD4<sup>+</sup> T cell numbers**

To determine the role of the IL-6 cytokine family on T cell responses during acute viral infection, we infected Cd4-cre Il6sr<sup>Δ9Δ10</sup> (T cell–specific gp130-deficient) mice and littermate control, Il6sr<sup>Δ9Δ10</sup> (cre-negative, or wild-type [WT]), mice with LCMV ARM.

During chronic LCMV infection, T cell–specific deletion of gp130 significantly reduces the survival of virus-specific CD4<sup>+</sup> T cells at later stages of infection. After acute LCMV ARM infection, the polyclonal virus-specific CD4<sup>+</sup> T cells responses, as marked by high expression of both CD11a and CD49d (32), in the blood were similar in Il6sr<sup>Δ9Δ10</sup> and Cd4-cd Il6sr<sup>Δ9Δ10</sup> mice (Fig. 1A). We did, however, find that by day 12 p.i. there was a significant reduction in the proportion and number of L<sup>A</sup> GP<sub>27-77</sub>-specific CD4<sup>+</sup> T cells in the spleen in the absence of gp130, despite similar numbers being present at day 8 p.i. (Fig. 1B). Reduced virus-specific CD4<sup>+</sup> T cell numbers remained observable out to day 60 p.i. Supporting this observation, the number of IFN-γ CD4<sup>+</sup> T cells present in the spleen after ex vivo GP<sub>27-77</sub> peptide stimulation at day 12 p.i., but not day 8 p.i., was significantly reduced in the absence gp130 (Fig. 1C). Production of IL-2 by virus-specific CD4<sup>+</sup> T cells was decreased in LCMV C113-infected animals that lack gp130 signaling in T cells (20). In LCMV ARM infection there also appeared to be a selective, but mild, alteration in cytokine production by virus-specific IFN-γ CD4<sup>+</sup> T cells by day 12 p.i. when stimulated ex vivo with GP<sub>07-77</sub> peptide (Fig. 1D). Specifically TNF-α production was similar between WT and gp130-deficient animals, whereas IL-2–producing CD4<sup>+</sup> T cells were slightly yet significantly reduced, and IL-2–producing CD4<sup>+</sup> T cells were increased. Overall, these data indicate that gp130 signaling influences both virus-specific CD4<sup>+</sup> T cell numbers and cytokine production after LCMV ARM infection.
gp130 regulates T<sub>FH</sub> function

gp130 signaling is also known to affect a number of other CD4<sup>+</sup> T cell differentiation fates (1). Furthermore, IL-21 can be thought of as a canonical cytokine produced by T<sub>FH</sub> and IL-2 via STAT-5 signaling, and it has recently been identified as inhibiting T<sub>FH</sub> fate (33–35), raising the possibility that gp130 induction of IL-21 during LCMV ARM infection (Fig. 1D) would result from regulation of T<sub>FH</sub> differentiation. We therefore evaluated CD4<sup>+</sup> T cell differentiation during acute LCMV infection in the absence of gp130 signaling in T cells at day 12 p.i., the earliest time point at which altered CD4<sup>+</sup> T cell responses were seen. We observed that the number of splenic Foxp3<sup>+</sup> Tregs was identical in WT and gp130-deficient animals (Fig. 2A). Additionally, despite the reported role of IL-27 in regulating the canonical Th1 transcription factor T-bet (10), the expression of T-bet was similar in WT and gp130-deficient I-Ab<sup>+</sup> tetramer<sup>+</sup> CD4<sup>+</sup> T cells (Fig. 2B). Despite the IL-21/IL-2 skew seen previously (Fig. 1C), the proportion of virus-specific CD4<sup>+</sup> T cells that were T<sub>FH</sub> (Bcl6<sup>+</sup>CXCR5<sup>+</sup>) was similar, although there was a trend toward decreased numbers of virus-specific T<sub>FH</sub> in the absence of gp130, which reached significance in one of three independent experiments. This was most likely due to the reduced number of total virus-specific CD4<sup>+</sup> T cells in these mice (Fig. 1B). Within the T<sub>FH</sub> population Bcl6 expression was unchanged; however, gp130-deficient T<sub>FH</sub> did express less ICOS (Fig. 2D, 2E).

