HHS Public Access

Author manuscript

Nat Immunol. Author manuscript; available in PMC 2011 June 01.

Published in final edited form as:

Nat Immunol. 2010 December ; 11(12): 1110–1118. doi:10.1038/ni.1954.

Plasma cells negatively regulate the follicular helper T cell program

Nadege Pelletier, **Louise J. McHeyzer-Williams**, **Kurt A. Wong**, **Eduard Urich**, **Nicolas Fazilleau**, and **Michael G. McHeyzer-Williams**

Department of Immunology and Microbial Sciences The Scripps Research Institute, La Jolla CA 92037

Abstract

B lymphocytes differentiate into antibody-secreting cells under the antigen-specific control of follicular helper T (T_{FH}) cells. Here, we demonstrate that isotype-switched plasma cells expressed MHCII, CD80 and CD86 and intracellular machinery required for antigen presentation. Antigen-specific plasma cells could access, process and present sufficient antigen *in vivo* to induce multiple T_H cell functions. Importantly, antigen-primed plasma cells failed to induce interleukin 21 or Bcl-6 in naïve T_H cells and actively shut down these key molecules in antigen-activated T_{FH} cells. Mice lacking plasma cells displayed altered T_{FH} activity, providing evidence for this negative feedback loop. Hence, antigen presentation by plasma cells defines a new layer of cognate regulation that limits the antigen-specific T_{FH} program controlling ongoing B cell immunity.

INTRODUCTION

Protein vaccination induces high-affinity B cell memory and persistent circulating antigen-specific antibodies for long-lasting humoral immunity1,2. Follicular helper T (T_{FH}) cells have emerged as a new class of immune regulatory cells3–5 specialized to control the stepwise development of antigen-specific B cell immunity. Within the first week after priming, antigen-specific T_{FH} cells emerge6–8 to initiate antibody secretion, isotype switch and the germinal center (GC) reaction9. Within the GC, T_{FH} cells regulate high-affinity memory B cell development10–12 and the production of long-lived plasma cells13. Upon antigen re-challenge, memory T_{FH} cells promote antigen-specific memory B cell expansion and the rapid induction of high-affinity plasma cells7,14. Thus, antigen-specific T_{FH} function is central to multiple facets of B cell immunity, but how this cognate regulatory activity is controlled remains poorly understood.

Correspondence should be addressed to M.G.M, (mcheyzer@scripps.edu).

Author contributions N.P., L.J.M-W. and M.G.M-W. conceived and designed the project. N.P. executed and analyzed the data for all experiments except Figure 3a–b and Figure 6. K.A.W. designed, completed the experiments and analyzed the data on pMHCII HEL-specific plasma cells (Fig. 3a–b). E.U. laid the experimental foundation for staining HEL-specific B cells. N.F. prepared the activated T_H cell samples for quantitative PCR analysis (Fig. 4b) and provided expertise in setting-up the T cell in vitro experiments. L.J.M-W. and M.G.M-W. conducted the experiments and analyzed the data on the cKO-Blimp1 mice (Fig. 6). K.A.W. and N.F. contributed important ideas and participated in the manuscript preparation. N.P., L.J.M-W. and M.G.M-W. wrote the manuscript.

Current affiliations: Roche, Basel Switzerland (EU) and INSERM, U563, Toulouse, F-31300, France (NF).

