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## **Cytokine-mediated regulation of immunity during persistent viral infection**

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# Chapter 3

## B cell-derived IL-27 promotes control of persistent LCMV infection

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## Abstract

Recent studies have identified a critical role for B cell-produced cytokines in regulating both humoral and cellular immunity. Here, we show that B cells are an essential source of IL-27 during persistent Lymphocytic choriomeningitis virus (LCMV) Clone 13 infection (Cl-13). By using conditional knockout mouse models with specific IL-27p28 deletion in B cells, we observed that B cell-derived IL-27 promotes survival of virus-specific CD4 T cells and supports functions of T follicular helper (Tfh) cells. Mechanistically, B cell-derived IL-27 promotes CD4 T cell function, antibody class switch and the ability to control persistent LCMV infection. Deletion of IL-27ra in T cells demonstrated that T cell-intrinsic IL-27R signaling is essential for viral control, optimal CD4 T cell responses and antibody class switch during persistent LCMV infection. Collectively, our findings identify a cellular mechanism whereby B cell-derived IL-27 drives antiviral immunity and antibody responses through IL-27 signaling on T cells to promote control of LCMV Cl-13 infection.

## Significance Statement

Persistent viral infection remains a major source of global morbidity and mortality. Studies using a persistent clone of lymphocytic choriomeningitis virus (LCMV) revealed that in addition to optimal functions and interactions of T and B cells, production of cytokines is essential in promoting and long-term control of infection. Here we report that B cells are an indispensable source of IL-27 during persistent LCMV infection. B cell-derived IL-27 promotes viral control via supporting accumulations of virus-specific CD8 and CD4 T cells. During later stages of infection, B cell-derived IL-27 promotes production of IFN- $\gamma$  and IL-21 by virus-specific CD4 T and Tfh cells, respectively. Our study unveils the critical role of a B cell-secreted cytokine in controlling a persistent infection.

## Introduction

Persistent viral infections, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) cause devastating disease burden around the globe. Viral persistence leads to sustained viremia, prolonged immune activation, disorganized microarchitecture of secondary *lymphoid* organs, reduced numbers and compromised functions of virus-specific T and B cells as well as aberrant production of virus-specific antibodies (1-5). Infected hosts are confronted with long-term health challenges which can ultimately result in medical diseases and in some cases lead to death. Research efforts using persistent Lymphocytic choriomeningitis virus (LCMV) Clone 13 (Cl-13) as a model of persistent viral infection in mice primarily led to our mechanistic understanding of how interactions between different immune cell populations contribute to virus clearance. There are 3 major stages of LCMV Cl-13 infection; initial infection and establishment of virus persistence, maintenance of persistence and finally immune control and eventual purging of the virus from the host. Initially upon infection, LCMV Cl-13 induces higher production of type 1 interferon (IFN-I) and expression of immunoregulatory molecules such as IL-10 and programmed cell death ligand 1 (PD-L1), which restrains T cell functions and sets up virus persistence. Blockade of IFN-I, PD-1/PD-L1, and IL-10 were shown to improve functions of virus-specific T cells to kill infected cells and produce effector cytokines such as interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-2 (IL-2) (6-12). During the later stages of persistent LCMV infection, virus-specific CD4 T cells display differentiation bias towards T follicular helper (Tfh) cells and produce the immune stimulatory cytokine interleukin-21 (IL-21) to provide help to both CD8 T cells and B cells. IL-21 signaling

leads to improved CD8 T cells functions and generation of LCMV-specific neutralizing antibodies that drives viral control (13-17). A recent study identified interleukin-27 (IL-27) as an essential immune regulator required for the control of persistent LCMV infection (18). Ablation of IL-27ra led to increased viral replication, reduction of both virus-specific CD4 T cells and LCMV-specific IgG antibodies at later stages of infection (18, 19).

IL-27 is a pleiotropic cytokine composed of the IL-27p28 and Epstein Barr-Virus Inducible protein-3 (EBI3) subunits. It signals through a heterodimeric receptor complex consisting of WSX-1 (IL-27ra) and gp130 to activate both STAT1 and STAT3 transcription factors (20). Although dendritic cells and macrophages were identified as major producers of IL-27 required for maintenance of early antiviral immunity, the cellular source of IL-27 required for long term control of persistent LCMV infection is unknown (19). Previous studies demonstrated that B cells secrete cytokines to provide signals to other cells to modulate both humoral and cellular immunity (21-25). Intriguingly, in addition to IL-6, IL-10, IL-12, and IL-35, B cells were shown to independently secrete EBI3 and IL-27p28 upon *in vitro* activation (23, 26). Taken together, these studies are highly suggestive that B cells may play a key role in IL-27 production during persistent LCMV infection. In the present study, we identified B cells as an essential source of IL-27 that promotes survival and functions of virus-specific CD8 and CD4 T cells. B-cell-derived IL-27 induces IFN- $\gamma$  and IL-21 production by virus-specific CD4 T cells and T<sub>fh</sub> cells at later stages of LCMV infection that contribute to virus clearance. Our findings reveal the central role of B cells in the regulation of T cell functions and describes IL-27-dependent cellular interactions between T and B cells necessary for control of persistent LCMV infection.

## Results and Discussion

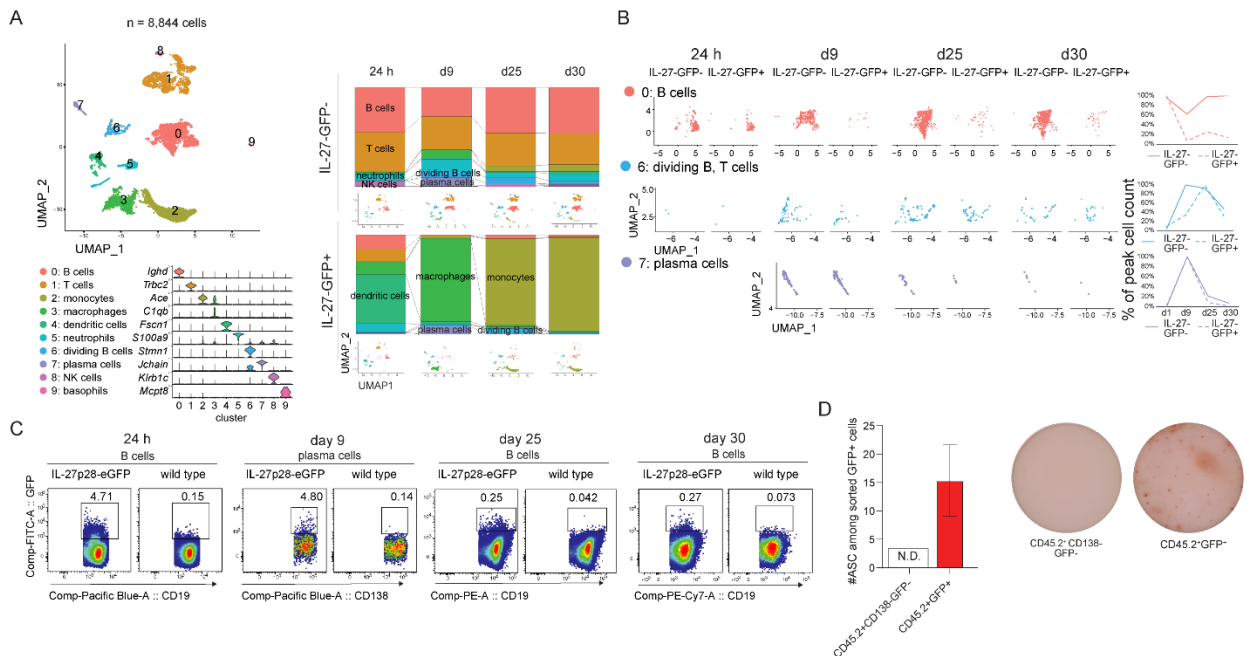
### Various B cell populations express IL-27 during persistent LCMV infection

Following persistent virus infection, IL-27 is necessary to purge the virus from animals (18, 27). However, the specific cellular sources of IL-27 required for viral clearance are currently unknown. To generate a complete picture of the cellular and temporal production of IL-27 following LCMV CI-13 infection we characterized the landscape of IL-27p28-expressing and IL-27p28-non-expressing cells using IL-27p28-eGFP reporter mice by sequencing transcriptomes of single sorted IL-27p28-GFP<sup>+</sup> and IL27p28-GFP<sup>-</sup> splenocytes at days 1, 9, 25 and 30 post infection. The cells were reliably separated into clusters associated with known cell types (Fig. 1A, Fig. S1A). As expected, myeloid cells were the dominant source of IL-27p28: early IL-27p28<sup>+</sup> cells displayed transcriptomic signatures of dendritic cells whereas macrophages and monocytes predominated at later times following infection (Fig. 1A, Fig. S1B). Dendritic cells (DCs) and myeloid cells have previously been identified as major producers of IL-27 required for early control of persistent LCMV infection (19). Given that chronic infection persists for several weeks and B cells were shown to secrete IL-27 upon *in vitro* activation, we were particularly interested in the possibility that IL-27 could be produced at later stages of infection and potentially by cellular sources other than DCs and myeloid cells. From our single cell data, we observed that B cells and plasma cells comprised a minor but significant fraction of IL-27p28-GFP<sup>+</sup> cells at all times following infection (Fig. 1A). The combined percentage of B cells and plasma cells of total IL-27p28-GFP<sup>+</sup> splenocytes ranged from 14.4% on day 1 to 10.5, 9.5 and 3.8% on days 9, 25 and 30 p.i., respectively. At day 1 post infection, IL-27p28-GFP<sup>+</sup> B cells were predominantly from the *Ighd*<sup>+</sup> naïve-like cluster but later B-cell IL-27p28 was associated with mitotic B cells and plasma cells (Fig. 1A-B). Interestingly, IL27p28-GFP<sup>+</sup> B cells in Cluster 0 expressed lower levels of genes

associated with naïve B cells and higher levels of genes associated with germinal center B cells compared to IL-27p28-GFP<sup>-</sup> B cells in the same cluster (Fig. S1C-D). Further, whereas the percentage of IL-27p28-GFP<sup>+</sup> cells of non-dividing B cells peaked on day 1 p.i., the highest percentage of IL-27p28-GFP producing cells among dividing B cells occurred on day 25 p.i. (Fig. 1B). These observations are consistent with the association of IL-27p28 expression with activated B cells.