Protein expression levels of T-bet and Bcl6 were confirmed by gene expression in FACS-isolated CD4<sup>+</sup> T effector cells (CD19<sup>+</sup>CD4<sup>+</sup>CD44<sup>+</sup>PD1<sup>+</sup>CXCR5<sup>+</sup>), T<sub>FH</sub> (CD19<sup>+</sup>CD4<sup>+</sup>CXCR4<sup>+</sup>PD1<sup>+</sup>CXCR5<sup>+</sup>), and virus-specific CD4<sup>+</sup> T cells (CD19<sup>+</sup>CD4<sup>+</sup>I-Ab<sup>+</sup>Gp<sub>67–77</sub>) from the spleen of day 12 p.i., with both Tbx21 and Bcl6 expression being similar between WT and Cd4<sub>-</sub>-cre Il6stfl/fl mice in all three cell populations (Fig. 2F). In contrast, the expression of Il21 was significantly reduced in isolated T<sub>FH</sub> and virus-specific CD4<sup>+</sup> T cells, but not in effector T cells. This was associated with a significant decrease in the expression of Maf in the T<sub>FH</sub> and virus-specific CD4<sup>+</sup> T cells, a transcription factor known to play a role in IL-21 production in T cells (7) (Fig. 2F).

FIGURE 1. gp130 signaling regulates virus-specific CD4<sup>+</sup> T cell numbers and cytokine production. Il6st<sup>fl/fl</sup> or Cd4-cre Il6st<sup>fl/fl</sup> mice were infected i.v. with 2 × 10<sup>6</sup> PFU LCMV ARM. (A) The number of CD11a<sup>hi</sup>CD49<sup>+</sup> CD4<sup>+</sup> T cells in the blood was determined at indicated days after infection. (B) I-A<sup>B</sup> GP<sub>67–77</sub> tetramer<sup>+</sup> CD4<sup>+</sup> T cells were monitored in the spleen at indicated days after infection. (C) The proportion and number of IFN-γ<sup>+</sup>CD4<sup>+</sup> T cells after GP<sub>67–77</sub> peptide stimulation were determined at days 8 and 12 p.i. (D) The frequency of TNF-α–, IL-21–, and IL-2–producing cells within IFN-γ<sup>+</sup>CD4<sup>+</sup> T cells after GP<sub>67–77</sub> peptide stimulation was quantified at day 12 p.i. Representative FACS plots are gated on CD4<sup>+</sup> cells (A–C) or CD4<sup>+</sup>IFN-γ<sup>+</sup> cells (D). Data are representative of n = 3 experiments with four or more mice per group. *<i>p</i> < 0.05, **<i>p</i> < 0.01.
Taken together, these data indicated that signaling through gp130 did not directly affect T<sub>FH</sub> differentiation but controlled specific T<sub>FH</sub> features such as IL-21 production as well as ICOS and Maf expression after acute LCMV infection.

CD4<sup>+</sup> T cell-specific gp130 deficiency alters B cell responses

CD4<sup>+</sup> T cell help is vital for subsequent humoral immune responses to infections and vaccination. We therefore wanted to determine whether the differences observed in T<sub>FH</sub> responses in the absence of gp130 signaling in T cells affected this process.

Analysis of B cell responses at day 12 p.i. showed that the number of GC B cells (IgD<sup>-</sup>IgM<sup>-</sup>CD38<sup>+</sup>GL7<sup>+</sup>) was decreased (Fig. 3A). There was an even greater reduction in the number of CD138<sup>+</sup> cells among isotype-switched IgD<sup>-</sup>IgM<sup>-</sup>B cells, indicating a strong effect of T cell–gp130 signaling on plasmablast formation (Fig. 3B). The low levels LCMV-specific IgG and the IgG1 and IgG2 subtypes detected in the serum at day 12 p.i. were similar in WT and gp130-deficient mice (data not shown). However, flow cytometric analysis of IgG2a/c expression on B cells revealed a profound reduction in the proportion of isotype-switched IgD<sup>-</sup>IgM<sup>-</sup>B cells that were IgG2a/c<sup>+</sup> in the absence of T cell–specific gp130 at day 12 after ARM infection (Fig. 3C).

