Vaccine-elicited CD4 T cells prevent the deletion of antiviral B cells in chronic infection

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Edited by Rafi Ahmed, Emory University, Atlanta, GA, and approved October 8, 2021 (received for review May 3, 2021)

Chronic viral infections subvert protective B cell immunity. An early type I interferon (IFN-I)–driven bias to short-lived plasmablast differentiation leads to clonal deletion, so-called "decimation," of antiviral memory B cells. Therefore, prophylactic countermeasures against decimation remain an unmet need. We show that vaccination-induced CD4 T cells prevented the decimation of naïve and memory B cells in chronically lymphocytic choriomeningitis virus (LCMV)-infected mice. Although these B cell responses were largely T independent when IFN-I was blocked, preexisting T help assured their sustainability under conditions of IFN-I–driven inflammation by instructing a germinal center B cell transcriptional program. Prevention of decimation depended on T cell–intrinsic Bcl6 and Tfh progeny formation. Antigen presentation by B cells, interactions with antigen-specific T helper cells, and costimulation by CD40 and ICOS were also required. Importantly, B cell–mediated virus control averted Th1-driven immunopathology in LCMV-challenged animals with preexisting CD4 T cell immunity. Our findings show that vaccination-induced Tfh cells represent a cornerstone of effective B cell immunity to chronic virus challenge, pointing the way toward more effective B cell–based vaccination against persistent viral diseases.

chronic viral infection | B cell decimation | T cell help | T follicular helper cells | interferon

CD4 T cell induction represents a key goal of vaccination and an important correlate of immunity to diseases of global importance such as tuberculosis (1). Moreover, genetic deficiency in CD4 T cell defense or the loss thereof in AIDS allows the emergence of various microorganisms, resulting in life-threatening opportunistic infections (2, 3). Significant gaps remain, however, in our understanding of how CD4 T cells contribute to protection against chronic viruses. While essential to contain established HIV or cytomegalovirus infection (4–6), the potential role of preexisting CD4 T cell immunity in the prevention of viral persistence remains a matter of debate (7). Observations made in the model of chronic lymphocytic choriomeningitis virus (LCMV) infection of mice indicate that vaccination-induced CD4 T cell immunity, similarly to adoptively transferred virus-specific CD4 T cells, can result in severe immunopathological disease instead of protection (8–10). Th1-biased CD4 T cell memory—a type of CD4 T cell immunity commonly induced by bacterially and virally vectored vaccine delivery systems (11–13)—was found to trigger a pathogenic cytokine storm upon chronic virus challenge, thus questioning the utility of CD4 T cell–targeted vaccination for the prevention of chronic viral infection.

Humoral immune protection against acute viral disease commonly requires a critical titer of preexisting serum antibodies, irrespective of B cell memory (14). As an important exception, recall responses by vaccination-induced memory B (memB) cells have the capacity to prevent hepatitis B virus (HBV) persistence even after serum antibody immunity has waned (15, 16). Similarly, rapid neutralizing antibody (nAb) responses to primary and, importantly, also secondary HCV infection are associated with spontaneous viral clearance (17–20). These findings highlight not only the tremendous potential of B cell–based protection specifically in the context of chronic viral diseases but also the need to better understand its functioning and the requirements for its optimal exploitation in vaccination.

When aiming to harness B cell–based immunity against persistent viral diseases, the subversion of early—and therefore timely—humoral immune responses to these viruses is a major hurdle. This is evident in a delayed and inadequate antibody response to HCV and HIV in man (21–23) and to LCMV in mice (24). Often associated therewith is an accumulation of so-called atypical memB cells, a common observation in persistent microbial diseases such as HIV, hepatitis B and C, malaria, schistosomiasis, and tuberculosis but also in systemic lupus

Significance

Vaccines against persisting viruses such as HIV or hepatitis C virus remain a significant unmet global need. Long-lived memory B cells can be induced by vaccination to form an important pillar of antiviral immunity, yet persisting viruses subvert both primary B cell responses and memory B cell protection. Studying a mouse model of persistent viral infection, we found that CD4 T memory cells averted the deletion of antiviral B cells upon chronic virus challenge. Memory T help promoted germinal center reactions, preventing the B cells' untimely, interferon-induced end differentiation. These observations indicate that B cell–based vaccination should be paired with potent memory CD4 T cell induction for memory B cells to prevail when confronted with a chronic viral intruder.

Author contributions: K.N., Y.I.E., B.F., K.C., M.D., A.-F.M., K.M., T.A.M., M.K., D.S., T.M.B., M.K., I.W., F.G., M.L., D.M., C.G.K., and D.D.P. designed research; K.N., Y.I.E., B.F., K.C., M.D., A.-F.M., K.M., T.A.M., M.K., D.S., T.M.B., M.K., I.W., F.G., and L.B. performed research; L.B. contributed new reagents/analytic tools; K.N., Y.I.E., B.F., K.C., M.D., A.-F.M., K.M., T.A.M., M.K., D.S., T.M.B., M.K., F.G., M.L., D.M., C.G.K., and D.D.P. analyzed data; and K.N., D.S., C.G.K., and D.D.P. wrote the paper.

Competing interest statement: D.D.P. is a founder, shareholder, owner of stock options, and consultant of Hookipa Pharma Inc. commercializing arenavirus-based vector technology. M.K., D.M., and D.D.P. are inventors on patents describing arenavirus-based vector technology.

This article is a PNAS Direct Submission.

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This article contains supporting information online at https://www.pnas.org/content/pnas/suppl/2021/11/11/2108157118.DCSupplemental/pnas.2108157118.sapp.pdf.

Published November 11, 2021.

erythematosus, all of which are characterized by chronic antigenic stimulation (25–32). While atypical memB cells are commonly defined by elevated expression levels of inhibitory receptors and hyporesponsiveness to in vitro stimulation (25, 28, 29, 31, 32), observations in the context of human malaria infection suggest atypical memB cells may represent a population of short-lived antibody-secreting cells (ASCs) and thus may resemble plasmablasts (33). We and others have reported that antiviral B cells, whether naïve or antigen-experienced memB cells, undergo rapid terminal differentiation to short-lived ASCs and near-total clonal deletion at the onset of chronic LCMV infection (34–36). This process, referred to as "B cell decimation," is driven by type I interferon (IFN-I)-induced inflammation and may explain delayed antibody responses to persisting viruses (21–24). Antibody-mediated blockade of the IFN-I receptor (IFNAR) prevented B cell decimation and restored B cell transcription factor profiles of mature B cell stages (34), thus enabling a sustainable germinal center (GC)-based antiviral B cell response. These findings suggested the utility of IFNAR blockade as a therapeutic strategy to counter B cell decimation. The same treatment given to SIV-infected nonhuman primates resulted, however, in the animals' rapid progression to AIDS, illustrating the superordinate importance of IFN-I signaling in antiviral defense (37). Strategies to prevent B cell decimation and to enable effective primary and/or secondary B cell responses to chronic virus challenge remain, therefore, an important unmet need.

CD4 T cell help, notably in the form of follicular T helper cells (Tfh), is essential for GC reactions and thereby for B cell affinity maturation and long-term antibody as well as B cell memory formation (38–40). Unlike protein-based immunization, several viruses have the capacity to trigger early antibody responses in a CD4-independent fashion (41, 42). Accordingly, we have reported that under conditions of IFNAR blockade, thus when B cell decimation was prevented, the early B cell response to chronic LCMV challenge was CD4 independent (34). In contrast to primary B cell responses, the dependence of memB cell recall responses on primary and/or secondary CD4 T cell help remains a subject of controversy (43, 44). Accordingly, the differential availability and quality of memory CD4 T cell help (45, 46) has been identified as a likely confounding variable across several recent studies on memB cell recall responses (47–52).

Here, we investigated the role of vaccination-induced CD4 T cell immunity in supporting primary and secondary B cell responses to chronic viral challenge. We found that early, cognate interactions of antiviral B cells with vaccination-induced CD4 T cells reversed the cellular and molecular hallmarks of B cell decimation, thereby enabling a robust and sustainable antibody response and the formation of B cell memory. In return, effective B cell–mediated control of viral replication prevented immunopathological complications of CD4 recall responses. These observations establish vaccination-induced CD4 T cell memory as an essential pillar of effective B cell immunity against persistent viral challenge.

Results

Preexisting CD4 T Cell Help Prevents Decimation of Naïve and memB Cells upon Chronic Virus Challenge. To study the impact of preexisting CD4 T cell help on B cell responses to chronic LCMV challenge, we immunized mice with a recombinant *Listeria monocytogenes* (LM61) expressing from its transgene the immunodominant LCMV glycoprotein (GP)-derived CD4 T cell epitope GP61-80. Nontransgenic LM was used for controls. Both groups of mice were given monoclonal GP–specific B cells (KL25HL B cells) by adoptive transfer 4 wk later and were challenged with a chronic strain and dose of LCMV (Fig. 1*A*). At 5 d into the LCMV response, splenic GP66-77 (GP66) tetramer–binding CD4 T cells of LM61-immunized mice were significantly more abundant than in LM-vaccinated controls, as expected (Fig. 1*B* and *SI Appendix*, Fig. S1*A*). Intriguingly, however, LM61-immune mice harbored also >20-fold–higher numbers of KL25HL progeny B cells and >200-fold–higher numbers of ASCs (Fig. 1*C* and *SI Appendix*, Fig. S1*B*). Accordingly, they mounted >100-fold–higher LCMV-nAb titers than LM-immunized controls (Fig. 1*D*). Altogether, these findings suggested that preexisting GP-specific CD4 help prevented GP-specific B cell decimation upon chronic viral challenge. When B cell transfer and LCMV challenge were conducted only 9 d after LM61 preimmunization, thus shortly after its clearance (53), GP66-specific CD4 T cell as well as KL25HL B cell responses were similarly augmented (Fig. 1*E*), and this effect was sustained for at least 20 d after chronic virus challenge (Fig. 1 *E*–*G*). Importantly, LM61 immunization also augmented the number of KL25HL B cells with a GC phenotype (GL7+CD95+, Fig. 1 *G* and *H*) and promoted their participation in GC reactions as evident from histological sections (Fig. 1*I*). As both naïve and memB cells are susceptible to decimation (34), we next assessed whether preexisting CD4 T help enables robust memB cell recall responses to chronic viral challenge. We transferred KL25HL cells into LCMV-infected primary recipients, where they underwent robust virus-induced proliferation as evident from uniform and complete carboxyfluorescein succinimidyl ester (CFSE) dilution within only a couple of days (Fig. 1*J* and *SI Appendix*, Fig. S1 *C*–*E*). The resulting population of antigen-experienced $B220^+GL7^-CD138^-$ KL25HL memB cells consisted of isotype-switched (IgM^-IgD^-) as well as unswitched IgM^+IgD^- and IgM^+IgD^+ memB cells (48, 50, 54, 55) (*SI Appendix*, Fig. S1 *D* and *E*) and was sorted for adoptive transfer into either LM61- or LM-immune secondary recipients (Fig. 1*J*). At 7 d after LCMV challenge, substantial progeny populations of KL25HL B cells and ASCs were recovered from LM61-immune mice, while KL25HL memB cells in LM-immune control recipients were largely decimated (Fig. 1*K* and *SI Appendix*, Fig. S1*F*). This effect translated into ∼100-fold–higher LCMV-nAb and GP-binding antibody responses by memB cell progeny on day seven (Fig. 1 *L* and *M*), which at this early time point of analysis were mostly found outside B cell follicles (Fig. 1*N*). Altogether, these observations indicated that preexisting CD4 T help prevented the decimation of both naïve and memB cells. Analogous effects on KL25HL B cell and antibody responses were observed when GP66-specific T cell receptor (TCR)-transgenic CD4 T cells (SM cells) from naïve donor animals were engrafted in numbers to approximate LM61-induced CD4 T cell responses (Fig. 1 *O*–*Q*). RAG-deficient SM cells were similarly effective (*SI Appendix*, Fig. S1 *G* and *H*), indicating that truly naïve CD4 T cells, when provided in sufficient numbers (56), can help B cells and prevent their decimation.