We next verified that B cells derived from IL-27p28-eGFP mice were able to express IL-27p28 upon *in vitro* activation by TLR4 and CD40 as previously described (Fig. S1E) (23). In addition, we observed that IL-27p28 production in B cells was markedly enhanced in the presence of IL-21 which was accompanied by sustained elevated levels of EB13 (Fig. S1E-F).

To confirm our observation from single-cell RNA sequencing, we performed flow cytometric analyses of fluorescence signals in splenocytes derived from IL-27p28-eGFP mice at 24 hr, day 9, day 25 and day 30 p.i. B cells from wild type mice were examined concomitantly as negative controls to confirm the specificity of the GFP reporter signal. Consistently, total B cells and plasma B cells from IL-27p28-eGFP reporter mice were significant producers of IL-27p28 at 24 h p.i. and day 9 p.i. respectively whereas wild type B cells showed negligible GFP fluorescence (Fig. 1C, Fig. S1G). By performing IgG B cell ELISpot of GFP<sup>+</sup> cells derived from IL-27p28eGFP mice at day 9 p.i., we observed that some of IL-27 producers were capable of secreting antibodies (Fig. 1D), suggesting that plasma cells are significant sources of IL-27 during LCMV CI-13 infection. Taken together, these data demonstrate that, in addition to DCs and macrophages, B cell populations and plasma cells produce IL-27 during persistent LCMV infection.



**Figure 1. B cell populations express IL-27**

IL-27p28-eGFP mice were infected with  $2 \times 10^6$  PFU LCMV CI-13 i.v. (A, B, D). IL-27p28-eGFP mice and wild type C57BL/6 mice were infected with  $2 \times 10^6$  PFU LCMV CI-13 (C). **A**) Analysis of single-cell RNA sequencing of splenocytes at days 1, 9, 25 and 30 p.i. Left: dimensionality-reduced UMAP plot and expression of cluster marker genes; right: cluster distribution of IL-27p28-GFP<sup>+</sup> and IL-27p28-GFP<sup>-</sup> cells at each timepoint, identity of each cluster indicated by color. **B**) Time-resolved distribution of cells within each cluster, graph indicates relative abundance of IL-27p28-GFP<sup>+</sup> (denoted IL-27-GFP<sup>+</sup>) and IL-27p28-GFP<sup>-</sup> (denoted IL-27-GFP<sup>-</sup>) cells in each cluster scaled to peak relative abundance within each experimental group. **C**) GFP expression on B cells (1, 25 and 30 days p.i.) or plasma

cells (9 days p.i.) was determined by flow cytometry, gated on splenic B cells or plasma cells in IL-27p28-eGFP reporter mice and wild type mice (the latter as negative control for background fluorescence). **D**) Frequencies of antibody-secreting cells (ASCs) in CD45.2<sup>+</sup>GFP<sup>+</sup> and CD45.2<sup>+</sup>GFP<sup>-</sup>CD138<sup>-</sup> populations were determined using IgG B cell ELISPOT at day 9 p.i. Data in C-D are representative of two experimental replicates and error bars represent mean  $\pm$  SD from 9 mice per group; N.D., not detected.

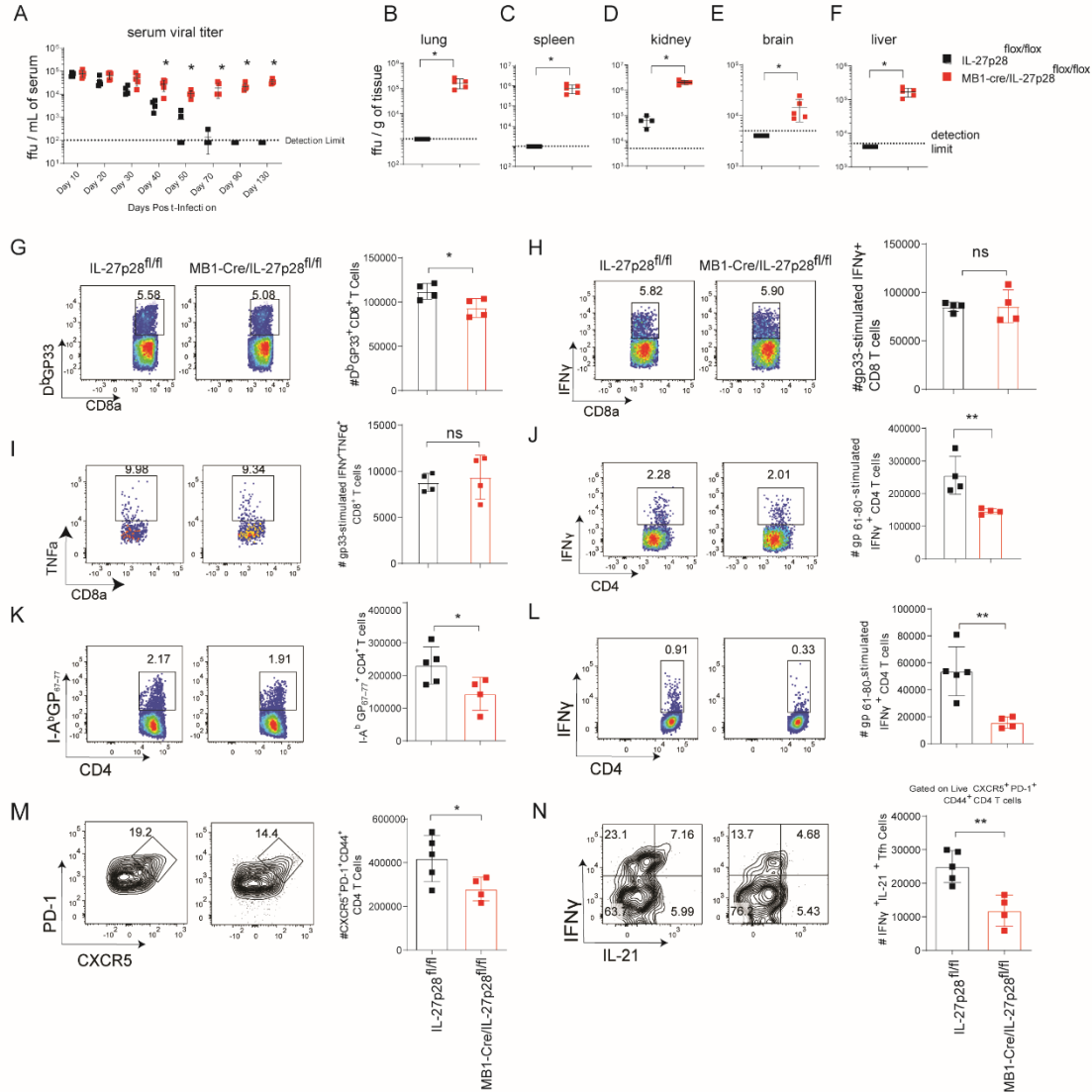
## **B-cell-derived IL-27 is required to control persistent LCMV infection**

Given that B cells are a significant cellular source of IL-27, we asked if B-cell-derived IL-27 promotes long-term control of viral persistence. To explore the impact of B cell-specific deletion of IL-27p28 during chronic LCMV infection, we interbred IL-27p28<sup>fl/fl</sup> mice to MB1-Cre mice and infected them with LCMV CI-13. Deletion efficiency and specificity were subsequently determined by assessing the expression of IL-27p28 in CD138<sup>+</sup> plasma B cells using flow cytometry and assessing the relative expression of *Il27* mRNA using quantitative polymerase chain reaction (qPCR) of sorted CD138<sup>+</sup> plasma cells, B cells, DCs and macrophages from MB1-Cre<sup>+/-</sup>/IL-27p28<sup>fl/fl</sup> and IL-27p28<sup>fl/fl</sup> mice at day 9 post-infection. We observed a significant reduction of relative *Il27* mRNA expression in B cells and plasma B cells but not in DCs and macrophages derived from MB1-Cre<sup>+/-</sup>/IL-27p28<sup>fl/fl</sup> mice compared to IL-27p28<sup>fl/fl</sup> mice (Fig. S2A-E). Our results verified that MB1-Cre deletion strategy is specific to B cell populations. MB1-Cre<sup>+/-</sup>/IL-27p28<sup>fl/fl</sup> mice were unable to control viral loads in serum at various times post-infection and in multiple tissues including lungs, spleen, kidney, brain and liver at day 130 p.i. (Fig. 2A-F).