Skewing of Ab responses from IgG2a toward IgG1 has commonly been associated with increased Th2 immunity, and gp130 signaling has previously been reported to enhance Th2 to immune responses (27). However, we found that after LCMV ARM infection the expression of Gata3, the canonical transcription factor associated with Th2 differentiation, was not significantly altered in FACS isolated effector T cells, T<sub>FH</sub>, and virus-specific (I-<A b>Ab</A> GP67–77<sup>+</sup>) CD4<sup>+</sup> T cells in the absence of gp130 (Fig. 3D). Indeed, Gata3 ex-
expression in both WT and gp130-deficient cells remained similar to expression seen in naive CD4+ T cells, indicating an absence of Th2 immune cells (Fig. 3D).

Taken together, these data indicate that gp130 signaling in T cells is essential for mounting optimal GC and plasmablast responses as well as IgG2a/c switching memory B cells after acute LCMV infection.

gp130 signaling in T cells influences long-lasting TFH and B cell responses

We next investigated whether the aforementioned changes observed in TFH and B cells from gp130-deficient mice at day 12 p.i. were either transient or long-lasting. For that, we analyzed CD4+ T cell and B cell responses in Il6stα/β versus Cd4-cre Il6stα/β mice at day 60 after LCMV ARM infection. We observed that even at this late time point, the frequency of LCMV-specific CD4+ T cells that were Bcl6-CXCR5+ TFH was similar between WT and gp130-deficient mice (Fig. 4A). As by day 12 p.i., there was a nonsignificant trend to reduced numbers of virus-specific TFH in the spleen in the absence of gp130 as a result of the significantly reduced number of virus-specific CD4+ T cells in Cd4-cre Il6stα/β mice (Figs. 1B, 4A). Another phenotype maintained since day 12 p.i. was the reduced ICOS (but not Bcl6) expression in gp130-deficient compared with WT virus-specific (I-Aβ GP33–41) or polyclonal TFH (Fig. 4B, Supplemental Fig. 1). Activated CD4+CD44+ T cells were FACS isolated as a surrogate for virus-specific CD4+ T cells given the low number of I-Aβ GP33–41 tetramer+CD4+ T cells at this late time point. Consistent with an Il21 defect at day 12 p.i., expression of Il21 was significantly decreased in CD44+CD4+ T cells from Cd4-cre Il6stα/β compared with WT mice at day 60 p.i. (Fig. 4C).

By day 60 p.i. there were far fewer GC B cells or CD138+ plasmablasts in LCMV ARM-infected mice than at day 12 p.i. (Fig. 3A versus Fig. 4D); these numbers, however, were for the most part still above the levels detected in age-matched WT uninfected mice. Despite this contraction, T cell–specific deletion of gp130 continued the trend toward reduced numbers of GC B cells and significantly fewer plasmablasts compared with WT mice at this later time point (Fig. 4D). By day 60 p.i. the number of splenic memory B cells (IgD- IgM−CD138−GL7−CD38+) was only marginally elevated above uninfected animals and this was not dependent on T cell–gp130 signaling (Fig. 4D). Importantly, whereas the number of memory B cells was unchanged, the loss of T cell–gp130 signaling resulted in a dramatic bias of memory B cells toward IgG1 expression with a profound reduction in IgG2a/c memory B cells (Fig. 4E). This B cell phenotype was consistent with a significant shift in LCMV-specific Ab isotypes in the serum: there was decreased IgG2a/c and IgG2b and increased IgG1 in Cd4-cre Il6stα/β mice versus WT littermates at both day 30 and 60 after ARM infection (Fig. 5). Decreased total Ig levels were also observed in T cell–gp130-deficient mice in two of four experiments by day 60 p.i., correlating with fewer plasmablasts in these animals.

To confirm that the changes in B cell responses in Cd4-cre Il6stα/β mice were most likely via loss of gp130 on T cells, rather than deletion occurring on another Cd4-expressing cell, we analyzed gp130 expression in the spleen at day 60 p.i. gp130 is constitutively expressed by all immune cells; however, gp130 expression by B cells was considerably lower than that observed on the other cell types analyzed (Supplemental Fig. 2). Loss of gp130 in Cd4-cre Il6stα/β mice, even 60 d after LCMV ARM infection, appeared to be predominantly restricted to T cells with no decreased gp130 expression seen in any other cell type analyzed, even among CD4+ populations of DCs.