LM61-Induced CD4 Responses Are Th1-Biased but Comprise a Tfh-Like Population That Expands upon Recall. The prevention of B cell decimation by LM61-induced CD4 T cells was surprising in light of the reportedly pronounced Th1 bias of these responses (8), prompting us to investigate the heterogeneity of LM61-induced CD4 T cell responses by single-cell RNA sequencing (scRNAseq). We profiled the transcriptome of GP66 tetramer–binding CD4 T cells, which we collected 9 d after LM61 preimmunization (d9, Fig. 2*A*) and 5 d following subsequent KL25HL cell transfer and LCMV challenge (d 9 + 5, Fig. 2*A*). A principle component analysis and hierarchical clustering evidenced five and four main cell clusters on d 9 and d 9 + 5, respectively (Fig. 2 *B*, *C*, *F*, and *G*). As expected, three Th1-like clusters dominated the response at both time points, but 7 to 8% of cells fell into a fourth cluster, which by comparison to published scRNAseq data (57), exhibited a Tfh gene expression

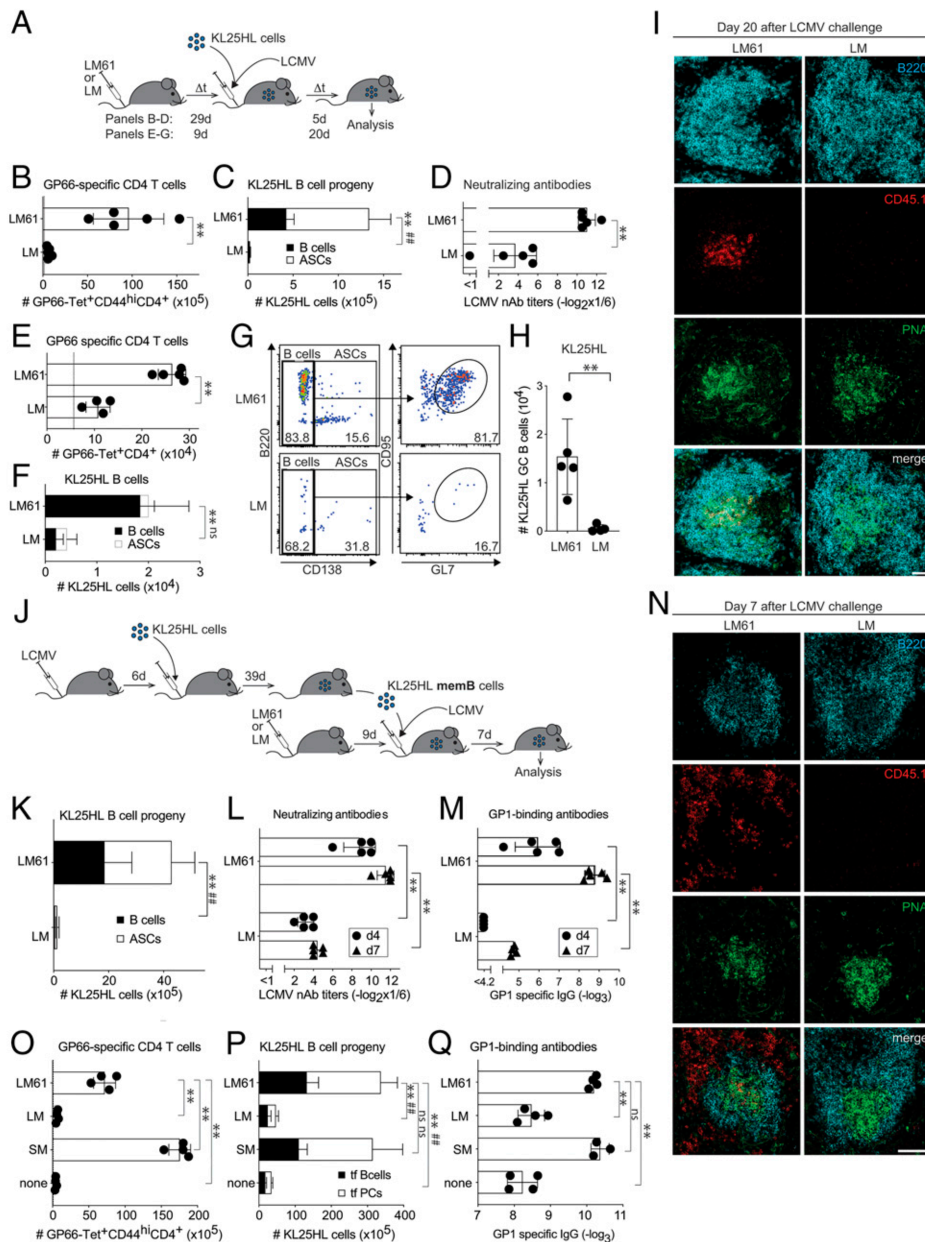

Fig. 1. Preexisting CD4 T cell help prevents decimation of naïve and memB cells upon chronic virus challenge. (*A–I*) Schematic of the experimental design to panels *B–I*. Recipients were preimmunized with LM61 or LM. 29 (*B–D*) or 9 d later (*E–I*), they received KL25 B cells transfer and LCMV infection. Immune responses were analyzed another 5 (*B–D*) or 20 d (*E–I*) later. GP66-Tet$^+$ CD4 T cells (*B* and *E*) and KL25HL B cell progeny (*C* and *F*) were enumerated, and nAb titers in serum (*D*) were determined. Exemplary flow cytometry (FACS) plots analyzing KL25HL B cell progeny are shown in (*G*). For details on flow cytometry gating, reference *SI Appendix*, Fig. S1 *A* and *B*. GL7$^+$CD95$^+$ KL25HL GC B cells are enumerated in (*H*). KL25HL B cell progeny (CD45.1+) inside GCs (PNA) of splenic B cell follicles (B220) were visualized by histology (*I*). (Scale bars, 100 μm.) The dotted line in (*E*) indicates the technical detection limit as determined using an irrelevant tetramer (*SI Appendix*, Fig. S1*A*). (*J–N*) Schematic of the experimental layout to panels *K–N*. KL25HL cells were adoptively transferred into LCMV-infected primary recipients (200 plaque forming units intravenous). Progeny memB cells were purified from spleen 39 d later (reference *SI Appendix*, Fig. S1 *C* and *D*) and were adoptively transferred into LM61- or LM-preimmunized secondary recipients, followed by LCMV challenge. KL25HL memB cell progeny were analyzed 7 d later (*K*), and neutralizing (*L*) and GP-1–binding (*M*) antibody titers were determined 4 and 7 d later. Histological spleen sections (*N*) localized KL25HL memB cell progeny (CD45.1$^+$) mostly outside B cell follicles (B220) and GCs. (PNA; Scale bars, 100 μm.) (*O–Q*) SM CD4 T cells were adoptively transferred to recipients 1 d prior to KL25HL cell transfer and LCMV challenge. Instead of SM transfer, controls were LMgp61 preimmunized (LM61), LM preimmunized (LM), or received neither CD4 T cell transfer nor preimmunization (none). GP66-Tet–binding CD4 T cells (*O*), KL25HL B cell progeny (*P*), and GP1-binding antibodies (*Q*) were analyzed 5 d after LCMV challenge. Symbols and bars represent means ± SD. Number of biological replicates (n) = 5 (*B–D* and *K–N*), n = 4 to 5 (*E–I*), and n = 4 (*O–Q*), Number of independent experiments (N) = 2 to 3. Unpaired two-tailed Student's t test (*B*, *D*, *E*, and *H*), two-way ANOVA with Bonferroni's (*C*, *F*, and *K–M*) or Dunnett's (*P*) posttest for multiple comparison, and ordinary one-way ANOVA with Dunnett's posttest for multiple comparisons (*O* and *Q*). ns: not significant; **, $^{\#\#}P < 0.01$; when used next to each other ** compares B cells, and ## compares ASCs.

signature (Fig. 2 *D*, *E*, *H*, and *I* and *SI Appendix*, Fig. S2 *A* and *B*). This Tfh cell cluster was characterized by elevated messenger RNA(mRNA) levels of the master transcriptional regulators *Bcl6*, *Tcf1* (encoded by the *Tcf7* locus) and *Id3*, and of Tfh hallmark genes such as *Cxcr5* and *Il21* (Fig. 2 *E* and *I* and *SI Appendix*, Fig. S2 *A* and *B*). In contrast, the expression of Th1-related genes such as *Tbx21* (encoding T-bet) and *Selplg* (encoding PSGL1) was lower in these cells. A fifth yet very minor Treg-like subset was only detected on day nine.

When analyzed by flow cytometry on day nine, vaccination with LM61 induced a population of ∼1 to 2% GP66-Tet–binding CD44^{hi} cells (Fig. 2 *J–L*). Within this population, a small but distinct subpopulation of cells coexpressed the Tfh markers CXCR5 and PD-1, mostly in combination with ICOS (Fig. 2 *J–L*). The GP66-specific recall response to LCMV on d 9 + 5 contained ∼15 to 20% CXCR5$^+$PD-1$^+$ cells (Fig. 2 *J–L*). In keeping with a persisting Th1-bias, this proportion of Tfh-like cells was lower than in the primary GP66-specific response of LM control–immunized animals (∼40 to 60%, Fig. 2*L*). Still, the total number of GP66-specific CD4 T cells in LM61-preimmunized mice on d 9 + 5 exceeded the primary response of LM control–vaccinated animals ∼15-fold. By consequence, the CXCR5$^+$PD-1$^+$ Tfh–like subpopulation was also ∼5-fold more abundant than in the respective population of LM-immune controls (Fig. 2*L*). When mice were vaccinated with LM61 26 d prior to LCMV challenge and analyzed 5 d later (d 26 + 5), GP66-specific Tfh recall responses were comparable to animals analyzed on d 9 + 5, both in magnitude and phenotype (Fig. 2 *J–L*). Experiments in IL-21^{gfp} reporter mice demonstrated that approximately two-thirds of the Tfh-like (CXCR5$^+$) GP66-specific cells expressed IL-21 (Fig. 2*M* and *SI Appendix*, Fig. S2*C*), an important Tfh signature cytokine