Antigen-specific T_{FH} development and function emerges progressively with separable requirements for cognate control. Initial T_{FH} programming occurs upon first contact with peptide-MHC class II (pMHCII) expressing dendritic cells (DCs) in the T cell zones of draining lymphoid tissue15. Loss of CCR7 and expression of CXCR5 relocates T_{FH} cells to B cell zones6 and facilitates contact with antigen-primed pMHCII-expressing B cells16–18. Bcl-6 [http://www.signaling-gateway.org/molecule/query;jsessionid=c6ca4b34229c15d89939cccc445b981f9b070d6997a2?afcsid=A000369] is expressed by the early pre-GC T_{FH} cells8 and is sufficient and necessary to induce this program *in vivo*19–21. Long duration intercellular contact17 with signals promoted by SLAM receptors17 and ICOS engagement through ICOS-L on B cells is required for pre-GC T_{FH} cell entry into the GC environment22,23. Ectopic ICOS expression by naïve T_H cells promotes excessive T_{FH} expansion, enlarged GCs and autoantibody production9. IL-21 [http://www.signaling-gateway.org/molecule/query?afcsid=A001258] and IL-21R [http://www.signaling-gateway.org/molecule/query?afcsid=A001259] interactions are central to effective B cell immunity24,25. While T_{FH} cells can develop in the absence of IL-21R10,11, the lack of IL-21 signaling in B cells substantially compromises the GC reaction and post-GC plasma cell production10–12. PD-1 expressed by T_{FH} cells has also been implicated in PD-L2 mediated control of GC B cell survival and post-GC plasma cell production13. Hence, clonal expansion and the cognate delivery of specific T_{FH} cell functions also require a highly-regulated program of progressive development *in vivo*.

Antibody secreting cells (ASCs) are the critical effector B cell compartment produced following antigen-specific T_{FH} regulation *in vivo*. Plasma cells are defined as post-mitotic ASCs and represent the terminally differentiated component of the ASC compartment1,2. Outside the GC, plasma cell development requires T_{FH} regulation and occurs rapidly during the first week after protein vaccination. These non-GC plasma cells do not somatically hypermutate26,27 but can antibody class switch and typically have short half-lives of 3–5 days28. In contrast, GC plasma cell development involves affinity maturation and antibody class switch resulting in long-lived compartments of high-affinity plasma cells1,2,29. The transcription factor Blimp-1 [http://www.signaling-gateway.org/molecule/query?afcsid=A003268] initiates plasma cell development in both pathways30 exerting its control largely through repression of Bcl-6 and Pax-5 (ref. 31) allowing de-repression of XBP-1 (ref. 32,33). Moreover, IL-21 abundantly produced by T_{FH} cells8,10–12, potently induces Blimp-1 expression34 and plasma cell development in a STAT3 and IRF4 dependent manner35. XBP-1 controls the unfolded protein response and supports many facets of the cellular secretory apparatus32. Decreased surface expression of B cell receptor and down-regulation of the MHCII transactivator CIITA36 suggest that plasma cells remain highly specialized for antibody secretion *in vivo* and have a diminished capacity for immune regulation and responsiveness.

Contrary to these expectations, we demonstrate continued high expression of MHCII, CD80, CD86 and the intracellular machinery for antigen presentation in antigen-specific isotype-switched plasma cells directly ex vivo. Importantly, after priming *in vivo* antigen-specific plasma cells expressed pMHCII complexes and were able to activate antigen-specific T_H cells. Antigen-pulsed plasma cells induced proliferation and effector cell differentiation *in*

vitro from naive antigen-specific T_H cells but promoted Blimp-1 in favor of Bcl-6 and IL-21 induction in the T_H cell compartment. Furthermore, plasma cells shut down IL-21 production and decreased Bcl-6 expression in activated T_H cells in an antigen-specific manner. In support of this negative regulatory function, $CXCR5^+PD\text{-}1^+$ T_{FH} cells accumulated to exaggerated numbers in draining and distal lymphoid tissues following immunization of mice lacking B cell-expressed Blimp-1 that do not produce plasma cells *in vivo*. Large numbers of plasma cells appear interspersed with T_H cells at the T-B borders and throughout the lymphoid tissue during the second week after antigen priming with evidence for T_H cell contact with plasma cells *in situ*. Finally, we diminished Bcl-6 and IL-21 expression in antigen-specific T_{FH} cells *in vivo* through adoptive transfer of antigen-pulsed plasma cells. These data reveal an antigen presentation function for plasma cells during adaptive immunity that serves to limit ongoing antigen-specific T_{FH} function. Hence, we propose a new layer of negative regulation during adaptive immunity that is a functional sensor of plasma cell production that can refine the development of antigen-specific B cell memory.