Analysis of the anti-viral T cell responses at day 9 p.i. revealed a modest but significant reduction in GP33 tetramer positive CD8 T cells (Fig. 2G) however, no defect in cytokine production was observed in virus-specific CD8 T cells (Fig. 2H & I). Interestingly, we observed a significant reduction in the numbers of virus-specific IFN- $\gamma$ -producing CD4 T cells at day 9 p.i. (Fig. 2J). Deleterious effects of MB1-Cre deletion during persistent LCMV infection were ruled out as MB1-Cre<sup>+/-</sup> mice did not display defects in T cells and were able to control persistent LCMV infection as efficiently as WT mice (Fig S2F-I).

Since LCMV CI-13 infection persists for several weeks and is associated with dysfunctional exhausted CD8 T cells and differentiation bias of CD4 T cells towards Tfh cells (17), we wondered whether B-cell-derived IL-27 affects T cell responses at later stages of infection. At day 40 p.i., we observed that LCMV infected MB1-Cre<sup>+/-</sup>/IL-27p28<sup>fl/fl</sup> displayed fewer virus-specific I-A<sup>b</sup> GP<sub>67-77</sub> tetramer<sup>+</sup> CD4 T cells and IFN- $\gamma$ -producing CD4<sup>+</sup> T cells compared to IL-27p28<sup>fl/fl</sup> littermate controls, suggesting that B-cell-derived IL-27 is essential for the maintenance of both the expansion and function of virus-specific CD4 T cells during chronic LCMV infection (Fig. 2K-L). In addition to regulating survival and functions of virus-specific CD4 T cells, IL-27 has been shown to play an important role in supporting Tfh functions during Ovalbumin priming (28). Recent findings also indicated that Tfh cells are essential for the generation of LCMV-specific antibodies that drive clearance of persistent LCMV infection (7, 16). Therefore, we investigated the impact of B-cell-derived IL-27 on the numbers and function of Tfh cells during the chronic phase of LCMV infection. At day 40 p.i., MB1-Cre<sup>+/-</sup>/IL-27p28<sup>fl/fl</sup> displayed fewer Tfh cells compared to IL-27p28<sup>fl/fl</sup> littermate controls (Fig. 2M). We stimulated splenocytes derived from B cell-specific IL-27p28 knockout mice and littermate controls *ex vivo* with either PMA and ionomycin or LCMV GP<sub>61-80</sub> peptide and observed knockout Tfh cells produced markedly fewer IFN- $\gamma$ <sup>+</sup> IL-21<sup>+</sup> compared to WT cells (Fig. 2N, S2J). Moreover, mice lacking IL-27p28 in B cells displayed similar levels of LCMV-specific IgG1 but reduced IgG2a/2c and neutralizing antibodies (Fig. S2K-M) which have been previously demonstrated to promote control

of CI-13 infection (16, 29, 30). These results jointly suggest that B-cell-derived IL-27 is required for the maintenance of Tfh function, antibody production and clearance of a persistent virus but also that IL-27 produced by other cells is insufficient to compensate for loss of the B-cell-derived IL-27 pool.



**Figure 2. IL-27-producing B cells promotes control of persistent LCMV infection**

IL-27p28<sup>flox/flox</sup> (WT) and MB1-Cre/IL-27p28<sup>flox/flox</sup> (B cell-specific IL-27p28 KO) mice were infected with  $2 \times 10^6$  PFU LCMV CI-13. A) Serum viral loads were determined throughout infection. At day 130 p.i. viral loads were measured in B) lungs C) spleens D) kidneys E) brain and F) liver. At day 9 p.i. splenocytes were analyzed by flow cytometry to determine total number of G) H2-Db GP33-41 virus-specific CD8 T cells, H) IFN- $\gamma$ -producing GP33-41 virus-specific CD8 T cells, I) Polyfunctional IFN- $\gamma$ - and TNF- $\alpha$ -producing GP33-41 virus-specific CD8 T cells, and J) IFN- $\gamma$ -producing GP61-80 virus-specific CD4 T cells. At day 40 p.i., splenocytes were analyzed by flow cytometry to determine the number of K) I-Ab GP67-77+ CD4 T cells, L) IFN- $\gamma$ + CD4 T cells after GP61-80 peptide stimulation, M) Tfh cells, N) IL-21+IFN- $\gamma$ + Tfh cells after PMA and ionomycin stimulation. Data are representative of three experimental replicates and error bars represent mean  $\pm$  SD from 4-5 mice per group. Statistical analyses of experimental groups were performed using Mann-Whitney U test (A-F) or Student's two-tailed t test (G-N): not significant (ns),  $P > 0.05$ ; \* $P \leq 0.05$ , \*\* $P \leq 0.01$ .

## T-cell-intrinsic IL-27 signaling is required to the control persistent LCMV infection

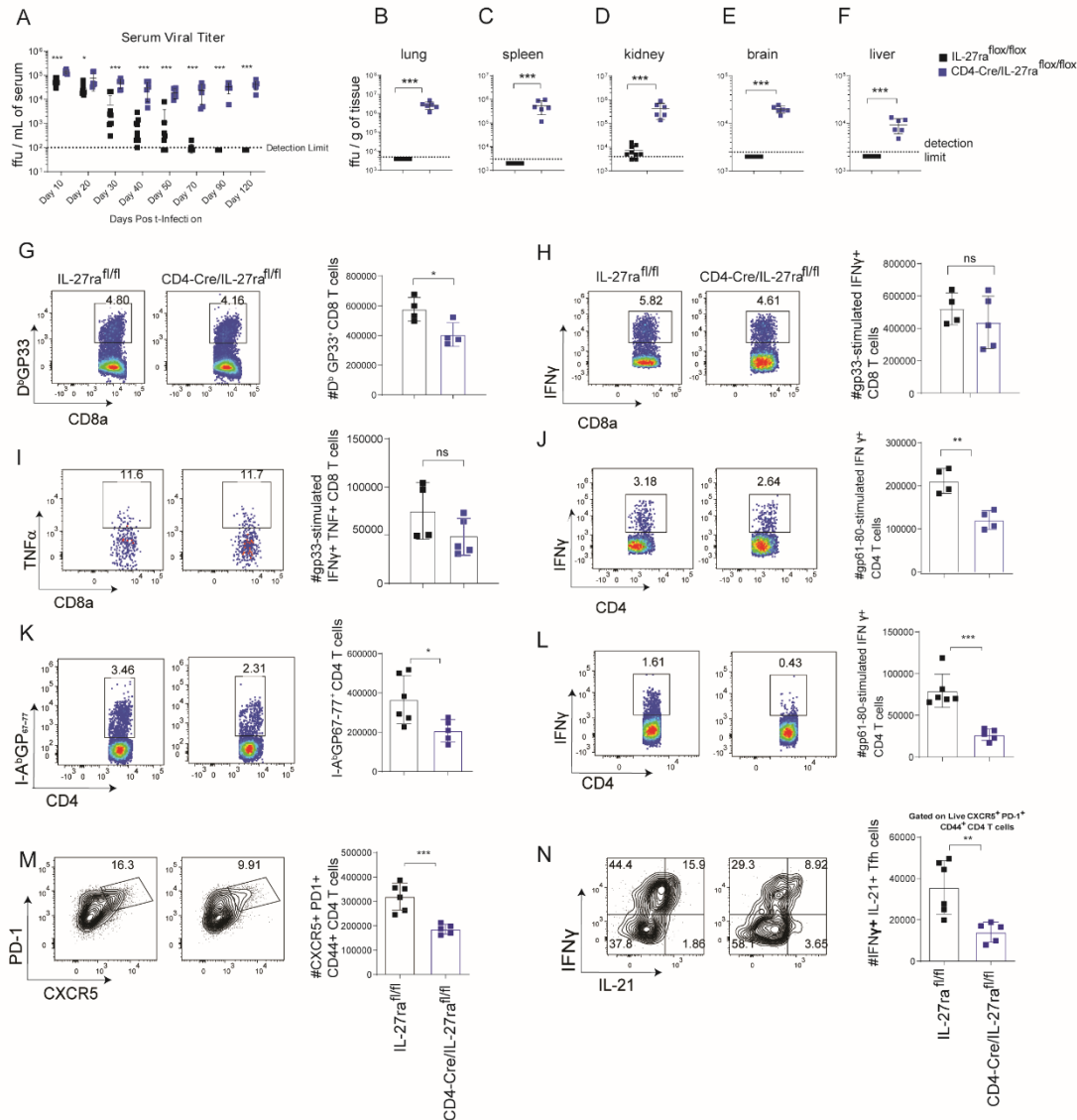
To address mechanistically how B-cell-derived IL-27 facilitates host defense against persistent viral infection, we next investigated the cellular populations that require IL-27 signaling to promote viral control. We first investigated the possibility that B-cell-intrinsic IL-27R signaling may be required to control the virus. To test this, IL-27ra<sup>fl/fl</sup> mice were crossed with MB1-Cre mice (MB1-Cre<sup>+/-</sup>/IL-27ra<sup>fl/fl</sup>) to generate conditional knockout mice with B cell-specific IL-27ra deletion before infecting them with LCMV Cl-13. We assessed serum viral titers from day 9 until day 120 p.i. and observed that mice deficient in IL-27ra specifically in B cells were able to control Cl-13 infection similar to littermate controls (Fig. S3A-C) suggesting that B cell-intrinsic IL-27R signaling is not required for the control of persistent LCMV infection. Besides controlling virus as effectively as IL-27ra<sup>fl/fl</sup> (WT) controls, mice lacking IL-27ra in B cells displayed normal LCMV-specific IgG2a/2c production (Fig. S3D). In addition, B cell-specific deletion of IL-27ra did not alter numbers of germinal center (GC) B cells, virus-specific CD4 T cells and virus-specific CD8 T cells at day 9 p.i. (Fig. S3E-G).