https://doi.org/10.1073/pnas.2108157118

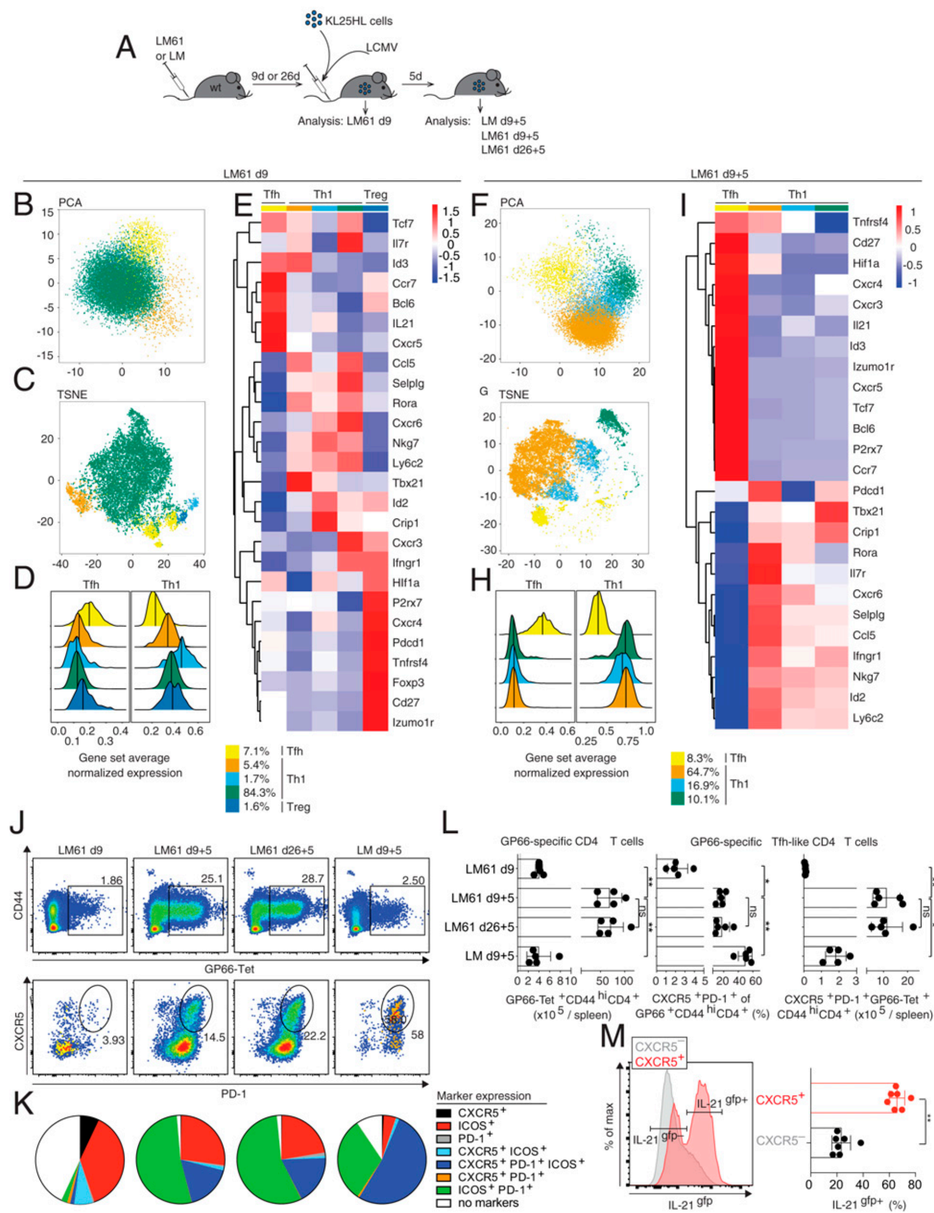

Fig. 2. LM61-induced CD4 responses are Th1 biased but comprise a Tfh-like population that expands upon recall. (*A*) Schematic of the experimental design to panels *B–L*. Mice were preimmunized with LM61 or LM on day 0, followed by KL25HL cell transfer and LCMV challenge on day 9. GP66-Tet+ CD4 T cell responses in spleen were analyzed on day 9 and day 9 + 5 of the experiment. (*B–I*) scRNAseq data from cells recovered on day 9 (*B–E*; 14,459 cells from two mice) or day 9 + 5 (*F–I*; 13,478 cells from two mice). PCA (*B* and *F*) and t-distributed stochastic neighbor embedding (*C* and *G*), normalized expression of gene sets (57) (*D* and *H*) and expression levels of select Tfh and Th1 hallmark genes (*E* and *I*) are shown. (*J–L*) Representative FACS plots of the indicated immunization groups and time points show CD44^{hi}GP66-Tet^{+} CD4 T cells (pregated as shown in *SI Appendix*, Fig. S1*A*) and these cells' expression of CXCR5 and PD-1 (*J*). Coexpression of these markers together with ICOS is displayed in (*K*). Total CD44^{hi}GP66-Tet^{+} CD4 T cells, the percentage thereof expressing both CXCR5 and PD-1, and the total number of the latter subset are displayed in (*L*, *Left* to *Right*). (*M*) We immunized IL-21^{gfp} reporter mice with LM61 and challenged them with LCMV as outlined in (*A*), and on d9 + 5 compared the IL-21 reporting by the CXCR5^{+} Tfh-like and CXCR5^{-} Th1-like subsets of CD44^{hi}GP66-Tet^{+} CD4 T cells. The percentage of IL-21^{gfp}-positive cells in each subset is indicated in the bar chart. Numbers in exemplary FACS plots indicate percentages of gated cells. Symbols and bars represent means± SD, Number of biological replicates (n) = 5 (*J–K*) and n = 7 (*M*). Number of independent experiments (N) = 1 (*B–I*) and n = 2 (*J–M*). Ordinary one-way ANOVA with Tukey's posttest for multiple comparisons (*L*), unpaired two-tailed Student's t test (*M*). $^{**}P < 0.01$.

that is not normally expressed by Th1 cells (58). Altogether, these observations indicated that the LM61-induced GP66 response was Th1 biased but also contained a subpopulation of Tfh-differentiated CD4 T cells, which expanded upon LCMV challenge.

Bcl6-Dependent CD4 Response and Th2- but Not Th1-Polarized SM CD4 T Cells Prevent B Cell Decimation. We aimed to establish a functional link between CD4 T cell subset differentiation and the cells' ability to prevent B cell decimation. To assess whether Th1 differentiation was required for the prevention of B cell decimation, we performed adoptive transfer experiments with SM cells lacking T-bet (SMxTbx21$^{-/-}$), the master transcriptional regulator of Th1 differentiation. These cells' expansion upon LCMV challenge and, importantly, also the expansion of cotransferred KL25HL B cells was comparable to control mice receiving T-bet–sufficient SM cells (Fig. 3 *A* and *B*). This finding indicated that T-bet–mediated Th1 differentiation was not required for CD4 T cells to prevent B cell decimation.

To test whether the Tfh-like subpopulation in the LM61-induced GP66 response was required for the prevention of B cell decimation, we exploited Bcl6$^{fl/fl}$CD4^{Cre} mice with a T cell–specific deficiency in Bcl6, the master transcriptional regulator of Tfh differentiation (59–61). When immunized with LM61 and subsequently challenged with LCMV, Bcl6$^{fl/fl}$CD4^{Cre} mice mounted a CD4 recall response that significantly exceeded the primary response of LM control–vaccinated wild-type (wt) and Bcl6$^{fl/fl}$CD4^{Cre} mice (Fig. 3*C*). However, as expected, the number of CXCR5^{+}PD-1^{+} Tfh–like CD4 T cells in Bcl6$^{fl/fl}$ CD4^{Cre} mice did not exceed the respective primary responses in LM-immune controls (Fig. 3*D*). Consistent with the lack of Tfh cells in Bcl6$^{fl/fl}$CD4^{Cre} mice, there was an almost complete absence of the T-bet$^{low/neg}$CXCR5^{+} subpopulation (Fig. 3 *F* and *G*), which in control mice expressed elevated levels of Bcl6 (Fig. 3*H*). Most importantly, Bcl6-deficient T help failed to prevent B cell decimation. Unlike in wt mice where LM61 preimmunization augmented KL25HL B cell and ASC progeny ~10- and ~50-fold, respectively, immunization of Bcl6$^{fl/fl}$ CD4^{Cre} mice failed to exert any discernible effect on KL25HL B cell progeny (Fig. 3*E*).

To formally determine whether bona fide Th1 cells have the capacity to prevent KL25HL B cell decimation we subjected