Collectively, these data suggest that when T cells do not receive signaling via gp130 there is significant alteration in long-lasting TFH and B cells, both in the scale and quality of the responses.

gp130 signaling promotes formation of virus-specific memory CD8+ T cells

As Cd4-cre Il6stα/β mice also lack gp130 on their CD8+ T cells, we next analyzed the formation of virus-specific CD8+ T cells after LCMV ARM infection. The absence of gp130 signaling did not affect the generation of immunodominant virus-specific D8 NPb NPb NPb Np0–84a and D8 GP6–121 CD8+ T cells, with similar numbers observed in the blood at day 9 p.i., although there was a trend for slightly more gp130-deficient virus-specific CD8+ T cells upon contraction of the response at days 15 and 30 p.i. whereas the numbers at day...
60 p.i. were similar (Supplemental Fig. 3A, 3B). Similarly in the spleen, absence of gp130 signaling in T cells had little effect on the proportion or number of I-A<sup>β</sup> GP<sub>67–77</sub> +CXCR5<sup>+</sup>Bcl6<sup>+</sup>CD4<sup>+</sup> T cells, and (B) their Bcl6 and ICOS expression by flow cytometry. Mean fluorescence intensities (MFI) of Bcl6 and ICOS on naive CD4<sup>+</sup> T cells from uninfected mice are depicted by the broken lines. (C) The expression of IL21 was determined in FACS-isolated CD4<sup>+</sup>CD4<sup>+</sup> T cells by quantitative PCR. Expression levels in naive (CD62L<sup>+</sup> CD4<sup>+</sup>) CD4<sup>+</sup> T cells from uninfected mice are depicted by the broken lines. (D and E) The number of GC (GL7<sup>+</sup>CD38<sup>−</sup>CD138<sup>+</sup>), plasmablast (CD138<sup>+</sup>), and memory (CD38<sup>−</sup>GL7<sup>−</sup>CD138<sup>−</sup>) B cells among switched (IgM<sup>+</sup> IgD<sup>−</sup> CD19<sup>−</sup>B220<sup>−</sup>) B cells was determined by flow cytometry, as was the proportion of switched memory CD38<sup>+</sup> B cells expressing IgG1 and IgG2a/c. The numbers and proportion of each B cell compartment from uninfected mice are depicted by the broken lines. Data represent two experimental repeats with n ≥ 3 mice per group. *p < 0.05, **p < 0.01, ***p < 0.001.
and proportion of $D^b$ NP$_{396-404}$ memory CD8$^+$ T cells at day 60 p.i. (Fig. 6C). To determine whether this affected CD8$^+$ T cell recall responses, we then rechallenged these mice between days 60 and 70 with the more virulent LCMV Cl13 strain. Despite similar quantities of total $D^b$ NP$_{396-404}$CD8$^+$ T cells immediately prior to rechallenge (Fig. 6A), the number of $D^b$ NP$_{396-404}$CD8$^+$ T cells in the spleen was significantly reduced in the absence of gp130 upon rechallenge (Fig. 6D), consistent with a reduced presence of memory CD8$^+$ T cells seen in Fig. 6C. These results indicate that gp130 signaling in T cells influences the formation of memory CD8$^+$ T cells and can affect the virus-specific CD8$^+$ T cell recall response on rechallenge. Tcf1, encoded by $Tcf7$, is

**FIGURE 5.** T cell–gp130 signaling promotes increased circulating IgG2a/c over IgG1. Il6st$^{fl/fl}$ or Cd4$^-cre$ Il6st$^{fl/fl}$ mice were infected i.v. with $2 \times 10^6$ PFU LCMV ARM. Anti-LCMV–specific Abs in the serum at (A) days 30 and (B) 60 p.i. were determined by ELISA. Data are representative of $n = 4$ experiments with $n \geq 3$ mice per group. **$p < 0.01$, ***$p < 0.001$.