Since CD8 T cells are essential effectors that drive the antiviral immune response during chronic viral infection, we next tested whether IL-27 signaling in effector CD8 T cells was required for protection against persistent LCMV infection. We generated conditional knockout mice where IL-27ra was deleted in granzyme B expressing cells (including effector CD8 T cells and NK cells) by crossing IL-27ra<sup>fl/fl</sup> mice to Granzyme B Cre mice (GZMB-Cre<sup>+/-</sup>/IL-27ra<sup>fl/fl</sup>). Following LCMV Cl-13 infection, GZMB-Cre<sup>+/-</sup>/IL-27ra<sup>fl/fl</sup> controlled viral loads as efficiently as GZMB-Cre<sup>-</sup> littermate controls (Fig. S3H-J). Moreover, we did not observe defects in anti-viral T cells responses nor was there a difference in virus-specific IgG2a/2c production (Fig. S3K). Numbers of virus-specific CD8 T cells, IFN- $\gamma$ -producing CD8 T cells, and IFN- $\gamma$ -producing CD4 T cells were not affected by the lack of IL-27ra in granzyme B expressing cells (Fig. S3L-N). Together, these results indicate that IL-27R signaling in granzyme B expressing cells is not required for control of persistent LCMV infection.

Since our findings indicated that both B cell- and effector CD8 T cell-specific IL-27R signaling are not required to control LCMV viremia, we further tested whether IL-27R signaling in all T cells was required for protection against persistent LCMV infection. By crossing IL-27ra<sup>fl/fl</sup> mice with CD4-Cre mice (CD4-Cre<sup>+/-</sup>/IL-27ra<sup>fl/fl</sup>), we generated conditional knockout mice with IL-27ra deletion in both CD4 and CD8 T cells. Deletion efficiency and specificity were verified by flow cytometry analyses of splenocytes at day 9 and 40 p.i. Our findings revealed that IL-27ra expression was specifically reduced in CD8 and CD4 T cells in CD4-Cre/IL-27ra<sup>fl/fl</sup> mice at both time points when B cells, DCs and macrophages displayed WT levels of IL-27Ra (Fig. S3O-P). Notably, CD4-Cre<sup>+/-</sup>/IL-27ra<sup>fl/fl</sup> mice were unable to clear LCMV Cl-13 from serum at various times p.i. and lung, spleen, kidney, brain and liver 120 days p.i. (Fig. 3A-F). Analysis of anti-viral T cell responses revealed a small but significant reduction in GP33 tetramer positive CD8 T cells (Fig. 3G) however, no significant reductions in cytokine production were observed in GP33-specific CD8 T cells (Fig. 3H-I). Analysis of anti-viral CD4 T cells revealed significant reductions in IFN- $\gamma$ -producing CD4 T cells at day 9 p.i. (Fig. 3J). In accordance with MB1-Cre/IL-27p28<sup>fl/fl</sup> mice, CD4-Cre/IL-27ra<sup>fl/fl</sup> mice displayed reduced virus-specific I-A<sup>b</sup> GP<sub>67-77</sub><sup>+</sup> CD4<sup>+</sup> T cells, IFN- $\gamma$ -producing CD4<sup>+</sup> T cells, Tfh cells, and IFN- $\gamma$ <sup>+</sup> IL-21<sup>+</sup> Tfh cells at day 40 p.i. (Fig. 3K-N, Fig. S3Q). Taken together, these data suggest that IL-27p28 is produced by B cells and signals on CD4 T cells to promote expansion and functional responses of virus-specific CD4 T cells and Tfh cell



differentiation and function at later times post-LCMV infection that result in control of persistent LCMV infection. In line with the observed defects in Tfh responses, mice lacking IL-27ra in T cells also displayed reduced LCMV-specific IgG2a/2c but not IgG1 titers (Fig. S3R-S).

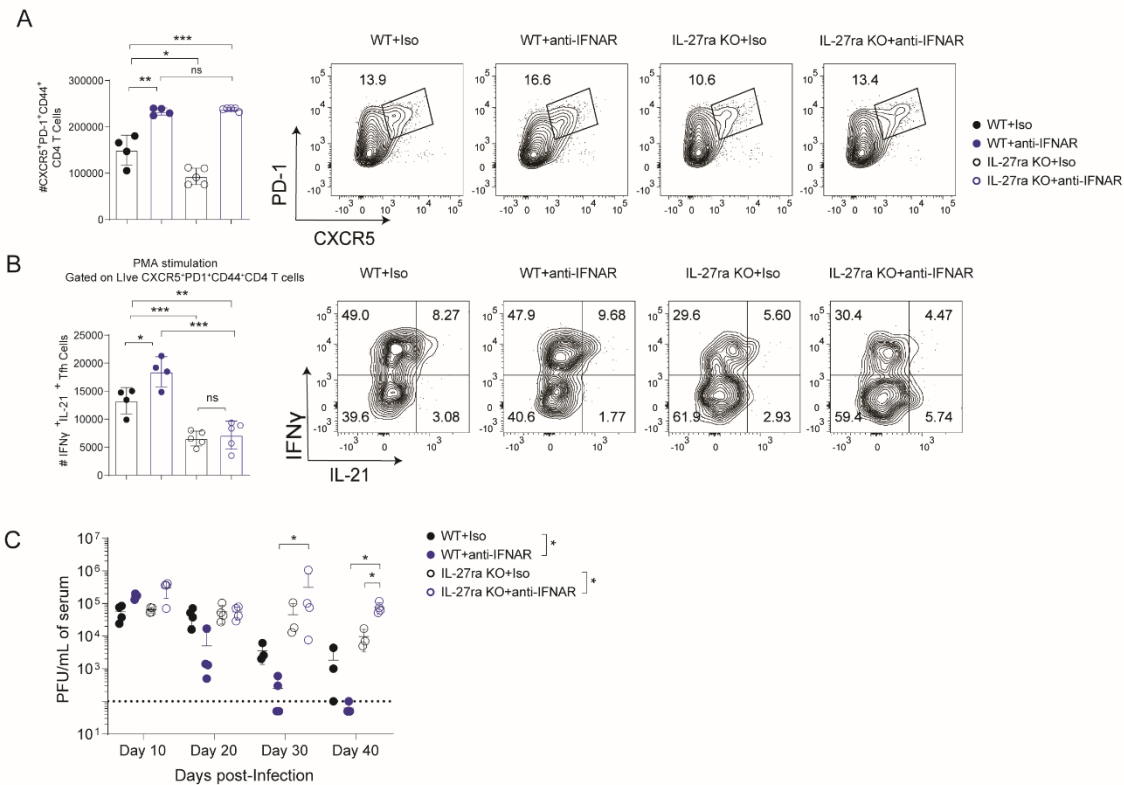


**Figure 3. Intrinsic IL-27 Signaling to T cells mediates control of persistent LCMV infection**

IL-27ra<sup>flox/flox</sup> (WT) and CD4-Cre/IL-27ra<sup>flox/flox</sup> mice (T cell specific IL-27ra KO) were infected with  $2 \times 10^6$  PFU LCMV Cl-13. A) Serum viral loads were determined throughout infection. At day 120 p.i. viral loads were measured in B) lung C) spleen D) kidney E) brain and F) liver. At day 9 p.i. splenocytes were analyzed by flow cytometry to determine total number of G) H2-Db GP33-41 virus-specific CD8 T cells, H) IFN- $\gamma$ -producing GP33-41 LCMV-specific CD8 T cells, I) polyfunctional IFN- $\gamma$ - and TNF $\alpha$ -producing GP33-41 LCMV-specific CD8 T cells, and J) IFN- $\gamma$ -producing GP61-80 LCMV-specific CD4 T cells. At day 40 p.i. splenocytes were analyzed by flow cytometry to determine the number of K) I-A $\alpha$  GP67-77+ CD4 T cells, L) IFN- $\gamma$ -producing CD4 T cells after GP67-80 peptide stimulation, M) Tfh cells, N) IL-21+IFN- $\gamma$ + Tfh cells after PMA and ionomycin stimulation. Data are representative of three experimental replicates and error bars represent mean  $\pm$  SD from 4-10 mice per group. Statistical analyses of experimental replicates were performed using Mann-Whitney U test (A-F) or Student's two-tailed t test (G-N): not significant (ns),  $P > 0.05$ ; \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

## Type 1 interferon blockade requires IL-27 signaling for enhanced control of persistent LCMV infection.

Our group recently showed that blockade of Type I interferon signaling led to increased numbers of Tfh cells in both WT and IL-27ra knockout (KO) mice during persistent LCMV infection (6-8, 31), thus we further tested whether anti-IFNAR1 immunotherapy is able to improve functions of CD4 T cells and promote viral clearance. We first assessed Tfh numbers and function following anti-IFNAR1 antibody treatment at day 40 p.i.. IL-27ra KO mice treated with anti-IFNAR1 displayed similar numbers of Tfh cells to WT controls that received anti-IFNAR1 antibody (Fig. 4A). However, we observed significantly less IFN- $\gamma$ <sup>+</sup>IL-21<sup>+</sup> Tfh cells in IL-27ra KO mice even after anti-IFNAR1 blockade (Fig. 4B, Fig. S4A). Consequently, unlike anti-IFNAR1 blockade in WT mice which leads to hastened control of C1-13 infection (Fig. 4C) (6, 8), anti-IFNAR1 treated IL-27ra KO mice were unable to control the virus and even displayed elevated serum C1-13 titers compared to isotype treated animals at day 40 post-infection (Fig. 4C). Taken together, these findings emphasize the importance of IL-27 for maintaining and enhancing the function of Tfh cells during later stages of LCMV infection.