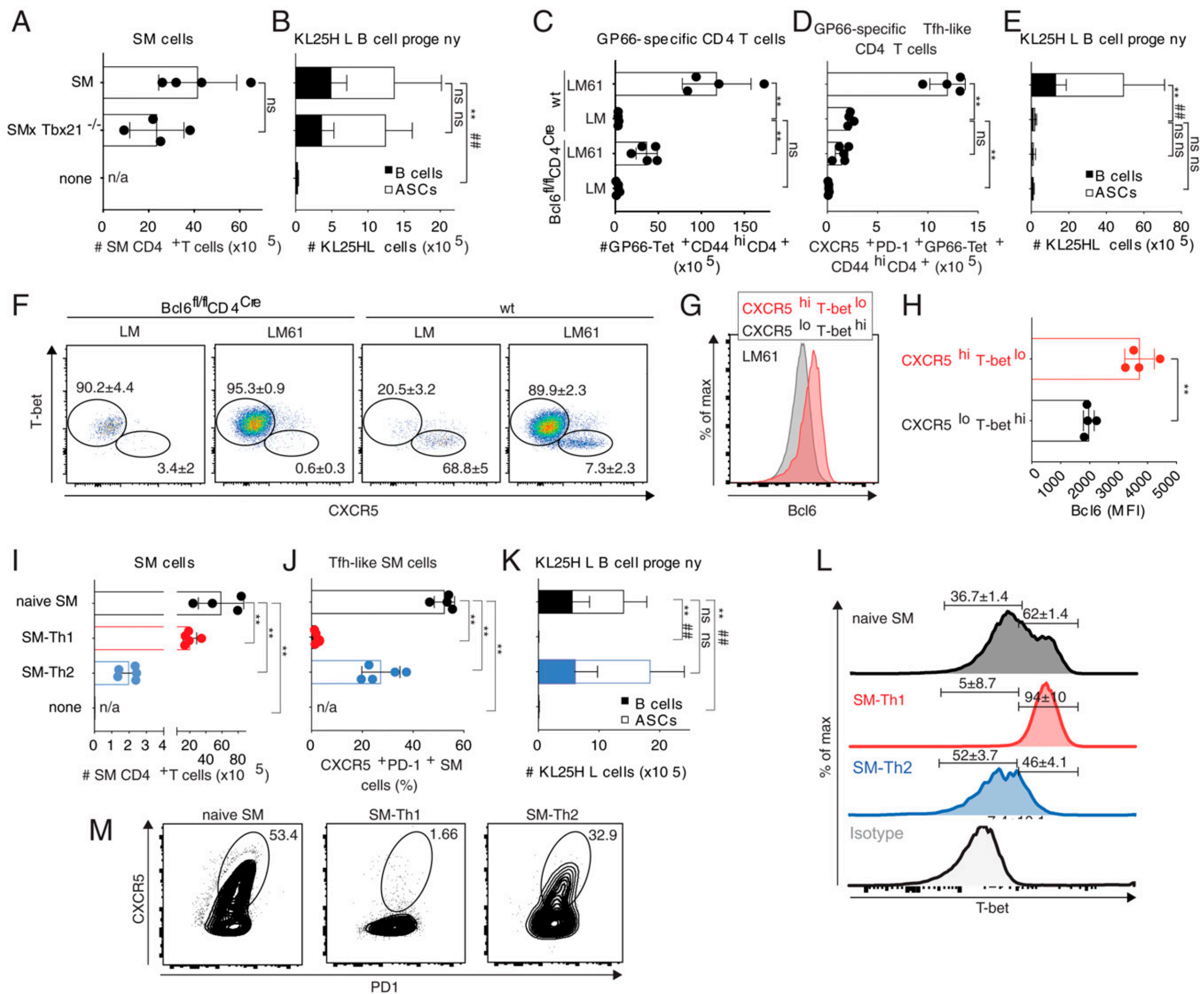

Fig. 3. Bcl6-dependent CD4 response and Th2- but not Th1-polarized SM CD4 T cells prevent B cell decimation. (*A* and *B*) On day −1 we transferred either T-bet–sufficient or T-bet–deficient SM CD4 T cells (SMxTbx21$^{-/-}$) to naïve recipients, followed by KL25HL cell transfer and LCMV challenge on d 0. Controls were left without CD4 T cell transfer. After 5 d, we enumerated the progeny of adoptively transferred CD4 T cells (*A*) and KL25HL B cells (*B*) in spleen. (*C*–*H*) CD4^{Cre}Bcl6^{fl} mice and wt controls were preimmunized with either LM61 or LM on day −9, followed by KL25HL cell transfer and LCMV challenge on d 0. On d 5, we determined CD44^{hi}GP66-Tet^{+} CD4 T cells (*C*), the CXCR5^{+}PD-1^{+} Tfh subset comprised therein (*D*), as well as KL25HL cell progeny (*E*) in spleen. (*F*) Exemplary FACS plots characterizing T-bet^{hi}CXCR5^{lo} Th1 and T-bet$^{low/neg}$CXCR5^{hi} Tfh cell subsets among the CD44^{hi}GP66-Tet^{+} CD4 T cells enumerated in *C*. Bcl6 levels of the aforementioned Th1 and Tfh cell subsets in LM61-preimmunized wt control mice are displayed (*G*) and quantified (*H*). (*I*–*M*) SM cells were polarized to either a Th1 (SM-Th1) or a Th2 phenotype (SM-Th2) using the appropriate in vitro culture conditions (*SI Appendix*, Fig. S3) and were adoptively transferred to syngeneic recipients on d −1. Control mice were either given naïve SM cells or were left without SM cell transfer ("none"). On d 0, all mice were given KL25HL cells and were challenged with LCMV. We analyzed the expanded SM cells (*I*), Tfh-differentiated CXCR5+PD-1+ SM cells (*J*), and KL25HL progeny (*K*) from spleen on d 5. T-bet expression levels (*L*) of transferred SM cells including an isotype control stain as well as exemplary FACS plot of CXCR5 and PD-1 expression are shown (*M*). Percentage values in *L* are shown as mean ± SD. Numbers in exemplary FACS plots indicate the mean ± SD (*F*) or the percentage of gated cells (*M*). Symbols and bars represent means ± SD, number of biological replicates (n) = 4 (*A* and *B*) and n = 4 to 5 (*C*–*M*). Number of independent experiments (N) = 2 (*A*–*M*). Unpaired two-tailed Student's t test (*A* and *H*), two-way ANOVA with Dunnett's posttest for multiple comparison (*B*, *E*, and *K*). Ordinary one-way ANOVA with Dunnett's posttest for multiple comparisons (*C*, *D*, *I*, and *J*). ns: not significant; **, $^{##}P < 0.01$; when used next to each other, **compares B cells, and $^{##}$compares ASCs.

SM CD4 T cells to two consecutive rounds of in vitro activation and polarization to either a Th1 or a Th2 phenotype (SM-Th1, SM-Th2; *SI Appendix*, Fig. S3). When transferred into naïve recipients alongside with KL25HL B cells and challenged with LCMV, SM-Th2 cells expanded substantially less than SM-Th1 cells (Fig. 3*I*). Yet, SM-Th2 but not SM-Th1 cells formed a sizeable population of CXCR5+PD-1+ Tfh–like progeny (Fig. 3 *J* and *M*), and they acquired only low to intermediate T-bet levels, whereas SM-Th1 cells were uniformly T-bet^{hi}, as expected (Fig. 3*L*). In keeping with superior plasticity and the resulting Tfh phenotype, SM-Th2 but not SM-Th1 cells prevented the decimation of KL25HL B cells (Fig. 3*K*).

Altogether, these observations support the concept that the LM61-induced CD4 T cell response comprised a Bcl6-dependent Tfh-like population of CXCR5^{+}PD-1^{+}T-bet$^{low/neg}$ CD4 T cells, which was accountable for the prevention of B cell decimation upon LCMV recall.

https://doi.org/10.1073/pnas.2108157118

T Help Prevents B Cell Decimation in an Antigen-Specific and MHC Class II–Dependent Manner. Antigen-specific but also nonspecific bystander interactions between T and B cells can contribute to humoral immune responses (62). To test whether activated CD4 T cells prevent B cell decimation in a bystander fashion, we transferred either vesicular stomatitis virus (VSV)-specific TCR-transgenic CD4 T cells (L7 cells) or SM cells, followed by KL25HL B cell transfer and coinfection with VSV and LCMV (Fig. 4*A*). L7 cells expanded vigorously and secreted cytokines upon peptide restimulation (Fig. 4*B*), a response that equaled or exceeded the response of SM cells (Fig. 4*C*). Still, the KL25HL B cell response in L7 cell recipients did not exceed the one of control recipients without CD4 T cell transfer, indicating that bystander L7 T help failed to prevent B cell decimation (Fig. 4*D*).

To investigate whether KL25HL B cells were juxtaposed to virus-specific SM CD4 T cells, likely reflecting cellular contacts, we analyzed histological sections of spleens prepared 24 and 48 h after LCMV challenge (*SI Appendix*, Fig. S4 *A–D*). SM cells and KL25HL cells were frequently juxtaposed (Fig. 4*E*). A computational assessment confirmed that the juxtaposition ratio of KL25HL cells with virus-specific SM cells in the T–B border area exceeded the same B cells' juxtaposition ratio with polyclonal endogenous and thus mostly virus-unspecific CD4 T cells (Fig. 4*F*), altogether suggesting early cognate T–B cell interactions.

T help induced by LM61 immunization targets the same epitope as adoptively transferred SM cells. To determine whether another antiviral CD4 T cell specificity could prevent B cell decimation similar to GP61-specific SM cells, we assessed the effect of adoptively transferred LCMV nucleoprotein (NP)-specific TCR-transgenic CD4 T cells (NIP cells). NIP cells are predicted to provide help to KL25HL B cells that capture entire LCMV particles and present virus-internal NP fragments on MHC class II (MHC-II) (63). When challenged with LCMV, NIP cells expanded somewhat less than SM cells (Fig. 4*G*). Nevertheless, they were at least partially effective in preventing B cell decimation (Fig. 4*H*).

We next assessed whether the prevention of B cell decimation required direct antigen presentation by the respective antiviral B cells (64). LM61 preimmunization (Fig. 4*I*) prevented the decimation of MHC-II–sufficient but not of MHC-II–deficient KL25HL cells (Fig. 4*J*). In contrast, MHC-II–deficient KL25HL cells expanded vigorously when LCMV challenge was conducted in nondecimating IFNAR$^{-/-}$ hosts (Fig. 4*J*), confirming their intrinsic ability to mount T-independent responses to LCMV (34). Taken together, these observations indicated that the prevention of B cell decimation by preexisting T help relies on cellular interactions that involve MHC-II on the responding B cell.

T Help Prevents B Cell Decimation in a CD40- and ICOS-Dependent Manner. Since CD4 T cells provide help to B cells in the form of costimulatory receptor engagement as well as cytokines, we examined these signals in the context of T help–mediated prevention of B cell decimation. ICOS blockade during LCMV challenge did not interfere with the expansion of LM61-preprimed CD4 T cells (Fig. 5 *A* and *B*), and as expected (65), it did not prevent the juxtaposition of KL25HL cells and SM cells in the T–B border area either (*SI Appendix*, Fig. S4*E*), but it largely abrogated the ability of preprimed CD4 T cells to prevent B cell decimation (Fig. 5*C*). This indicated an essential role for ICOS–ICOSL interactions in the prevention of B cell decimation. Similarly and despite GP66-specific CD4 T cell responses of normal magnitude, LM61 preimmunization of CD40L-deficient recipients failed to prevent the decimation of KL25HL cells (Fig. 5 *D* and *E*). Consistent with these observations, CD40L-deficient SM cells also responded vigorously to LCMV challenge but could not restore KL25HL B cell responses (*SI Appendix*, Fig. S5*A*). Importantly, LM61 preimmunization failed to prevent the decimation of CD40-deficient KL25HL B cells (Fig. 5 *F* and *G*), although CD40 deficiency did not prevent the cells' juxtaposition to SM cells in the T–B border area (*SI Appendix*, Fig. S4*E*). Altogether, these observations established an essential role for direct CD40–CD40L interactions by virus-specific CD4 T cells and B cells, respectively, to prevent decimation of the latter.

We also assessed a potential role of individual helper T cell cytokines in this process. Adoptively transferred IL-4– or IFN-γ–deficient SM CD4 T cells were as effective at preventing B cell decimation as their wt counterparts (*SI Appendix*, Fig. S5 *B* and *C*). In a complementary approach, we performed LM61 preimmunization experiments in either IL-10– or IL-21–deficient hosts but failed to reveal an essential individual contribution of either one of these cytokines to the prevention of B cell decimation (*SI Appendix*, Fig. S5 *D* and *E*). These observations indicated that IL-4, IL-10, IL-21, and IFN-γ were either dispensable for T help–mediated prevention of B cell decimation or played a largely redundant role in this process.

The experiments in Fig. 5 *D–G* and *SI Appendix*, Fig. S5*A* identified CD40L as a key signal preventing the decimation of naïve B cells, but the CD40L dependence of primary immune responses can differ from recall responses (66). Hence, we tested whether the prevention of memB cell decimation by T help (compare Fig. 1 *J–N*) was similarly dependent on CD40L signals. We generated KL25HL memB cells in LCMV-infected primary recipients, then sorted and adoptively transferred the cells into LM61-preimmunized secondary recipients, which were either wt or CD40L deficient (Fig. 5*H*). GP66-specific CD4 T cell responses of LM61-preprimed CD40L-deficient mice were of near-normal magnitude, but they failed to prevent the decimation of KL25HL memB cells upon LCMV challenge (Fig. 5 *I* and *J*). Hence, prevention of memB cell decimation by T help depended on CD40L signaling, analogously to primary B cell responses.

Taken together, our results reveal that preexisting CD4 T help prevents B cell decimation in an antigen-specific manner, which involves direct B cell–T cell contact with essential engagement of the costimulatory receptors ICOS and CD40 as well as MHC class II–dependent antigen presentation by B cells.

Preexisting CD4 T Help Antagonizes Inflammation and Instructs a GC B Cell Program. We previously reported that IFNAR blockade promotes transcription factor expression profiles of mature B cell stages, thereby antagonizing ASC differentiation and decimation (34). Here, we profiled the transcriptome of KL25HL B cells 4 d after LCMV challenge (Fig. 6 *A* and *B* and *SI Appendix*, Fig. S6). Preexisting T help, provided either by LM61 preimmunization or by SM cell transfer, augmented the expression of transcription factors characteristically up-regulated in GC B cells (67). Among them were master regulators of the GC B cell program such as Bcl6 and Mef2b (68, 69). Intriguingly, these effects of T cell help on GC transcription factor profiles closely mimicked analogous effects of IFNAR blockade on the B cell transcriptome (Fig. 6 *A* and *B*). Both T help and IFNAR blockade repressed inflammation-related hallmark gene sets such as interferon-α response, interferon-γ response, and TNF-α signaling (Fig. 6*C* and *SI Appendix*, Fig. S7). In addition, they augmented proliferation-related gene sets such as E2F targets and G2M checkpoints but also metabolism-related gene sets such as genes associated with oxidative phosphorylation (Fig. 6*C* and *SI Appendix*, Fig. S7). To further investigate the functional impact of preexisting CD4 T help on the GC program of B cells undergoing chronic viral challenge, we made use of KL25HL donor mice expressing an activation-induced deaminase (AID) fate mapping reporter [KL25HL-AID^{rep} (70)]. KL25HL-AID^{rep}