**FIGURE 6.** gp130 signaling on T cells promotes memory CD8$^+$ T cell responses. Il6st$^{fl/fl}$ or Cd4$^-cre$ Il6st$^{fl/fl}$ mice were infected i.v. with $2 \times 10^6$ PFU LCMV ARM. (A) $D^b$ NP$_{396-404}$ tetramer$^+$ and $D^b$ GP$_{33-41}$ tetramer$^+$ CD8$^+$ T cells were monitored in the spleen at days 8, 12 and 60 p.i. (B) The proportion of circulating $D^b$ NP$_{396-404}$ tetramer$^+$CD8$^+$ T cells expressing KLRG1, CD127, or both was examined throughout infection, and (C) the number of splenic $D^b$ NP$_{396-404}$ tetramer$^+$ memory (KLRG1$^-$CD127$^+$) cells 60 d p.i. with LCMV ARM was calculated. (D) $D^b$ NP$_{396-404}$ tetramer$^+$CD8$^+$ T cells in the spleen at day 5 after rechallenge with $2 \times 10^6$ PFU LCMV Cl13 were determined. (E) The expression of Tcf7 was determined in FACS-isolated KLRG1$^-$CD127$^+$ splenic $D^b$ NP$_{396-404}$CD8$^+$ T cells at day 60 after LCMV ARM infection. Data are representative of $n \geq 2$ experiments with at least three mice per group. *$p < 0.05$, **$p < 0.01$. **
a transcription factor known to be important in maintaining long-lasting KLRG1^2 CD127^+, but not KLRG1^+CD127^−, CD8^+ T cells, and Tcf7^−/− CD8^+ T cells fail to expand on secondary challenge (37). In the absence of gp130, Tcf7 expression was significantly reduced in KLRG1^+CD127^CD8^+ T cells, which may help explain both the reduced frequency of these cells and their limited expansion on rechallenge (Fig. 6E).

There was also a reduced number of both polyclonal (CD11a^+ CD49d^+) and virus-specific (I-A^b GP_67–77^) CD4^+ T cells at day 5 after challenge (Supplemental Fig. 4). This could be indicative of a common failure of gp130-deficient memory cells in expansion on rechallenge; however, unlike virus-specific CD8^+ T cells (Fig. 6A), there were also fewer virus-specific CD4^+ T cells prior to challenge (Fig. 1B).

**Intrinsic gp130 signaling is critical for CD4^+ T cell numbers and function**

T cell–specific deletion of gp130 resulted in long-lasting alterations to both the CD4^+ and CD8^+ T cell compartments. CD4^+ T cell help can be essential in maintenance of CD8^+ T cell memory. Indeed, IL-21, which is predominantly produced by CD4^+ T cells in vivo and reduced in the absence of gp130, has been shown to potently upregulate Tcf1 in CD8^+ T cells (38). To determine whether gp130 signaling on CD4^+ T cells, CD8^+ T cells, or both was responsible for the T cell defects observed in Cd4^cre Il6stfl/fl mice, we generated WT:Cd4^cre Il6stfl/fl mixed bone marrow chimeras and infected them with LCMV ARM. At day 12 p.i. the proportion of gp130-deficient virus-specific (I-A^b GP_67–77^) CD4^+ T cells was significantly lower than that observed in WT mice (Fig. 7A). Additionally, whereas the per cell production of IFN-γ by each virus-specific CD4^+ T cell appeared similar, virus-specific CD4^+ T cells had reduced levels of TNF-α and IL-21 when stimulated ex vivo (Fig. 7B). Consistently, Il21 expression in I-A^b GP_67–77 tetramer^+CD4^+ T cells was also lower in gp130-deficient cells than in WT cells directly ex vivo (Fig. 7C) and was associated with lower expression of Maf in T FH cells (Fig. 7D). We also observed that significantly fewer gp130-deficient CD4^+
T cells were T FH (CXCR5+Bcl6+) or alternatively gated as CXCR5+PD1+ than WT CD4+ T cells (Fig. 7E), possibly due to a reduced proportion of virus-specific CD4+ T cells in the gp130-deficient compartment (Fig. 7A). Importantly, gated T FH exhibited significantly reduced levels of ICOS (Fig. 7F, 7G), demonstrating the intrinsic nature of ICOS downregulation described above in the nonchimeric setting (Figs. 2E, 4B). Of note, in contrast to the nonchimeric mice, Bcl6 expression was also reduced in gp130-deficient versus WT gated T FH when analyzed in the competitive chimeric setting (Fig. 7G). There was, however, no observable defect in the proportion of B NP396-404+CD8+ T cells or the frequency of those cells that were CD127+KLRL1+ in the absence of gp130 (Supplemental Fig. 4C, 4D). These data support a direct role for gp130 signaling in regulating the quantity and quality of CD4+ T cell responses after an acute viral infection.