**Figure 4. IL-27R signaling is required for anti-IFNAR immunotherapy to promote IL-21 production by Tfh cells and viral clearance**

C57BL/6 WT and IL-27ra KO mice were treated with isotype control or anti-IFNAR1 antibody 1 day prior to LCMV-C113. At day 40 p.i. spleens were analyzed by flow cytometry to determine the number of A) Tfh cells and B) IL-21+IFN- $\gamma$ <sup>+</sup> Tfh cells after PMA and ionomycin stimulation. C) Serum viral loads were measured at different time points. Data are representative of three experimental replicates and error bars represent mean  $\pm$  SD from 4-5 mice per group. For panels A-B, statistical comparisons of experimental groups were performed using Student's

two-tailed t test; for panel C experimental groups were compared by two-way ANOVA (group-level) and Mann-Whitney U test (specific time points): not significant (ns),  $P > 0.05$ ; \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ .

Persistent LCMV infection is maintained for several months while the presence and optimal functions of both virus-specific T and B cells are required to purge the infection. The role of Tfh cells in driving production of LCMV-specific antibodies required for viral clearance has recently been established (16). IL-27 is an immunostimulatory cytokine that drives expansion of virus-specific stem-like CXCR5<sup>+</sup>Tim3<sup>-</sup> CD8 T cells, accumulation of virus-specific CD4 T cells and promotes IgG2a/2c class switch (18, 31, 32). Given that IL-27 can influence multiple cell types to drive viral clearance, it is crucial to understand how cell-specific IL-27 signaling mediates control of persistent viral infection.

In this study, we identified B cells as an essential source of IL-27 and provide insights into how B cell-derived IL-27 drives antiviral immunity and antibody response by regulating virus-specific CD4 T cells and Tfh cell functions during late stage of persistent viral infection. During persistent LCMV infection, B cell-derived IL-27 promotes the accumulation and function of both virus-specific CD4 T cells and Tfh cells. Importantly, our data suggests that IL-27R signaling on Tfh cells drives IFN- $\gamma$  and IL-21 production at later stages of chronic viral infection which in turn results in generation of antiviral antibodies that keeps the viral titers in check during the phase of antiviral CD8 T cell exhaustion. Intriguingly, although myeloid cells comprise the majority of IL-27p28<sup>+</sup> splenocytes during Clone 13 infection, they are unable to compensate for the loss of B-cell-derived IL-27 suggesting a possible requirement for IL-27 specifically in areas where B cell-T cell interactions occur. Given that IL-27p28 can be secreted without EB13 and modulate immune responses (26), we cannot definitively conclude that IL-27 (p28 + EB13) is the active cytokine required. However, we find that activated B cells also produce significant levels of EB13 (Fig. S1F), suggesting that IL-27 is the active cytokine in our system.

Given there are multiple sources of IL-27 during persistent LCMV infection, we speculate that the spatial-temporal production of IL-27 serves distinct non-redundant roles. Early IL-27 during persistent LCMV infection, which is produced mainly by DCs and myeloid cells, was shown to signal on both innate and adaptive immune cells to mediate early viral containment as IL-27ra KO mice showed higher viral titer than WT mice at day 9 p.i.(19). In contrast, B cell-derived IL-27 did not play a role in early viral containment (Fig. 2A), but instead we observed reduction of Tfh and Tfh-derived IL-21 and IFN- $\gamma$  in the absence of B cell-derived IL-27 at later times post-infection (Fig. 2N and S2J). Given that stable cognate interactions between T-B cells are required for Tfh to drive differentiation of GC B cells into plasma and memory B cells (33, 34), our finding suggests that while cognate Tfh provides IL-21 to support B cell differentiation, local B cell produced IL-27 maintains Tfh functions.

Overall, we demonstrate the ability of B cells to produce IL-27p28, which orchestrates a variety of important T cell functions that lead to optimal antiviral antibody responses during persistent LCMV infection. Given these results, we hypothesize that B cell-derived IL-27 may play a key role in regulating immunity in other diseases where T cells are important for pathogenesis. Intriguingly, multiple recent studies have shown that B cells in human tumors' tertiary lymphoid structures (TLS) are associated with a better response to immunotherapy (35-37). Taken together, our study adds to the list of essential cytokines produced by B cells (38) and further underscores the importance of studying B cells and their cytokine production in promoting control of persistent viral infections and possibly cancer.

## Materials and methods

### *Mice*

C57BL/6 wild-type, conditional knockout and germline knockout mice were used for this study. IL-27p28-eGFP mice were generated by Dr. Ross Kedl (University of Colorado) and generously gifted to us by Dr. Ross Kedl and Dr. Zhenming Xu (University of Texas at San Antonio)(39). IL-27p28<sup>flox/flox</sup> were kindly provided by Dr. Li-Fan Lu (University of California San Diego)(40). The generation and characterization of IL-27ra<sup>flox/flox</sup> was described previously (41). CD45.1<sup>+</sup> GP66-77 TCR tg (SMARTA), *Ebi3*<sup>-/-</sup>, *WSX-1*<sup>-/-</sup>, and Cre expressing lines were purchased from Jackson Laboratory. To obtain *Il27ra*<sup>-/-</sup> SMARTA CD45.1<sup>+</sup> mice, *WSX-1*<sup>-/-</sup> mice were crossed to CD45.1<sup>+</sup> SMARTA mice. To generate conditional deletion of IL-27p28<sup>flox/flox</sup> and IL-27ra<sup>flox/flox</sup> mice were crossed to Cre expressing lines obtained from Jackson Laboratory: Mb1-Cre to target B cells, granzyme-B-Cre to target activated T cells, CD4-Cre to target T cells. All mice were bred and maintained under specific pathogen-free conditions.

### *In vivo* LCMV infection

7-12 week-old mice were injected intravenously with 2x10<sup>6</sup> focus forming units (FFU) LCMV Clone 13. Viral titers were assessed by focus assays using Vero cell monolayers as previously described (7, 42). All experiments were conducted in accordance with guidelines and approval of the Institutional Animal Care and Use Committee of Scripps Research.

### *Antibody treatments*

For blockade of type I IFN, mice were treated with 1 mg of anti-IFNAR1 antibody intraperitoneally (clone MAR1-5A3; Leinco Technologies) or a mouse IgG1 isotype control (clone MOPC21; Leinco Technologies) 1 day prior to infection.

### *B cell isolation, stimulation, and cytokine production measurement*

Splenic B cells were purified from naïve mice using EasySep™ Mouse B Cell Isolation Kit (STEMCELL™ technologies). Isolated B cells were cultured at 1x10<sup>5</sup> cells/well in a 96-well-plate in complete RPMI containing LPS (3 mg/mL; Invivogen) for 24 h before being stimulated with agonistic anti-CD40 antibody (5 mg/mL; Biolegend) and IL-21 (50 ng/mL; R&D Stems). After 24 h post-stimulation, B cells were harvested, stained with surface markers and viability dye (eBioscience) and analyzed using flow cytometry.

### *Cell staining for flow cytometry*

For surface staining, antibodies for cell surface markers were added to single-cell suspensions prepared from spleens at dilutions 1:200 in PBS supplemented with 2% fetal bovine serum (FBS) and 1 mM EDTA, followed by incubation for 30 min at 4°C. Staining of CXCR5 of Tfh was performed as previously described (7). Staining of CXCR5<sup>+</sup>CD8<sup>+</sup> T cells was performed as previously described (31). Tetramer staining was performed as previously described using MHC tetramers provided by the National Institutes of Health (8). Live/Dead Fixable Dead Cell Stain (Invitrogen) was used to identify live cells. Cells were fixed with 4% paraformaldehyde. Flow cytometric analysis was performed using BD LSR II (Becton Dickinson) and data were analyzed using FlowJo (Tree Star Inc., Ashland, OR).

### *Ex Vivo Cell Stimulation and Intracellular Cytokine Staining*

Splenocytes were stimulated with major histocompatibility complex (MHC) class I-restricted LCMV-GP33–41 (2 µg/ml) or MHC class II-restricted LCMV-GP61–80 peptide (5 µg/ml) for 1 h in the absence of brefeldin A and then 5 h in the presence of brefeldin A (4 µg/ml; Sigma-Aldrich, St. Louis, MO). Cells were fixed and permeabilized with 2% saponin, and intracellular staining was performed with antibodies to IFN-γ (XMG1.2), TNF-α (MP6-XT22), and IL-2 (JES6-5H4). For polyclonal stimulation, single cell suspensions were stimulated with Ebioscience™ Cell Stimulation Cocktail according to the manufacturer's protocol. Intracellular IL-21 staining was performed as previously described (18).

### *LCMV-specific IgG Enzyme-linked immunosorbent assay (ELISA)*

Serum antibody ELISAs were performed as previously described (7). Microplates were coated with baby hamster kidney (BHK) cell lysates infected with LCMV Cl-13. Serial dilutions of serum were carried out and antibody detected by using purified biotin-conjugated anti-mouse IgG, IgG1 or IgG2a (1030-08, 1070-08, 1080-08 Southern Biotech) antibodies. CLARIOstar Plus Microplate Reader was used to quantify the results.