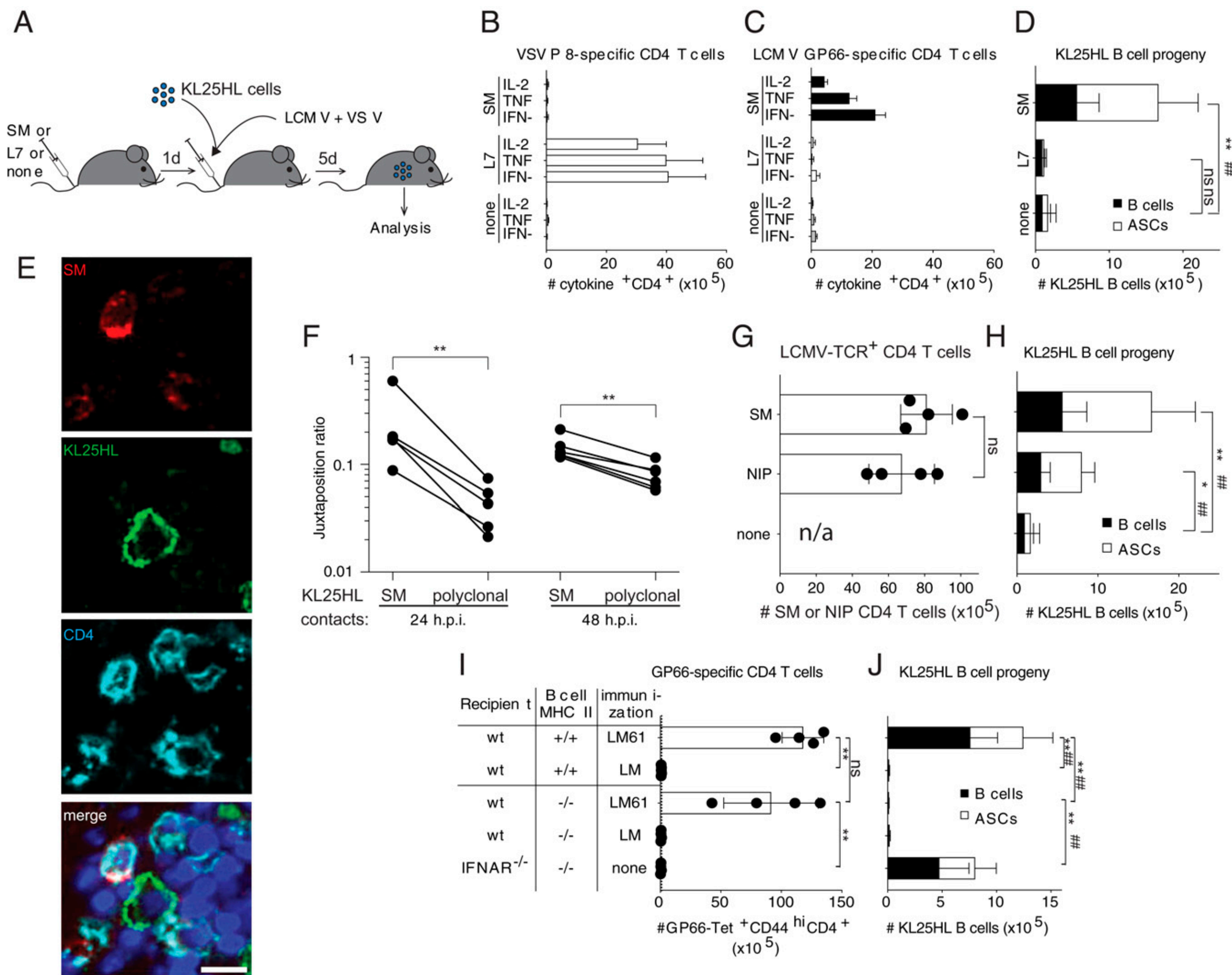

Fig. 4. T help prevents B cell decimation in an antigen-specific and MHC class II–dependent manner. (*A*) Schematic of the experimental design in *B–D*. Recipient mice were given either LCMV-specific SM CD4 T cells or VSV-specific L7 CD4 T cells or no CD4 T cells on −d 1. On day 0 they received KL25HL cells and were infected the same day with both LCMV and VSV. On day 5 we measured VSV P8–specific (*B*) and LCMV GP66–specific CD4 T cell responses (*C*) in spleen by intracellular cytokine assay and enumerated KL25HL progeny (*D*). (*E* and *F*) We transferred SM cells (Thy1.1$^+$) on day −1, followed by KL25HL cell transfer (CD45.1$^+$) and LCMV challenge on d 0. At 24 and 48 h later, we collected spleens for histological analysis. Exemplary image showing colocalization of SM and KL25HL cells (Magnification bar, 10 μm) (*E*). Juxtaposition ratios of KL25HL B cells with either LCMV-specific SM CD4 T cells or polyclonal endogenous CD4 T cells. Each dot represents one mouse (average juxtaposition ratio calculated from ∼100 to >1,000 KL25HL cells in the T–B-border area of each section) (*F*). (*G* and *H*) We transferred GP-specific SM CD4 T cells or NP-specific NIP CD4 T cells on d −1, followed by KL25HL cell transfer and LCMV challenge on d 0. Controls were left without T cell transfer ("none"). Adoptively transferred CD4 T cells (*G*) and KL25HL cell progeny (*H*) were enumerated on d 5. (*I* and *J*) Wt and IFNAR$^{-/-}$ recipients were preimmunized on d −9 as indicated (LM61, LM, or none), and on d 0 received either MHC-II–deficient or –sufficient KL25HL cells simultaneously with LCMV challenge. CD44^{hi}GP66-Tet$^+$ CD4 T cells (*I*) and KL25HL progeny (*J*) were enumerated on d 5 in spleen. Symbols and bars represent means ± SD, number of biological replicates (n) = 4 (*A–D*, *G*, and *H*), n = 5 to 6 (*E* and *F*), and n = 4 to 5 (*I* and *J*). Number of independent experiments (N) = 2 to 3 (*A–J*). Paired (*F*) or unpaired two-tailed Student's *t* test (*G*), two-way ANOVA with Dunnett's posttest for multiple comparison (*D* and *H*), two-way ANOVA with Tukey's posttest for multiple comparison (*J*), ordinary one-way ANOVA with Tukey's posttest for multiple comparisons (*I*). ns: not significant; $*P < 0.05$; **, $^{##}P < 0.01$; when used next to each other, ** compares B cells, and ## compares ASCs. Datasets in *A–D* are from the same experiment as (*G–H*).

mice carry one allele of an engineered *aicda* locus (encoding for AID), which drives a tamoxifen (TAM)-inducible Cre recombinase (Cre-ERT2). In conjunction with a Cre-inducible enhance yellow fluorescent protein (EYFP) reporter gene, TAM administration induces EYFP expression in ∼10 to 20% of AID^{rep} GC B cells (48, 70). KL25HL-AID^{rep} B cells were transferred into recipient mice immunized with either LM61 or LM, or into a third cohort that was given IFNAR blockade instead of preimmunization. Upon LCMV challenge, TAM was administered and KL25HL-AID^{rep} B cells were analyzed 5 d later (Fig. 6*D*). LM61 preimmunization as well as IFNAR blockade significantly augmented both the absolute number and the proportion of KL25HL-AID^{rep} B cell progeny that reported AID (EYFP, Fig. 6*E*). This effect of preexisting T help was also evident on histological sections (Fig. 6*F*). AID-reporting (EYFP+) B cells populated the B cell follicles of LM61-preprimed or IFNAR-blocked recipients, while only individual scattered cells were found in LM-immune controls (Fig. 6*F*). These results suggested that preexisting T help, analogous to IFNAR blockade (34), prevents B cell decimation by instructing a GC B cell transcriptional program.

KL25HL B Cell Transfer Results in Virus Control and Prevents CD4 T Cell–Driven Immunopathology upon Viral Challenge. Our results suggested that effective B cell immunity to chronic viral challenge requires not only a suitable repertoire of antiviral B cells

https://doi.org/10.1073/pnas.2108157118

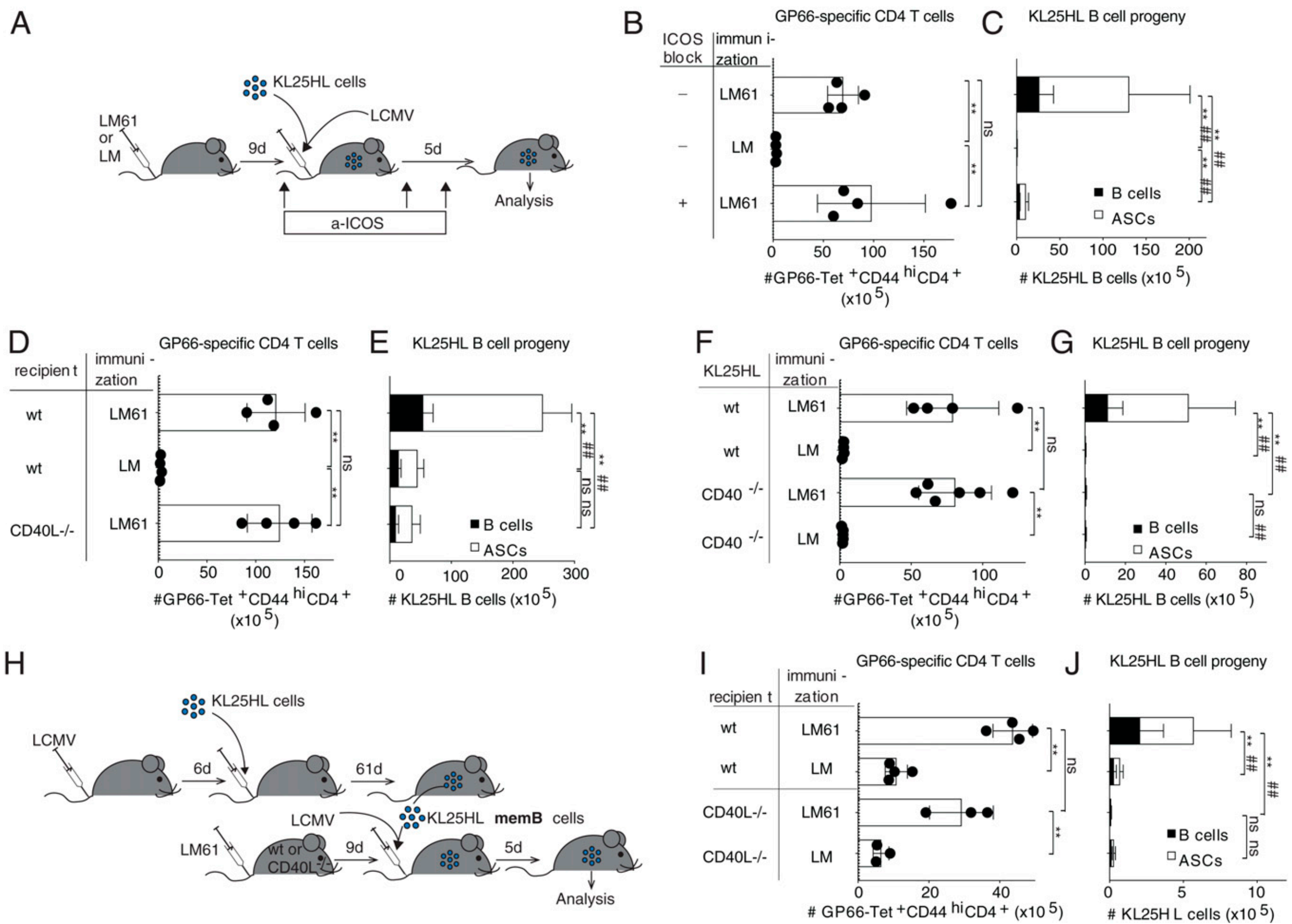

Fig. 5. T help prevents B cell decimation in a CD40- and ICOS-dependent manner. (*A*–*C*) Schematic of the experimental design to (*B* and *C*). On d −9 mice were preimmunized as indicated and challenged with LCMV on d 0. ICOS-blocking antibody was administered to the indicated group of mice on d −1, d 1, and d 3. $CD44^{hi}GP66\text{-}Tet^{+}$ CD4 T cells (*B*) and KL25HL progeny (*C*) were enumerated on d 5 in spleen. (*D* and *E*) On d −9, wt and $CD40L^{-/-}$ recipients were preimmunized as indicated and challenged with LCMV on d 0. $CD44^{hi}GP66\text{-}Tet^{+}$ CD4 T cells (*D*) and KL25HL progeny (*E*) were enumerated on d 5 in spleen. (*F* and *G*) Wt recipients were preimmunized on d −9 as indicated. On d 0, they received either CD40-sufficient or -deficient KL25HL cells simultaneously with LCMV challenge. $CD44^{hi}GP66\text{-}Tet^{+}$ CD4 T cells (*F*) and KL25HL progeny (*G*) were enumerated on d 5 in spleen. (*H*–*J*) Schematic of the experimental design to (*I* and *J*). KL25HL cells were adoptively transferred into LCMV-infected primary recipients. At 61 d later, progeny memB cells were purified from spleen (compare *SI Appendix*, Fig. S1 *C* and *D*) and were adoptively transferred into LM61-preimmunized secondary recipients, either wt or CD40L deficient, followed by LCMV challenge and analysis 7 d later. $CD44^{hi}GP66\text{-}Tet^{+}$ CD4 T cells (*I*) and KL25HL progeny (*J*) were enumerated in spleen. Symbols and bars represent means ± SD, number of biological replicates (n) = 4 (*A*–*E*), n = 4 to 6 (*F*–*G*), and n = 3 to 4 (*H*–*J*). Number of independent experiments (N) = 2 to 3 (*A*–*J*). Ordinary one-way ANOVA with Tukey's posttest for multiple comparisons (*B*, *D*, *F*, and *I*) and two-way ANOVA with Tukey's posttest for multiple comparison (*C*, *E*, *G*, and *J*). ns: not significant; **, $^{##}P < 0.01$; when used next to each other, ** compares B cells, and ## compares ASCs.