**T cell gp130 signaling is not essential for viral control during acute LCMV infection**

LCMV C13-infected Cd4-cre Il6stfl/fl mice fail to control virus, leading to prolonged persistence (20). In contrast, viremia after LCMV ARM infection was similar in WT and Cd4-cre Il6stfl/fl mice, and no virus was detectable by day 9 p.i. (Fig. 8A). Additionally, at day 5 p.i. tissue viral loads were also similar between WT and gp130-deficient animals (Fig. 8B). Because gp130 deficiency resulted in reduced memory CD8+ T cells and altered Ab immune response, we next assessed whether these effects could result in reduced immune protection. We rechallenged WT or Cd4-cre Il6stfl/fl mice with the more aggressive LCMV C13 virus 60–70 d after primary infection with LCMV ARM. By day 5 after challenge, both WT and gp130-deficient animals had no detectable virus in the serum (Fig. 8C). These results suggested that whereas gp130 signaling on T cells regulates both long-lasting humoral and CD8+ T cell memory, it is not essential for viral control during either primary or secondary viral infection with LCMV.

**Discussion**

The IL-6 cytokine family can influence a wide diversity of T cell functions and fates despite common use of the gp130 signal transduction protein. Therefore, to determine the direct role of signaling through gp130, we used T cell–specific gp130-deficient mice. Upon infection with acute LCMV, gp130 signaling was critical for the maintenance of splenic virus-specific CD4+ T cell, but not CD8+ T cell, numbers at later stages of infection. This signaling pathway intrinsically modulated expression of key molecules and genes associated with T FH, including Maf, ICOS, and IL-21, consequently influencing humoral immune responses. Notably, deficiency in gp130 signaling appeared to not dramatically affect other Th subsets. We also found that gp130 signaling in T cells regulated the ratio of effector to memory CD8+ T cells and impacted the recall of these cells during reinfection, which again appeared to be mediated through gp130 signaling events in virus-specific CD4+ T cells.

CD4+ T cell help is critical in both cytotoxic CD8+ T cell–mediated and humoral B cell–mediated immunity. In this study, we found that signaling through the gp130 pathway on CD4+ T cells is vital for the maintenance of higher virus-specific CD4+ T cell numbers during the contraction and memory phases of the T cell response. IL-6 can act in an antiapoptotic capacity in resting and primed CD4+ T cells, as well as CD4+ T cells during recall responses (39–41). Likewise, IL-27 signaling was found to be important in a T cell–mediated colitis model (42) and chronic LCMV infection (20) through promoting CD4+ T survival. IL-6–deficient mice have not previously been found to have a defect in virus-specific CD4+ T cell numbers during acute LCMV infection (17, 18), whereas in chronic LCMV infection we have previously shown that gp130 signaling drives survival of virus-specific CD4+ T cells via IL-27, and not IL-6, signaling (18, 20). Taken together, these data suggest that both intrinsic IL-27 and IL-6 likely play complementary roles in maintaining virus-specific CD4+ T cell numbers at optimal levels in a variety of situations.

Elevated IL-21 production, predominantly by CD4+ T cells, has been associated with improved responses during chronic infections in both mice and humans (24–26, 43–45). Contrastingly, IL-21 is thought to play a key role in inflammatory conditions such as diabetes, multiple sclerosis, Sjögren’s syndrome, and brain injury after stroke (46–50). Previously we showed that IL-21 production in chronic LCMV infection is driven by gp130 signaling (20). In the present study, we found that in the context of an acute stimulus, gp130 signaling on CD4+ T cells in part promotes IL-21 in vivo, although after restimulation ex vivo there was still significant IL-21 production. Nevertheless, this indicates the potential of this pathway to modulate IL-21 production in a number of different contexts.