### *LCMV Clone-13 neutralization assay*

LCMV neutralization *in vitro* was determined as previously described (42). Sera were prediluted 10-fold and inactivated at 56°C for 30 min. Serial 2-fold dilutions of sera were incubated 40 FFU LCMV Clone-13 at 37°C for 60 min before being transferred to a 96-well plate containing Vero cells. Neutralization capacities were determined at 16hr post-incubation as the relative reduction of infectious foci.

### *ELISPOT Assay*

Nitrocellulose Millititer Multiscreen plates were coated with BHK cell lysates infected with LCMV Cl-13. Nonspecific binding was blocked with RPMI supplemented with 10% FBS and 1% P/S. Serial 3-fold dilutions of IL-27p28eGFP<sup>+</sup> cells were carried out and plates were incubated overnight at 37°C incubator supplemented with 5% CO<sub>2</sub>. Plates were washed with PBS with 0.1% Tween (PBST) and then incubated with biotinylated anti-mouse IgGγ (Jackson ImmunoResearch) overnight at 4°C. The plates were washed with PBST and incubated with HRP-conjugated avidin-D (Vector) followed by incubation with a 3-amino-9-ethylcarbazole (AEC) substrate. The plates were subsequently washed and air-dried in the dark and then spots were counted.

### *Quantitative Polymerase Chain Reaction (qPCR)*

Total RNA extraction from sorted cells was performed using *RNeasy Plus Mini Kit* (Qiagen; 74134). Isolated RNA was reversetranscribed into cDNA using QuantiTect Reverse Transcription Kit (Qiagen; 205311). cDNA quantification was performed using Fast SYBR™ Green Master Mix (Applied Biosystems™; 4385612). The relative RNA levels were normalized to *Gapdh*. The following primers were used at 300 nM:

*I127p28* F, 5'-CTGTTGCTGCTACCCCTTGCTT-3' and

R, 5' CACTCCTGGCAATCGAGATTC-3'

*Gapdh* F, 5'-TCCCCTCTTCCACCTTCGA-3' and

R, 5'-AGTTGGGATAGGGCCTCTCTT-3.

## QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed using GraphPad Prism 9.0 software (San Diego, CA). R version 4.1.0 was used for RNA-seq analysis. ANOVA, Tukey's post-tests, Student's t-test and Mann-Whitney U test were used to assess differences between experimental groups as indicated in respective figure legends. Statistical significance is displayed as  $*P \leq 0.05$ ,  $**P \leq 0.01$ ,  $***P \leq 0.001$ , and  $****P \leq 0.0001$ .

### *Single-cell RNA sequencing and analysis*

Splenocytes from IL-27-p28-eGFP reporter mice infected with Clone 13 for 1, 9, 25 and 30 days were harvested and live cells sorted into IL-27p28-GFP<sup>+</sup> and IL-27p28-GFP<sup>-</sup> fractions by flow cytometry, then stained using TotalSeq-B cell hashing antibodies (Biolegend) and subjected to 3' single-cell transcriptome library preparation (v3.1, 10X Genomics). Libraries were sequenced on NextSeq 500 (Illumina) to an average depth of 8504 reads per cell. Cellranger version 4.0.0 was used for cell demultiplexing, reads-per-gene counting and hashing barcode counting. Seurat version 4.0.3 was used for cell filtering, sample demultiplexing, clustering, differential expression analysis, dimensionality reduction and plotting (43). R version 4.1.0 was used for Seurat analysis and additional transcriptome analyses (44). Packages ggplot2 and ComplexHeatmap were used for additional plotting. To analyze the expression profile of differentially expressed genes in B cell subsets, bulk RNA-sequencing data from Immgen (GSE109125) were used. Sequencing data have been deposited to GEO under accession GSE186898.

Reagents and resources	Source	Identifier
Antibodies		
PE/Cyanine7 anti-mouse CD8a Antibody	Biolegend	Cat# 100722
PerCP/Cyanine5.5 anti-mouse CD4 Antibody	Biolegend	Cat#116012
APC anti-mouse/human CD44 Antibody	Biolegend	Cat#103012
FITC anti-mouse CD279 (PD-1) Antibody	Biolegend	Cat#135214
Brilliant Violet 421™ anti-mouse IFN-γ Antibody	Biolegend	Cat#505830
PE anti-mouse TNF-α Antibody	Biolegend	Cat#506306
Alexa Fluor® 647 anti-mouse IL-27 p28 Antibody	Biolegend	Cat#516904
PE anti-mouse IL-27 p28 Antibody	Biolegend	Cat#516908
PE/Cyanine7 anti-mouse IL-27 p28 Antibody	Biolegend	Cat#516910
Brilliant Violet 421™ anti-mouse CD138 (Syndecan-1) Antibody	Biolegend	Cat#142508
Brilliant Violet 421™ anti-mouse CD185 (CXCR5) Antibody	Biolegend	Cat#145512
PE anti-mouse CD185 (CXCR5) Antibody	Biolegend	Cat#145504
Recombinant Mouse IL-6 (carrier-free)	Biolegend	Cat#575702

Purified anti-mouse CD40 Antibody	Biolegend	Cat#102802
PE-conjugated anti-mouse EB13 Antibody	R&D Systems	Cat#IC18341P
Alexa Fluor® 647 AffiniPure F(ab') <sub>2</sub> Fragment Goat Anti-Human IgG, Fcγ fragment specific	Jackson Immuno Research Labs	Cat#109-606-170
BV421 mouse anti-Bcl6 clone K112-91	BD	Cat#563363
PE Rat Anti-Mouse IL-27ra Clone 2918 (RUO)	BD	Cat#564337
Alexa Fluor® 647 Mouse Anti-Stat1 (pY701)	BD	Cat#612597
PE Mouse Anti-Stat3 (pY705)	BD	Cat#612569
TotalSeq B0301 Hashtag 1 antibody	Biolegend	155831
TotalSeq B0302 Hashtag 2 antibody	Biolegend	155833
TotalSeq B0303 Hashtag 3 antibody	Biolegend	155835
TotalSeq B0304 Hashtag 4 antibody	Biolegend	155837
TotalSeq B0305 Hashtag 5 antibody	Biolegend	155839
TotalSeq B0306 Hashtag 6 antibody	Biolegend	155841
TotalSeq B0307 Hashtag 7 antibody	Biolegend	155843
TotalSeq B0308 Hashtag 8 antibody	Biolegend	155845
Chemical, Peptides, and Recombinant Proteins		
Protein Transport Inhibitor (Containing Brefeldin A) BD GolgiPlug™	BD	Cat#555029
eBioscience™ Cell Stimulation Cocktail (plus protein transport inhibitors) (500X)	Thermo Fisher Scientific	Cat#00-4975-93
eBio Fix/Perm Diluent	Thermo Fisher Scientific	Cat#00-5223-56
eBioscience™ Fixation/Permeabilization	Thermo Fisher	Cat#00-5123-43



Concentrate	Scientific	
Recombinant Mouse IL-27 (NS0-expressed) Protein	R&D Systems	Cat# 2799-ML-010
Recombinant Mouse IL-21 R Fc Chimera Protein, CF	R&D Systems	596-MR-100
Standard lipopolysaccharide from <i>E. coli</i> K12 strain; TLR4 ligand	Invivogen	tlrl-eklps
<b>Experimental Models: Organisms/Strains</b>		
Mb1-Cre	Jackson Laboratory	Cat#20505
granzyme-B-Cre	Jackson Laboratory	Cat#003734
CD4-Cre	Jackson Laboratory	Cat# 022071
STAT1	Jackson Laboratory	Cat# 012606
Stat3 <sup>fl/fl</sup>	Jackson Laboratory	Cat#016923
SMARTA	Jackson Laboratory	Cat#030450
Ebi3	Jackson Laboratory	Cat# 008691
XCR1	Reiken	Cat# RBRC09929
IL-27eGFP	University of Colorado Anschutz	Ross.Kedl@cuanschutz.edu
IL-27ra <sup>fllox/fllox</sup>	Northwestern University	booki.min@northwestern.edu
IL-27p28 <sup>fllox/fllox</sup>	UCSD	lil034@ucsd.edu
<b>Software and Algorithms</b>		
GraphPad Prism 7	GraphPad Software	<a href="https://www.graphpad.com/">https://www.graphpad.com/</a>
FlowJo 10.4.2	Tree Star	<a href="https://www.flowjo.com/">https://www.flowjo.com/</a>
Cellranger 4.0.0	10X Genomics	<a href="https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger">https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger</a>
Seurat 4.0.3	Satija lab	(43)

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**Author Contributions:** I. P. and J. R. T. designed the project; I. P., J. Z. and Z. H. performed experiments and analyzed data; B.M. contributed reagents and advice; I. P. and J. R. T. wrote the paper; M. B. A. O. and J. R. T. supervised the project; J. R. T. obtained funding; all authors edited the manuscript.