but also preexisting CD4 T cells with the capacity to prevent decimation of the former. This concept raised the question of whether combined B cell– and CD4 T cell–based immunity was associated with a risk of severe immunopathological disease, as has been reported for vaccination-induced CD4 T cell memory in the absence of virus-neutralizing B cells (8). Mice were immunized with LM61 and were given KL25HL cells by adoptive transfer prior to LCMV challenge, while control animals were left without cell transfer (Fig. 7*A* and *SI Appendix*, Fig. S8*A*). The interval between LM61 immunization and LCMV challenge was either 9 (Fig. 7) or 28 d (*SI Appendix*, Fig. S8) with analogous outcomes. As expected (8), LM61-preimmunized and LCMV-challenged control mice evidenced signs of immunopathology such as weight loss as well as elevated serum alanine and aspartate aminotransferase (AST, ALT) levels (Fig. 7 *B*–*D* and *SI Appendix*, Fig. S8 *B*–*D*). Importantly, these signs of immunopathological disease were largely absent in mice that had received KL25HL B cells prior to LCMV challenge. A rapid and potent nAb response in LM61-preimmunized KL25HL cell recipients (Fig. 7*E* and *SI Appendix*, Fig. S8*E*) was associated with complete suppression of viremia (Fig. 7*F* and *SI Appendix*, Fig. S8*F*). This observation was in line with earlier findings that suppression of LCMV viral loads by passively administered KL25 antibody prevented immunopathological disease induction by vaccination-elicited CD4 T cells (8).

Discussion

The present work defines effective B cell immunity against chronic viral challenge as a synergy between two cellular compartments: B cells of a protective antiviral specificity as well as CD4 T cells with the capacity to provide early cognate help to the former. The mutual interdependence of these two cell types in the chronic infection context differs in key aspects from their cooperation in classical T-dependent B cell responses (71). In line with earlier CD4 T cell depletion data (34), we show that KL25HL B cell have the intrinsic capacity of responding to LCMV in a T-independent manner. It therefore appears that T help, which in classical T-dependent B cell responses is

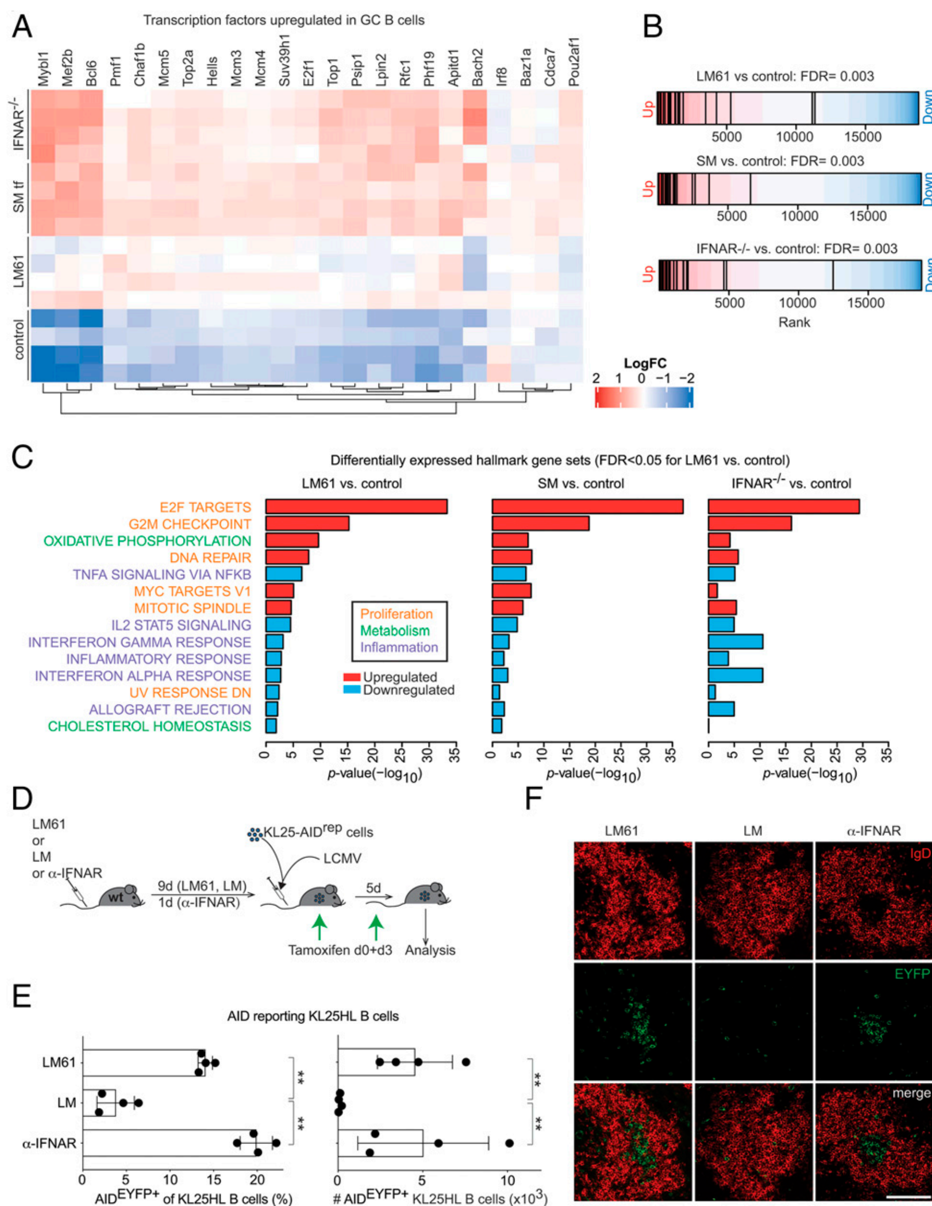

Fig. 6. Preexisting CD4 T help antagonizes inflammation and instructs a GC B cell program. (*A*–*C*) Wt recipients were either preimmunized with LM61 ("LM61") or LM ("control") on d −9 or were given SM CD4 T cells by adoptive transfer ("SM") on d −1. IFNAR$^{-/-}$ recipients were left untreated. KL25HL cell transfer and LCMV challenge were conducted on d 0. On d 4, KL25HL B cell progeny (CD45.1+B220+) were purified by FACS sorting (*SI Appendix*, Fig. S6) and subject to RNAseq. Heatmap displaying expression levels of GC B cell transcription factors as determined by Shi et al. (67). Pair-wise self-contained gene set testing for the same set of genes is shown in *B*. Hallmark gene sets, which are significantly differentially expressed (false discovery rate [FDR] < 0.05) in KL25HL B cells from LM61-preimmunized and LM-preimmunized control mice are displayed in *C*. *P* values obtained when assessing the same gene sets but comparing either SM versus control or IFNAR$^{-/-}$ versus control are also shown. (*D*–*F*) Schematic of the experimental design to (*E* and *F*). Recipients were preimmunized with LM61 or LM on d 9 or were given IFNAR-blocking antibody. On d 0, KL25HL-AID^{rep} B cells were adoptively transferred simultaneously with LCMV challenge. TAM was administered on d 0 and d 3, and AID^{EYFP}-reporting KL25HL-AID^{rep} cells were determined on d 5 in spleen (*E*) and are expressed as a percentage of all KL25HL-AID^{rep} cells and also in absolute numbers. Histological sections prepared at the same time point revealed clusters of AID^{EYFP}-reporting KL25HL-AID^{rep} cells in the splenic B cell zone (IgD) of LM61-preimmunized and IFNAR-blocked mice but not in LM-preimmunized controls (Magnification bar, 100 μm). Symbols and bars represent means ± SD, number of biological replicates (n) = 4 (*D*–*F*). Number of independent experiments (N) = 2 (*D*–*F*). Ordinary one-way ANOVA with Dunnett's posttest for multiple comparisons (*E*); $^{**}P < 0.01$.

essential for B cell activation, expansion, and affinity maturation, serves an additional key role in the context of chronic virus challenge. It assures the clonal survival and thereby the sustainability of antiviral B cell responses when at risk for IFN-I–driven decimation. However, vaccination-induced CD4 T cell immunity bears the risk of fatal immunopathology. Here, we show the latter can be averted by antiviral B cells that—with support from the same preexisting CD4 T cells—mount a timely and protective antibody response to control viral loads.

The finding that T help prevents B cell decimation by instructing a GC B cell program, analogously to IFNAR blockade, provides additional support to our postulate that B cell decimation reflects a biased, unsustainable plasmablast response (34). Studies in mice with B cell–specific Blimp1 deletion and a resulting defect in ASC differentiation have also demonstrated that in hapten-carrier immunization, ASC differentiation inevitably occurs at the expense of GC cellularity (72). It therefore appears that productive and sustainable B cell responses reflect a delicate balance of GC and ASC differentiation, a balance which is tilted by IFN-I–driven inflammation to culminate in B cell decimation. As reported herein, not only IFNAR blockade but also preexisting T help can readjust this balance to ensure both rapid antibody-mediated virus control as well as long-term sustained GC B cell responses. Whether and how preexisting T help may additionally affect other postulated pathways of B cell decimation remains to be investigated (35, 36).