Results

Antigen-specific plasma cells express MHCII, CD80 and CD86

The antigen-specific B cell response to nitrophenylacetyl (NP) coupled to keyhole limpet hemocyanin (KLH) as a protein carrier is regulated by T_{FH} cells and directly accessible by flow cytometry14,37. Following NP-KLH immunization, antibody-secreting cells can be quantified using intracellular labeling with antigen, cell surface antigen binding and antigen-specific antibody secretion by ELISPOT (Supplementary Fig. 1). Therefore, antigen-specific ASCs (IgM^-CD138^+) with distinct developmental histories can be isolated for subsequent analysis of function (Fig. 1a). By day 5 after secondary immunization using the TLR4 agonist based Ribi adjuvant system, >90% of isotype-switched antibody-secreting cells did not incorporate BrdU over the previous 24 h *in vivo* (Fig. 1b). Thus, the majority of antibody-secreting cells (specific and non-specific) used in this study can be considered non-cycling, terminally differentiated plasma cells.

Within the antigen-specific compartment, IgM^-CD138^+ plasma cells expressed high amounts of surface MHCII protein (Fig. 1c). All recently formed plasma cells (non-GC at day 7 primary; post-GC at day 14 primary and memory-response at day 5 memory) expressed equivalent amounts of MHCII as compared to naive B cells and $CD11c^+$ DCs. Long-lived plasma cells that persist in the spleen (> day14 memory) or bone marrow (BM) (> day14 memory; data not shown) expressed lower but readily demonstrable amounts of MHCII. Isotype-switched plasma cells also up-regulated the co-stimulatory molecules CD80 and CD86 and retained abundant expression at all developmental stages after antigen experience (Fig. 1d). These data suggested that antigen-experienced plasma cells could retain the capacity for cognate intercellular interaction with pMHCII-specific T_H cells.

The same expression pattern for MHCII, CD80 and CD86 was evident in switched plasma cells regardless of antigen specificity and across different mouse strains (Supplementary Fig. 2). In contrast, many IgM^+ plasma cells had lost MHCII expression with the majority also negative for CD80 and CD86 (Supplementary Fig. 3). The mRNAs encoding MHCII, its

transactivator CIITA, CD80 and CD86 were present at very low but detectable levels in the switched plasma cell compartment (Supplementary Fig. 4) as expected from the negative impact of Blimp-1 on CIITA during plasma cell development36. Thus, isotype-switched plasma cells express key surface proteins associated with MHCII-restricted antigen presentation even in the presence of low mRNA encoding these proteins *in vivo*.

Plasma cells retain antigen presentation machinery

Next, we investigated the mechanism used by plasma cells to control MHCII-restricted antigen processing and presentation. Invariant chain protein (Ii; also called CD74) and CLIP-I-A^b complexes were readily detected intracellularly in antigen-specific IgM^-CD138^+ plasma cells in quantities similar to resting B cells (Fig. 2a). Peptide editing complexes H2-M and H2-O were also found by direct intracellular labeling for these proteins (Fig. 2b). The CD74, H2-M and H2-O proteins were detected at similar abundance in non-specific switched $MHCII^{hi}$ and $MHCII^{int}$ plasma cells representing recently-formed and long-lived plasma cell compartments, respectively (Supplementary Fig. 5). The abundance of mRNAs encoding CD74, H2-M and H2-O were low but detectable in this switched plasma cell compartment (Supplementary Fig. 5b–d). The mRNA for carboxypeptidases Cathepsin (Cts) B and CtsZ associated with early endosomes and the aminopeptidases AEP and CtsH associated with lysosomes were present in switched plasma cells (Fig. 2c). Other cathepsin protease mRNAs were expressed and present in low abundance (GILT, CtsS, CtsL, CtsC and CtsD; Supplementary Fig. 6). Therefore, in addition to cell surface MHCII, CD80 and CD86, there is abundant evidence for intracellular machinery involved in antigen processing and presentation in the specialized MHCII peptide-loading pathway *in vivo*.