**Competing Interest Statement:** The authors declare no competing interests.

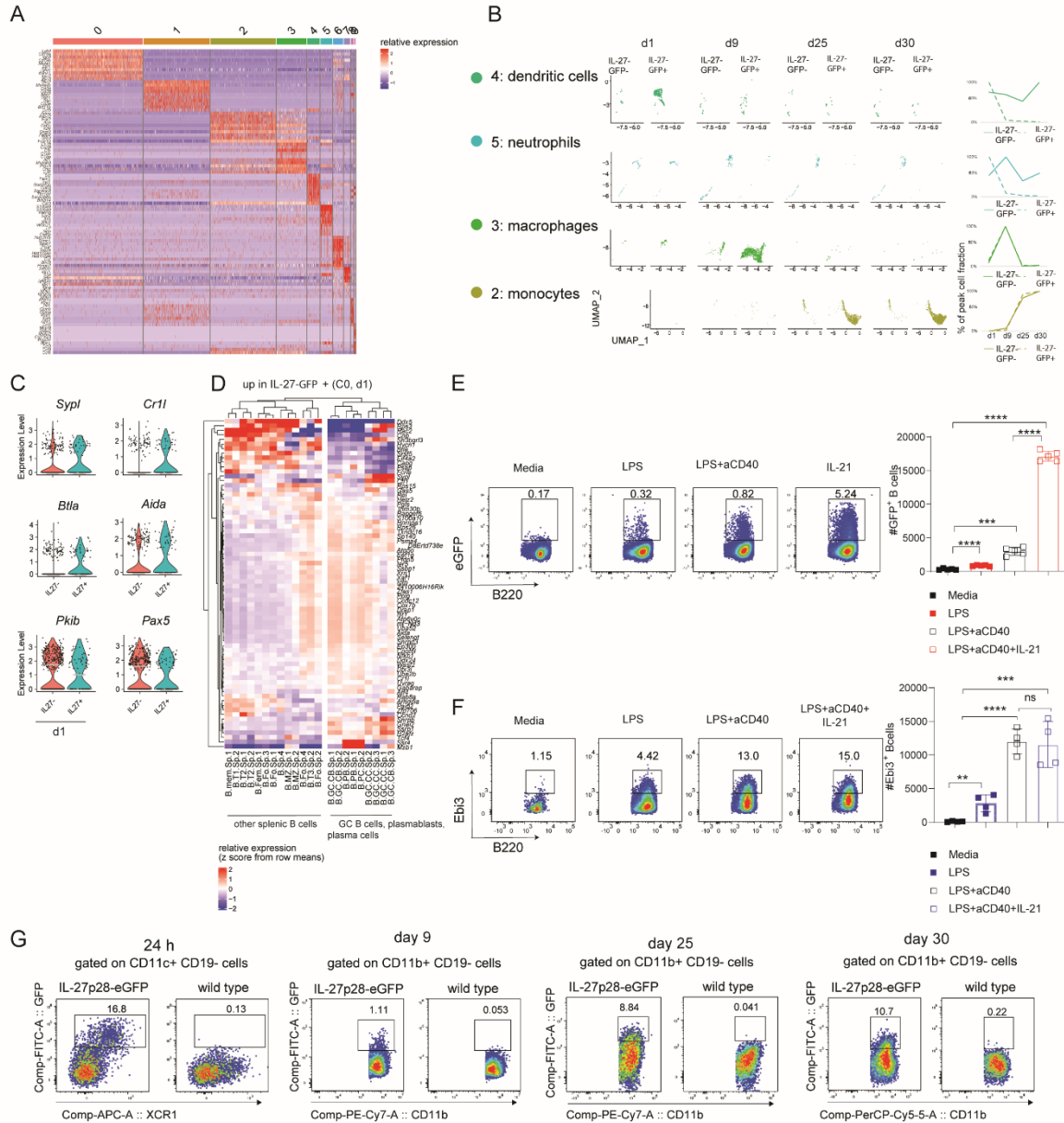
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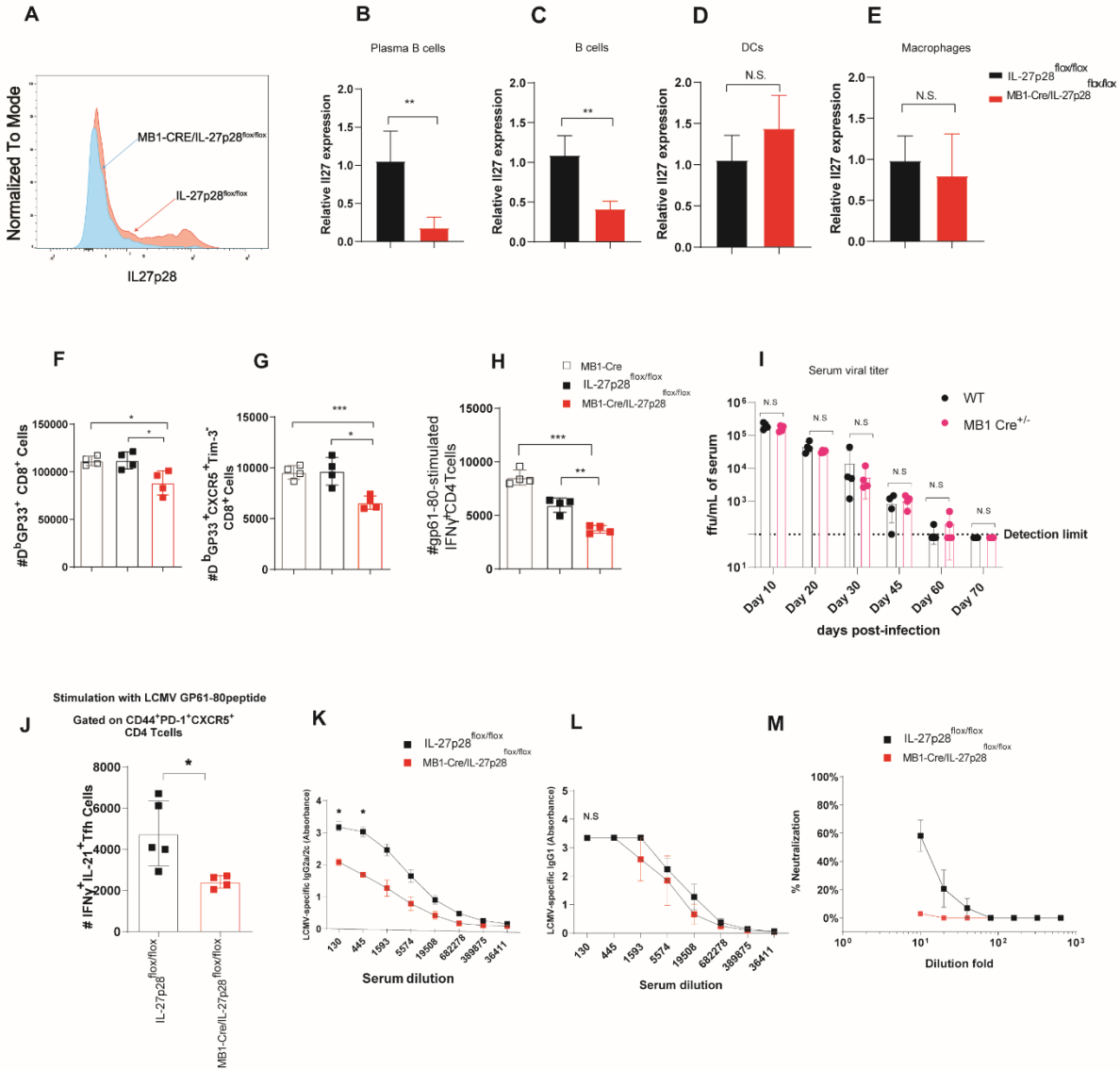
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## Supplementary materials



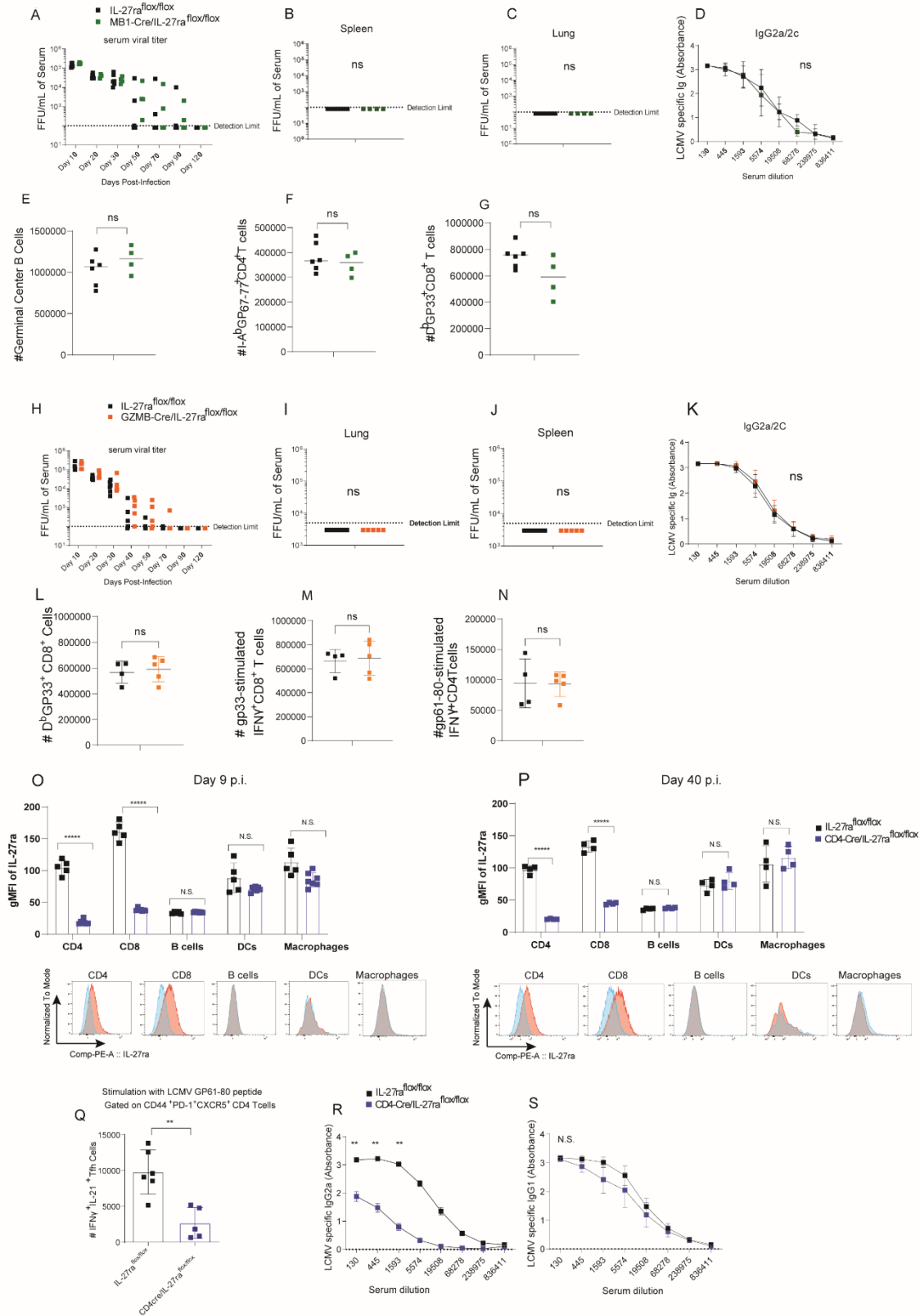
### Figure S1. B cell populations express IL-27