Thus far, IFNAR blockade has represented the only known intervention for the prevention of B cell decimation, but from a practical perspective, vaccination-induced CD4 T cell immunity offers key advantages. First, IFNAR blockade can result in unwanted side effects owing to inhibition of a main pathway of innate antiviral defense (37). Second, as our earlier studies suggested (34), IFNAR blockade would likely have to be administered within the first 2 d if not within hours after a viral encounter, an occurrence that commonly goes unnoticed. CD4 T cell memory can be induced prophylactically with potentially long-lived effects (73), and strategies for its induction in clinical vaccination programs are well established (74). The present findings suggest preexisting T help and memory Tfh cells in particular represent a rate-limiting component of protective B cell memory to persisting viruses. Vaccination strategies against

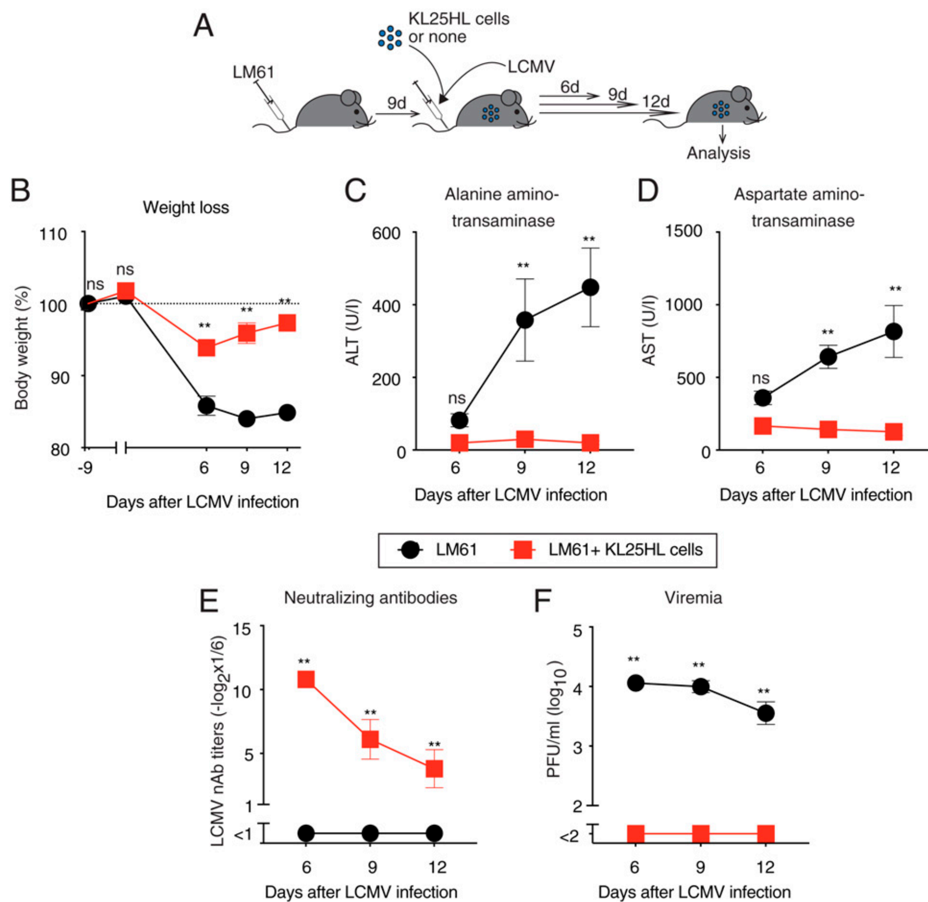

Fig. 7. KL25HL cell transfer suppresses viremia and prevents CD4 T cell–driven immunopathology upon LCMV challenge. (*A*) Schematic of the experimental design in *B–F*. Two groups of mice were preimmunized with LM61 on d −9 and on d 0 were challenged with LCMV. One of these groups was given KL25HL B cells simultaneously with LCMV challenge. Body weight was monitored on the indicated time points to determine weight loss (*B*). Serum alanine aminotransaminase activity (*C*), serum aspartate aminotransaminase activity (*D*), LCMV-nAb titers (*E*), and viremia (*F*) were determined on d 6, d 9, and d 12. Symbols represent means ± SEM; number of biological replicates (*n*) = 5 (*B–F*). Number of independent experiments (*N*) = 3 (*B–F*). Two-way ANOVA with Bonferroni's posttest for multiple comparisons (*B–F*); ns: not significant; $^*P < 0.05$; $^{**}P < 0.01$.

HIV or HCV that focus on the induction of affinity-matured, broadly neutralizing memB cells (75) may therefore only succeed if combined with modalities that elicit potent and long-lived Tfh memory. With critical support from Tfh memory cells during the earliest phase of infection, timely and sustained memB cell responses may critically curb or even eliminate chronic viruses (76) and thereby may prevent viral sequence diversification from outpacing the host GC response (77, 78).

The molecular interactions and signals we identified as essential for CD4 T cells to prevent B cell decimation at the onset of chronic infection—cognate antigen, MHC-II, CD40, and ICOS—closely resemble those required for classical T-dependent B cell responses (71). Somewhat more surprisingly, not only IFN-γ but also IL-4, IL-10, and IL-21 each ndividually appeared dispensable. Importantly however, this observation does not exclude a supportive-yet-redundant role of these cytokines in the prevention of early B cell decimation. Moreover, these cytokines and even combinations thereof clearly are essential for effective CD4 T help to B cells during later stages of chronic LCMV infection (79). Interestingly, the key role of CD40 signals in preventing B cell decimation aligns well with the observation that CD40 ligation potently antagonizes ASC differentiation in human B cell cultures (80).

In our standard experimental setting, the number of adoptively transferred naïve KL25HL B cells resulted in a splenic B cell precursor frequency of $\sim 2 \times 10^{-4}$ (*Materials and Methods*). This corresponds to the physiological range of B cells specific for proteins such as phycoerythrin, *Bacillus anthracis*–protective antigen, or influenza hemagglutinin, which range from $\sim 9 \times 10^{-4}$ to 6×10^{-5} (81, 82). In contrast, naïve precursor frequencies as low as $\sim 3 \times 10^{-6}$ have been reported for VRC01-class antibodies that target a specific neutralizing epitope on the HIV envelope (83). For such rare specificities, KL25 B cell precursor frequencies as established in our experiments may thus be more reflective of memB cell populations upon vaccination (84), which we show are similarly rescued from decimation by preexisting T help.

We confirm earlier observations that LM61-induced CD4 T cell responses are largely Th1 biased (8). In vivo generated polyclonal CD4 T cell responses, however, commonly comprise several differentiation patterns (73, 85). Accordingly, we detect a small but clearly discernible Tfh-like subpopulation in the primary response to LM61 and a population of ∼15 to 20% of such cells in the recall response to LCMV. It seems likely that the latter are descendants of the former analogously differentiated cells, with their Tfh differentiation likely supported by the antiviral B cell response ensuing in parallel (86). In light of the considerable plasticity of CD4 T cell subsets in the context of viral infection (87) we acknowledge, however, that Tfh transdifferentiation of other LM61-induced CD4 T cell subpopulations remains a not mutually exclusive possibility.

Taken together, our findings reveal and characterize very early yet essential interactions of antiviral memB cells with preexisting CD4 T helper cells, which provide key signals to prevent decimation and thereby subversion of the antiviral B cell response at the onset of chronic infection. These insights establish CD4 T cell memory as an essential cornerstone of B cell immunity to chronic viral challenge and should help in leveraging the full potential of B cell–based protection in the fight against persisting human pathogens such as HIV and HCV.

Materials and Methods

Materials and methods are provided as a part of the *SI Appendix* and detail mice and animal experimentation, bacteria, viruses, virus titrations, infections

and immunizations, flow cytometry and intracellular cytokine assays, immunohistochemistry and image analysis, next-generation RNA sequencing and bioinformatics data analyses, adoptive cell transfer, fluorescent cell labeling and FACS sorting, in vivo cell depletion, antibody blockade and tamoxifen administration, measurements of neutralizing and GP-binding antibody responses and serum transaminases, in vitro differentiation of SM-Th1 and SM-Th2 cells as well as statistical analysis. Animal Experiments were performed at the University of Basel in accordance with the Swiss law for animal protection and with authorization by the Cantonal Veterinary Office Basel-Stadt.

Data Availability. Bulk RNAseq and single-cell RNAseq data are deposited with the National Center for Biotechnology Information Gene Expression Omnibus under the accession nos. GSE160294 and GSE161356, respectively. Additional raw data reported in this study are deposited in Zenodo under the accession no. 10.5281/zenodo.5203142.

ACKNOWLEDGMENTS. We thank Christian Beisel and Tobias Schär from the Genomics Facility Basel of the University of Basel and of the Department of Biosystems Science and Engineering (D-BSSE) of the Eidgenössische Technische Hochschule Zurich for single-cell RNAseq library preparation and next-generation RNA sequencing; Julien Roux from the Department of Biomedicine (DBM) Bioinformatics Core Facility of the University of Basel for single-cell RNA-Seq analysis; Philippe Demougin from the Life Sciences Training Facility of the University of Basel Pharmacenter for bulk RNA sequencing; Karsten Stauffer for experimental support in animal handling and care; Karim Hammad for immunohistochemistry; Telma Lopes, Danny Labes, Lorenzo Raeli, and Emmanuel Traunecker from the DBM flow cytometry core facility for FACS sorting; Jean-Claude Weill, Claude-Agnès Reynaud, Peter Aichele, Shane Crotty, Manfred Kopf, and Stephen Nutt for generously donating mouse strains; Pablo Penaloza, Dan Barouch, and Hao Shen for providing recombinant Listeria; the NIH tetramer core facility for MHC class II tetramers; and the entire Experimental Virology laboratory for helpful discussions. All bioinformatic calculations were performed at the sciCORE (https://scicore.unibas.ch/) scientific computing center at University of Basel.