Antigen-specific plasma cells present antigen *in vivo*

While the machinery for antigen processing is in place, it remained important to address the capacity of plasma cells to present specific antigenic peptides *in vivo*. Using hen egg lysozyme (HEL) as the immunogen, we visualized directly HEL-specific plasma cells in polyclonal B10.BR mice (H-2^k–restricted) 5 days after a secondary boost with soluble antigen (Fig. 3a). Direct labeling with Aw3.18 (ref. 38), an antibody specific for the dominant helper T cell epitope $HEL_{48–61}$ in I-A^k, revealed substantial amounts of specific pMHCII on HEL-specific plasma cells (Fig. 3b). There was negligible background antigen binding or pMHCII complex expression when animals were primed with irrelevant pigeon cytochrome c (PCC) antigen before the soluble HEL boost (Fig. 3b). Thus, antigen-specific memory-response plasma cells present detectable amounts of the dominant pMHCII complex 5 days after soluble protein rechallenge *in vivo*.

Plasma cells drive specific T_H cell proliferation

Ten days after primary adjuvant-driven immunization, the vast majority of antigen-specific plasma cells express isotype-switched BCRs with point mutations and evidence for antigen-driven selection during the GC reaction27. To test the capacity of post-GC plasma cells to present antigen, IgM^-CD138^+ plasma cells were isolated 10 days following immunization with PCC and co-cultured with naïve PCC-specific 5C.C7αβ transgenic T_H cells (Fig. 3c). The switched plasma cells from PCC immunized animals (but not adjuvant only controls)

promoted substantial proliferation of naïve antigen-specific T_H cells without addition of exogenous antigen. The addition of exogenous peptide promoted no further CD44 up-regulation or CFSE dilution *in vitro*. Thus, antigen-specific post-GC plasma cells express sufficient pMHCII and adequate co-stimulation *in vivo* to drive naïve antigen-specific T_H cell proliferation *in vitro*.

Next, to assess whether plasma cells could activate naïve T_H cells *in vivo*, we adoptively transferred $HEL_{48–61}$-pulsed plasma cells into animals containing CFSE-labeled 3A9αβ HEL-specific naïve transgenic T_H cells (Fig. 3d). In this transfer, HEL-peptide–pulsed plasma cells, but not the same dose of soluble HEL peptide, promoted activation and proliferation of T_H cells. Thus, plasma cells likely gain physical access to naïve T_H cells *in vivo* by migrating through lymphatics to the T cell zones of the draining LNs. In this manner, pMHCII-expressing plasma cells can activate antigen-specific T_H cells *in situ*. Hence, antigen-specific plasma cells access protein antigen *in vivo*, process and present pMHCII complexes in sufficient abundance to promote naïve T_H cell activation and proliferation.

Plasma cells cannot induce pre-GC T_{FH} development

Based on the capacity of switched plasma cells as antigen presenting cells (APCs), it became important to compare and contrast this activity with conventional APCs. The same numbers of switched plasma cells and $CD11c^+$ DCs pulsed with either peptide or protein antigen, induced similar activation and proliferation in naïve antigen-specific T_H cells (Fig. 4a and Supplementary Fig. 7). Similar results were seen across two antigen systems (PCC and HEL) using different naïve antigen-specific T_H cell populations (Supplementary Fig. 8). Even substantially decreasing antigen dose or APC to T_H cell ratios revealed very little difference between the different types of APC (Supplementary Fig. 9). Furthermore, activated antigen-specific T_H cells purified by flow cytometry for qPCR after culture (Supplementary Fig. 10) indicated that both types of APC induced robust and equivalent expression of mRNA (Fig. 4b) and protein (Supplementary Fig. 11) for a series of known T_H cell derived cytokines. However, in stark contrast, antigen-pulsed DCs induced high expression of IL-21 and Bcl-6 in activated T_H cells that were notably absent from the T_H cells in antigen-pulsed plasma cell cultures (Fig. 4c). Switched plasma cells uniquely induced Blimp-1, the transcriptional repressor of Bcl-6 and the T_{FH} program. Thus, unlike DCs, switched plasma cells were unable to drive naïve antigen-specific T_H cells towards the T_{FH} cell fate *in vitro*.