A) Heatmap of marker genes, related to Figure 1A; B) time-resolved distribution of cells in myeloid clusters, analysis equivalent to Figure 1B; C) expression levels of genes differentially expressed between IL-27p28+ and IL-27p28- cells in Cluster 0 at d1 post infection; D) expression levels of genes upregulated in IL-27p28+ cells in Immgen splenic B cells (GSE109125). E) Purified splenic B cells from naïve IL-27p28GFP mice were stimulated with LPS for 24 h, and then with anti-CD40, or anti-CD40+IL-21rm for 24 h. IL-27p28GFP expression was measured by flow cytometry. F) Purified splenic B cells from naïve C57BL/6 WT mice were stimulated with LPS for 24 h, and then with anti-CD40, or anti-CD40+IL-21rm for 24 h. B cells were intracellularly stained with anti-Ebi3 antibody and measured by flow cytometry. G) IL-27p28-eGFP mice and wild type C57BL/6 mice were infected with  $2 \times 10^6$  PFU LCMV Cl-13, eGFP expression on splenic myeloid cells was determined by flow cytometry analyses at day 1, 9, 25 and 30, p.i. Data are representative of two experimental replicates and error bars represent mean  $\pm$  SD from 4-5 mice per group. Statistical analyses of experimental groups were performed using ANOVA and Tukey's multiple comparisons test: not significant (ns),  $P > 0.05$ ; \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$ , \*\*\*\* $P \leq 0.0001$ .



**Figure S2. IL-27p28 expressing B cells are essential for control of persistent LCMV infection**

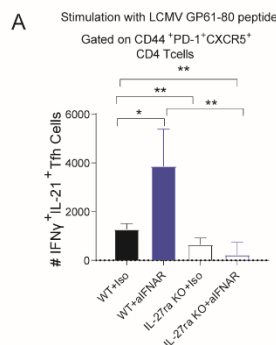
Mice with B cell-specific IL-27p28 deletion were generated by crossing IL-27p28<sup>flox/flox</sup> mice to MB1-cre<sup>+/-</sup> Mice. IL-27p28<sup>flox/flox</sup> and MB1-Cre/ IL-27p28<sup>flox/flox</sup> mice were infected with 2x10<sup>6</sup> PFU LCMV CI-13. At day 9 p.i. A) IL-27p28 express was determined by intracellular FACS staining in MB1-Cre/ IL-27p28<sup>flox/flox</sup> and IL-27p28<sup>flox/flox</sup> mice., B-E) Relative *Ii27* mRNA expression in sorted CD138+ plasma cells, B cells, DCs, and macrophages from MB1-Cre/ IL-27p28<sup>flox/flox</sup> and IL-27p28<sup>flox/flox</sup> mice was determined by qPCR. (F-I) MB1 cre<sup>+/-</sup> mice were infected with 2x10<sup>6</sup> PFU LCMV CI-13 together with IL-27p28<sup>flox/flox</sup> and MB1-cre<sup>+/-</sup> Mice. IL-27p28<sup>flox/flox</sup> and MB1-Cre/ IL-27p28<sup>flox/flox</sup> mice. Splenocytes were analyzed by flow cytometry to determine total number of F) H2-Db GP33-41 virus-specific CD8 T cells, G) virus-specific stem-like CXCR5<sup>+</sup>Tim3<sup>-</sup> CD8 T cells, H) IFN- $\gamma$ -producing GP61-80 LCMV-specific CD4 T cells. I) Serum viral loads of MB1 cre<sup>+/-</sup> and WT mice were determined at different time points p.i. J) At day 40 p.i. splenocytes from LCMV CI-13 infected from MB1-Cre/ IL-27p28<sup>flox/flox</sup> and IL-27p28<sup>flox/flox</sup> mice were analyzed by flow cytometry to determine the number of IL-21+IFN- $\gamma$ + Tfh cells after GP61-80 peptide stimulation. At day 130, serum LCMV-specific K) IgG2a/2c and L) IgG1 antibodies were determined by ELISA. M) Serum LCMV-neutralizing antibodies at day 70 p.i. was determined. Data are representative of two to three experimental replicates and error bars represent mean  $\pm$  SD (panel B) or mean  $\pm$  SEM (C-D) from 4-5 mice per group. Statistical analyses of experimental groups were performed using Student's two-tailed t test: not significant (ns), P > 0.05; \*P  $\leq$  0.05, \*\*P  $\leq$  0.01, \*\*\*P  $\leq$  0.001.



**Figure S3. B cells and Granzyme B<sup>+</sup> T cells are not required for control of persistent LCMV infection**  
 (A-G) IL-27ra<sup>flox/flox</sup> (WT) and MB1-Cre/IL-27ra<sup>flox/flox</sup> mice (B cell specific IL-27ra KO) were infected with 2x10<sup>6</sup> PFU LCMV CI-13: A) serum viral loads were determined at different time points p.i. At day 120 p.i. viral loads



were measured in B) spleen and C) lung. D) LCMV-specific IgG2a/2c antibody was measured in the serum by ELISA. At day 9 p.i., splenocytes were analyzed by flow cytometry to determine numbers of E) germinal center B cells, F) virus-specific CD4 T cells and G) virus GP33-specific CD8 T cells. (H-N) IL-27ra<sup>flox/flox</sup> (WT) and GZMB-Cre/ IL-27ra<sup>flox/flox</sup> (activated T cell specific IL-27ra KO) were infected with 2x10<sup>6</sup> PFU LCMV Cl-13. At day 120 p.i. H) serum viral loads were determined at different time points p.i. At day 120 p.i. viral loads were measured in I) spleen and J) lung. K) LCMV-specific IgG2a/2c antibody was measured in the serum. At day 9 p.i., splenocytes were analyzed by flow cytometry to determine numbers of L) virus-specific CD8 T cells, M) IFN- $\gamma$ -producing GP33-41 LCMV-specific CD8 T cells, and N) IFN- $\gamma$ -producing GP61-80 virus-specific CD4 T cells. (O-S) IL-27ra<sup>flox/flox</sup> and CD4-Cre/ IL-27ra<sup>flox/flox</sup> were infected with 2x10<sup>6</sup> PFU LCMV Cl-13. Splenocytes were stained with IL-27ra antibody and flow cytometry analysis was performed to determine the expression of IL-27ra in CD8 T cells, CD4 T cells, B cells, DCs and macrophages at O) day 9 and P) day 40 p.i. Q) At day 40 p.i. splenocytes were analyzed by flow cytometry to determine the number of IL-21+IFN- $\gamma$ +producing Tfh cells after GP61-80 peptide stimulation. R) At day 120, serum LCMV-specific IgG2a/2c and S) IgG1 antibody were determined by ELISA. Data are representative of two or three experimental replicates and error bars represent mean  $\pm$  SD from 4-5 mice per group. Statistical analyses of experimental groups were performed using Student's two-tailed t test: not significant (ns), P > 0.05; \*P  $\leq$  0.05, \*\*P  $\leq$  0.01.



**Figure S4. IL-27R signaling is required for anti-IFNAR immunotherapy to promote IL-21 production by Tfh cells**

C57BL/6 WT and IL-27ra KO mice were treated with isotype control or anti-IFNAR1 antibody 1 day prior to LCMV-C113 infection. At day 40 p.i. splenocytes were analyzed by flow cytometry to determine A) the number of IL-21+IFN- $\gamma$ + Tfh cells after GP61-80 peptide stimulation. Statistical analyses of experimental groups were performed using one-way ANOVA and Tukey's post-test: not significant (ns), P > 0.05; \*P  $\leq$  0.05, \*\*P  $\leq$  0.01.