1. C. Nunes-Alves *et al.*, In search of a new paradigm for protective immunity to TB. *Nat. Rev. Microbiol.* **12**, 289–299 (2014).
2. W. Reith, B. Mach, The bare lymphocyte syndrome and the regulation of MHC expression. *Annu. Rev. Immunol.* **19**, 331–373 (2001).
3. J. A. Kovacs, H. Masur, Prophylaxis against opportunistic infections in patients with human immunodeficiency virus infection. *N. Engl. J. Med.* **342**, 1416–1429 (2000).
4. S. Buranapraditkun *et al.*, Preservation of peripheral T follicular helper cell function in HIV controllers. *J. Virol.* **91**, e00497-17 (2017).
5. W. B. Dyer *et al.*, Mechanisms of HIV non-progression; robust and sustained CD4+ T-cell proliferative responses to p24 antigen correlate with control of viraemia and lack of disease progression after long-term transfusion-acquired HIV-1 infection. *Retrovirology* **5**, 112 (2008).
6. K. V. Komanduri *et al.*, Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nat. Med.* **4**, 953–956 (1998).
7. H. Streeck, M. P. D'Souza, D. R. Littman, S. Crotty, Harnessing $CD4^+$ T cell responses in HIV vaccine development. *Nat. Med.* **19**, 143–149 (2013).
8. P. Penaloza-MacMaster *et al.*, Vaccine-elicited CD4 T cells induce immunopathology after chronic LCMV infection. *Science* **347**, 278–282 (2015).
9. L. Hunziker *et al.*, Antagonistic variant virus prevents wild-type virus-induced lethal immunopathology. *J. Exp. Med.* **196**, 1039–1046 (2002).
10. A. Oxenius, R. M. Zinkernagel, H. Hengartner, Comparison of activation versus induction of unresponsiveness of virus-specific CD4+ and CD8+ T cells upon acute versus persistent viral infection. *Immunity* **9**, 449–457 (1998).
11. L. van der Fits *et al.*, Adenovector 26 encoded prefusion conformation stabilized RSV-F protein induces long-lasting Th1-biased immunity in neonatal mice. *NPJ Vaccines* **5**, 49 (2020).
12. B. Puissant-Lubrano *et al.*, Control of vaccinia virus skin lesions by long-term-maintained IFN-gamma+TNF-alpha+ effector/memory CD4+ lymphocytes in humans. *J. Clin. Invest.* **120**, 1636–1644 (2010).
13. C. S. Hsieh *et al.*, Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science* **260**, 547–549 (1993).
14. S. A. Plotkin, Correlates of protection induced by vaccination. *Clin. Vaccine Immunol.* **17**, 1055–1065 (2010).
15. European Consensus Group on Hepatitis B Immunity, Are booster immunisations needed for lifelong hepatitis B immunity? *Lancet* **355**, 561–565 (2000).
16. D. J. West, G. B. Calandra, Vaccine induced immunologic memory for hepatitis B surface antigen: Implications for policy on booster vaccination. *Vaccine* **14**, 1019–1027 (1996).
17. V. J. Kinchen *et al.*, Broadly neutralizing antibody mediated clearance of human hepatitis C virus infection. *Cell Host Microbe* **24**, 717–730.e5 (2018).
18. J. M. Pestka *et al.*, Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 6025–6030 (2007).
19. W. O. Osburn *et al.*, Clearance of hepatitis C infection is associated with the early appearance of broad neutralizing antibody responses. *Hepatology* **59**, 2140–2151 (2014).
20. S. Raghuraman *et al.*, Spontaneous clearance of chronic hepatitis C virus infection is associated with appearance of neutralizing antibodies and reversal of T-cell exhaustion. *J. Infect. Dis.* **205**, 763–771 (2012).
21. M. Chen *et al.*, Limited humoral immunity in hepatitis C virus infection. *Gastroenterology* **116**, 135–143 (1999).
22. A. L. Cox *et al.*, Prospective evaluation of community-acquired acute-phase hepatitis C virus infection. *Clin. Infect. Dis.* **40**, 951–958 (2005).
23. M. S. Cohen, G. M. Shaw, A. J. McMichael, B. F. Haynes, Acute HIV-1 infection. *N. Engl. J. Med.* **364**, 1943–1954 (2011).
24. D. D. Pinschewer *et al.*, Kinetics of protective antibodies are determined by the viral surface antigen. *J. Clin. Invest.* **114**, 988–993 (2004).
25. S. Moir *et al.*, Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J. Exp. Med.* **205**, 1797–1805 (2008).
26. G. E. Weiss *et al.*, Atypical memory B cells are greatly expanded in individuals living in a malaria-endemic area. *J. Immunol.* **183**, 2176–2182 (2009).
27. C. Wei *et al.*, A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. *J. Immunol.* **178**, 6624–6633 (2007).
28. E. D. Charles *et al.*, Clonal B cells in patients with hepatitis C virus-associated mixed cryoglobulinemia contain an expanded anergic CD21low B-cell subset. *Blood* **117**, 5425–5437 (2011).
29. B. Oliviero *et al.*, Enhanced B-cell differentiation and reduced proliferative capacity in chronic hepatitis C and chronic hepatitis B virus infections. *J. Hepatol.* **55**, 53–60 (2011).
30. B. Oliviero *et al.*, Skewed B cells in chronic hepatitis C virus infection maintain their ability to respond to virus-induced activation. *J. Viral Hepat.* **22**, 391–398 (2015).
31. L. A. Labuda *et al.*, Alterations in peripheral blood B cell subsets and dynamics of B cell responses during human schistosomiasis. *PLoS Negl. Trop. Dis.* **7**, e2094 (2013).
32. S. A. Joosten *et al.*, Patients with tuberculosis have a dysfunctional circulating B-cell compartment, which normalizes following successful treatment. *PLoS Pathog.* **12**, e1005687 (2016).
33. M. F. Muellenbeck *et al.*, Atypical and classical memory B cells produce Plasmodium falciparum neutralizing antibodies. *J. Exp. Med.* **210**, 389–399 (2013).
34. B. Fallet *et al.*, Interferon-driven deletion of antiviral B cells at the onset of chronic infection. *Sci. Immunol.* **1**, eaah6817 (2016).
35. S. Sammicheli *et al.*, Inflammatory monocytes hinder antiviral B cell responses. *Sci. Immunol.* **1**, eaah6789 (2016).
36. E. A. Moseman, T. Wu, J. C. de la Torre, P. L. Schwartzberg, D. B. McGavern, Type I interferon suppresses virus-specific B cell responses by modulating $CD8^+$ T cell differentiation. *Sci. Immunol.* **1**, eaah3565 (2016).
37. N. G. Sandler *et al.*, Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. *Nature* **511**, 601–605 (2014).
38. S. Crotty, Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* **29**, 621–663 (2011).
39. C. G. Vinuesa, M. A. Linterman, D. Yu, I. C. MacLennan, Follicular helper T cells. *Annu. Rev. Immunol.* **34**, 335–368 (2016).
40. H. Qi, T follicular helper cells in space-time. *Nat. Rev. Immunol.* **16**, 612–625 (2016).
41. B. O. Lee *et al.*, CD4 T cell-independent antibody response promotes resolution of primary influenza infection and helps to prevent reinfection. *J. Immunol.* **175**, 5827–5838 (2005).
42. A. F. Ochsenbein *et al.*, Protective T cell-independent antiviral antibody responses are dependent on complement. *J. Exp. Med.* **190**, 1165–1174 (1999).
43. P. Vieira, K. Rajewsky, Persistence of memory B cells in mice deprived of T cell help. *Int. Immunol.* **2**, 487–494 (1990).
44. B. J. Hebeis *et al.*, Activation of virus-specific memory B cells in the absence of T cell help. *J. Exp. Med.* **199**, 593–602 (2004).
45. A. Asrir, M. Aloulou, M. Gador, C. Pérals, N. Fazilleau, Interconnected subsets of memory follicular helper T cells have different effector functions. *Nat. Commun.* **8**, 847 (2017).
46. M. K. MacLeod *et al.*, Memory CD4 T cells that express CXCR5 provide accelerated help to B cells. *J. Immunol.* **186**, 2889–2896 (2011).
47. L. J. McHeyzer-Williams, C. Dufaud, M. G. McHeyzer-Williams, Do memory B cells form secondary germinal centers? Impact of antibody class and quality of memory T-cell help at recall. *Cold Spring Harb. Perspect. Biol.* **10**, a028878 (2018).
48. I. Dogan *et al.*, Multiple layers of B cell memory with different effector functions. *Nat. Immunol.* **10**, 1292–1299 (2009).
49. A. T. Krishnamurty *et al.*, Somatically hypermutated plasmodium-specific IgM(+) memory B cells are rapid, plastic, early responders upon malaria rechallenge. *Immunity* **45**, 402–414 (2016).
50. K. A. Pape, J. J. Taylor, R. W. Maul, P. J. Gearhart, M. K. Jenkins, Different B cell populations mediate early and late memory during an endogenous immune response. *Science* **331**, 1203–1207 (2011).
51. G. V. Zuccarino-Catania *et al.*, CD80 and PD-L2 define functionally distinct memory B cell subsets that are independent of antibody isotype. *Nat. Immunol.* **15**, 631–637 (2014).

52. L. J. McHeyzer-Williams, P. J. Milpied, S. L. Okitsu, M. G. McHeyzer-Williams, Class-switched memory B cells remodel BCRs within secondary germinal centers. *Nat. Immunol.* **16**, 296–305 (2015).
53. B. B. Porter, J. T. Harty, The onset of CD8+-T-cell contraction is influenced by the peak of Listeria monocytogenes infection and antigen display. *Infect. Immun.* **74**, 1528–1536 (2006).
54. S. M. Anderson, M. M. Tomayko, A. Ahuja, A. M. Haberman, M. J. Shlomchik, New markers for murine memory B cells that define mutated and unmutated subsets. *J. Exp. Med.* **204**, 2103–2114 (2007).
55. C. A. Reynaud *et al.*, IgM memory B cells: A mouse/human paradox. *Cell. Mol. Life Sci.* **69**, 1625–1634 (2012).
56. D. Duffy, C. P. Yang, A. Heath, P. Garside, E. B. Bell, Naive T-cell receptor transgenic T cells help memory B cells produce antibody. *Immunology* **119**, 376–384 (2006).
57. Y. S. Choi *et al.*, LEF-1 and TCF-1 orchestrate T(FH) differentiation by regulating differentiation circuits upstream of the transcriptional repressor Bcl6. *Nat. Immunol.* **16**, 980–990 (2015).
58. S. G. Tangye, Advances in IL-21 biology - Enhancing our understanding of human disease. *Curr. Opin. Immunol.* **34**, 107–115 (2015).
59. D. Yu *et al.*, The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. *Immunity* **31**, 457–468 (2009).
60. R. I. Nurieva *et al.*, Bcl6 mediates the development of T follicular helper cells. *Science* **325**, 1001–1005 (2009).
61. R. J. Johnston *et al.*, Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* **325**, 1006–1010 (2009).
62. Z. Wan, Y. Lin, Y. Zhao, H. Qi, T_{FH} cells in bystander and cognate interactions with B cells. *Immunol. Rev.* **288**, 28–36 (2019).
63. P. A. Scherle, W. Gerhard, Functional analysis of influenza-specific helper T cell clones in vivo. T cells specific for internal viral proteins provide cognate help for B cell responses to hemagglutinin. *J. Exp. Med.* **164**, 1114–1128 (1986).
64. A. Lanzavecchia, Antigen-specific interaction between T and B cells. *Nature* **314**, 537–539 (1985).
65. D. Liu *et al.*, T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. *Nature* **517**, 214–218 (2015).
66. M. G. Hernandez, L. Shen, K. L. Rock, CD40 on APCs is needed for optimal programming, maintenance, and recall of CD8+ T cell memory even in the absence of CD4+ T cell help. *J. Immunol.* **180**, 4382–4390 (2008).
67. W. Shi *et al.*, Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. *Nat. Immunol.* **16**, 663–673 (2015).
68. P. Brescia *et al.*, MEF2B instructs germinal center development and acts as an oncogene in B cell lymphomagenesis. *Cancer Cell* **34**, 453–465.e9 (2018).
69. A. L. Dent, A. L. Shaffer, X. Yu, D. Allman, L. M. Staudt, Control of inflammation, cytokine expression, and germinal center formation by BCL-6. *Science* **276**, 589–592 (1997).
70. B. Fallet *et al.*, Chronic viral infection promotes efficient germinal center B cell responses. *Cell Rep.* **30**, 1013–1026.e7 (2020).
71. J. G. Cyster, C. D. C. Allen, B cell responses: Cell interaction dynamics and decisions. *Cell* **177**, 524–540 (2019).
72. M. Shapiro-Shelef *et al.*, Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. *Immunity* **19**, 607–620 (2003).
73. M. Künzli *et al.*, Long-lived T follicular helper cells retain plasticity and help sustain humoral immunity. *Sci. Immunol.* **5**, eaay5552 (2020).
74. D. L. Hill *et al.*, The adjuvant GLA-SE promotes human Tfh cell expansion and emergence of public TCRβ clonotypes. *J. Exp. Med.* **216**, 1857–1873 (2019).
75. D. R. Burton *et al.*, A blueprint for HIV vaccine discovery. *Cell Host Microbe* **12**, 396–407 (2012).
76. J. Liu *et al.*, Antibody-mediated protection against SHIV challenge includes systemic clearance of distal virus. *Science* **353**, 1045–1049 (2016).
77. N. A. Doria-Rose *et al.*, Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature* **509**, 55–62 (2014).
78. H. X. Liao *et al.*, Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature* **496**, 469–476 (2013).
79. G. Xin *et al.*, Single-cell RNA sequencing unveils an IL-10-producing helper subset that sustains humoral immunity during persistent infection. *Nat. Commun.* **9**, 5037 (2018).
80. C. Arpin *et al.*, Generation of memory B cells and plasma cells in vitro. *Science* **268**, 720–722 (1995).
81. K. A. Pape *et al.*, Naive B cells with high-avidity germline-encoded antigen receptors produce persistent IgM^+ and transient IgG^+ memory B cells. *Immunity* **48**, 1135–1143.e4 (2018).
82. M. Kuraoka *et al.*, Complex antigens drive permissive clonal selection in germinal centers. *Immunity* **44**, 542–552 (2016).
83. J. G. Jardine *et al.*, Minimally mutated HIV-1 broadly neutralizing antibodies to guide reductionist vaccine design. *PLoS Pathog.* **12**, e1005815 (2016).
84. D. Huang *et al.*, B cells expressing authentic naive human VRC01-class BCRs can be recruited to germinal centers and affinity mature in multiple independent mouse models. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 22920–22931 (2020).
85. A. L. Marzo, V. Vezys, K. Williams, D. F. Tough, L. Lefrançois, Tissue-level regulation of Th1 and Th2 primary and memory CD4 T cells in response to Listeria infection. *J. Immunol.* **168**, 4504–4510 (2002).
86. W. Ise *et al.*, Memory B cells contribute to rapid Bcl6 expression by memory follicular helper T cells. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 11792–11797 (2014).
87. A. N. Hegazy *et al.*, Interferons direct Th2 cell reprogramming to generate a stable GATA-3(+)T-bet(+) cell subset with combined Th2 and Th1 cell functions. *Immunity* **32**, 116–128 (2010).