Plasma cells decrease IL-21 and Bcl-6 in T_{FH} cells

Antigen-specific T_{FH} cells are required for the generation of isotype-switched plasma cells *in vivo*. In this context, we considered it unlikely that naïve T_H cells would be the primary target of switched plasma cell APC activity *in vivo*. Therefore, we assessed whether antigen-primed plasma cells had an impact on the development or function of previously differentiated antigen-specific T_{FH} cells. To this end, we established primary T_H cell cultures using DCs and protein to induce T_{FH} cells (as above) and then subsequently divided the activated antigen-specific T_H cells across secondary cultures with isotype-switched plasma cells or $CD11c^+$ DCs, with or without antigen. $CD11c^+$ DCs supported further

accumulation of antigen-specific T_H cells regardless of antigen addition (Fig. 5a). In contrast, plasma cells supported no further accumulation of T_H cells in the absence of antigen and significantly decreased T_H cell numbers in the presence of antigen (Fig. 5a). The antigen-dependent decrease occurred without changes in the abundance of Bcl2, Bcl-X_L, Bax or Bak mRNA in the T_H cells suggesting no substantial changes by apoptosis in the culture (Supplementary Fig. 12a). Thus, in contrast to naïve T_H cell activation, switched plasma cells had a broad negative impact on previously activated effector T_H cells.

While IL-21 is produced across multiple effector T_H cell subtypes8 it has been a useful indicator of selective T_{FH} differentiation *in vitro*20–23. Importantly, there was a striking and antigen-dependent decrease in IL-21 controlled by switched plasma cells (Fig. 5b). This functional deviation was selective for IL-21 without impact on the production of other cytokines, such as tumor necrosis factor (TNF) and IL-10 (Supplementary Fig. 12b) demonstrating antigen-specific down-regulation of a major T_{FH} function. Furthermore, Bcl-6 is a major inducer of the T_{FH} developmental program *in vivo* and *in vitro*19–21. In the secondary cultures, plasma cells significantly reduced Bcl-6 expression in the antigen-specific T_H cells in an antigen-specific manner (Fig. 5b). We extended these results to *in vivo* antigen-activated 5C.C7αβ transgenic T_H cells that produced demonstrable amounts of IL-21 after priming with antigen. Within 24 h of short-term co-culture, in contrast to antigen-pulsed $CD11c^+$ DC, switched plasma cells decrease IL-21 expression and diminished Bcl-6 expression (Fig. 5c). This effect was selective as IL-4 and the transcription factor GATA3 were unaffected and T-bet expression similarly impacted with DC and PC presentation (Supplementary Fig. 12c). These trends were recapitulated using non-transgenic polyclonal $V_\alpha11^+V_\beta3^+$ antigen-specific T_H cells at day 7 after priming (Fig. 5d; Supplementary Fig. 12d). Therefore, antigen-specific plasma cells negatively regulate the function and developmental programming of antigen-specific T_{FH} cells *in vitro*.

T_{FH} cells accumulate in the absence of plasma cells

Towards understanding the role of plasma cell presentation *in vivo*, we immunized C56BL/6 mice lacking Blimp-1 in the B cell compartment. An antigen-specific primary and memory B cell response develops in these mice without antibody-secreting cells from all stages of the immune response30. Interestingly, the primary GC reaction is also significantly enlarged in these mice for reasons that remain unresolved (30; Supplementary Fig. 13a). Here, we quantified the numbers of $CXCR5^+$ $PD\text{-}1^+$ T_{FH} cells within the activated $CD44^{hi}CD62L^{lo}$ $ICOS^{hi}$ subset of $CD4^+$ T cells that accumulated after primary immunization (Fig. 6a and Supplementary Fig. 13b). In the absence of plasma cells, $CXCR5^+PD\text{-}1^+$ T_{FH} cells accumulate to significantly higher numbers in the draining lymph nodes (LN) and spleen at all time-points tested (D9, D11 and D15). Within the T_{FH} compartment, the brightest T_{FH} cells ($CXCR5^{++}PD\text{-}1^{++}$) were significantly higher (D11; $P < 0.01$) in the absence of plasma cells, suggesting a preferential accumulation of GC T_{FH} cells in these mice5.