In particular, IL-21 production by T FH appeared to be compromised in the absence of gp130 signaling, and no effect on I221 expression was observed in non-T FH. Fitting with this, although in T cell–gp130-deficient animals the generation of CXCR5+Bcl6+ T FH was not significantly reduced, the numbers of GC B cells and plasmablasts were dramatically reduced, potentially resulting from the reduced ICOS expression and IL-21 production by T FH at day 12 p.i. Intriguingly, in a competitive setting, gp130 deficiency adversely affected Bcl6 expression in T FH, indicating that there may be a progressive requirement for gp130 signaling in optimal T FH formation and function. Importantly, analysis of mice at 2 mo p.i. indicated that the aforementioned defects are in great part carried on.

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**FIGURE 8.** T cell–gp130 signaling does not affect viral control during acute primary LCMV infection or secondary chronic LCMV infection. Il6stfl/fl or Cd4-cre Il6stfl/fl mice were infected i.v. with 2 × 10^6 PFU LCMV ARM. (A and B) Viral loads were determined by plaque assay in serum at days 5 and 9 p.i. (A) or indicated tissues at day 5 p.i. (B). (C) At 2 mo p.i., mice were rechallenged with 2 × 10^6 PFU LCMV C13 i.v. Viral loads were determined in the serum at 5 d after challenge and compared with WT mice receiving LCMV C13 that had not previously been infected with LCMV ARM (1` WT). Data represent two independent experiments. n.d., Not detected.
to the memory phase and that gp130 signaling is required for optimal lasting CD4 T cell immunity after acute viral infection. Importantly, in the absence of T cell–gp130 signaling, there was a significant shift in the type of Abs produced, with increased IgG1 and reduced IgG2a/c and IgG2b in Cd4-cre Il6st<sup>fl/fl</sup> mice, and this defect also lasted up to 2 mo p.i. T cell–specific gp130 signaling has been associated with an increase in Th2 cell formation, which might account for the skewed Ab response; in the present study, however, we found that no apparent increase in Gata3 expression within the virus-specific CD4<sup>+</sup> T cell population, suggesting an alternative T cell–specific role of gp130 in IgG2 versus IgG1 switch.

gp130 signaling also regulated other Th17-specific features that could influence B cell responses, including the expression of ICOS. Signaling through ICOS has been linked to T<sub>FH</sub> migration and differentiation (51, 52). Although we observed no difference in CXCR5 or Bcl6 expression, indicative that T<sub>FH</sub> migration and differentiation were unaffected, in the nonchimeric setting, Bcl6 was downregulated in total gp130-deficient T<sub>H4</sub> when competing with WT cells in mixed chimeras. ICOS has also been implicated in c-Maf–dependent transcription of IL-21 (53), and it is therefore possible that ICOS downregulation contributes to the reduced expression of Maf and IL-21 observed in gp130-deficient CD4<sup>+</sup> T cells. Recently it has also been shown that ICOS is required to act in a “feed-forward” loop that allows the T<sub>FH</sub> to continuously provide B cell help once the GC has formed (54), and this could be related to the B cell defects observed in the T cell–gp130-deficient mice after acute LCMV infection. Overall, these results highlight the role of the gp130 pathway in modulating humoral immunity through functional changes in T<sub>FH</sub> responses.

In addition to alteration in virus-specific CD4<sup>+</sup> T cell responses, we also observed a preferential bias toward terminal effector KLRG1<sup>+</sup>CD127<sup>−</sup> CD8<sup>+</sup> T cells with reduced generation of virus-specific KLRG1<sup>+</sup>CD127<sup>−</sup> CD8<sup>+</sup> T cells in the absence of gp130 signaling. Fitting with this, there was reduced number of D<sub>P</sub> NP<sub>96–404</sub>-specific CD8<sup>+</sup> T cells before secondary infection. In line with this, IL-21 has been linked to maturation of memory CD8<sup>+</sup> T cells via an IL-10– and STAT3-enabled pathway, during infection. In line with this, IL-21 has been linked to maturation of virus-specific CD4<sup>+</sup> T cells. Recently it has also been shown that ICOS is required to promote differentiation of IL-10-producing Tr1 cells.

Modulation of CD4<sup>+</sup> T lymphocyte lineage outcomes with targeted, nanoparticle-mediated cytokine delivery. Mol. Pharm. 8: 143–152.