After local development in the GC, high-affinity post-GC plasma cells migrate to distal tissues that favor longevity and/or function of the secreting compartment. In the absence of plasma cells, $CXCR5^+PD\text{-}1^+$ T_{FH} cells accumulate to significantly higher numbers in the mesenteric LNs, consistent with the loss of a local regulatory function for plasma cells in

this region (Supplementary Fig. 13c). Exaggerated numbers of $CXCR5^{-}PD\text{-}1^{+}$ cells accumulated within the $CD44^{hi}CD62L^{lo}$ compartment of $CD4^{+}$ T_H cells in the BM without plasma cells (Supplementary Fig. 13d). No change occurred within the $Ly6C^{+}$ memory $CD4^{+}$ T_H cell compartment, suggesting a selective impact on $PD\text{-}1^{+}$ T_H cells within this typically plasma cell rich environment. These data suggested an additional function for plasma cells within favored survival niches and perhaps for extended periods after initial development within local GC environments.

Next, we attempted to reverse the T_{FH} accumulation in the day 11 immunized Blimp-1 cKO mice. We transferred unfractionated LN and spleen cells from C57BL/6 animals 4 days after a secondary immunization with the same antigen-adjuvant combination. Transferring this source of antigen-specific plasma cells restored T_{FH} cell numbers in the immunized Blimp-1 cKO model without impacting cell numbers within littermate controls in the spleen (Fig. 6b) and significantly reducing elevated T_{FH} cell numbers in the LNs (Supplementary Fig. 14a). At day 7 after priming, the majority of antigen-specific T_H cells are within the follicular B cell areas but not within the GCs. At this early time-point, there was significant elevation of $CXCR5^{+}PD\text{-}1^{+}$ T_{FH} cells in both spleen and LN of Blimp-1 cKO animals (Fig. 6c and Supplementary Fig. 14b,c). Thus, in the absence of plasma cell development, the accumulation of elevated T_{FH} cells is consistent with the loss of a plasma cell-controlled local negative regulatory loop *in vivo*.

Plasma cells negatively impact T_{FH} cells in vivo

Following PCC immunization, $IgD^{-}CD138^{+}$ plasma cells are found in large numbers interspersed with T cells outside GCs at T-B borders and throughout T cell zones and the medullary regions (Fig. 7a–e). Furthermore, we provide evidence for co-localization of $CD138^{+}$ plasma cells with $V_{\beta}3^{+}$ T cells that include the dominant antigen-specific responder T_H cell compartment7,8 with evidence for cell contact at multiple points between $V_{\beta}3^{+}$ T cells and $CD138^{+}$ plasma cells at the T-B regions of these LNs (Fig. 7f–h). Adoptive transfer of plasma cells pulsed with MCC peptide two days before the peak of the local antigen-specific T_{FH} response induced a selective decrease in Bcl-6 and IL-21 expression and not IL-4 or GATA-3 (Fig. 7i) on a per cell basis within purified $CD44^{hi}CD62L^{lo}CXCR5^{+}$ $V_{\alpha}11^{+}V_{\beta}3^{+}$ T_{FH} cells as previously described7,8. There was no influence on the splenic T_{FH} response in the lymphoid tissues that did not directly drain the site of adoptive transfer (Supplementary Fig. 15). Thus, excessive numbers of plasma cells can negatively impact the local T_{FH} program within antigen-responsive T_{FH} cells *in vivo*.

DISCUSSION

The current findings contest the existing paradigm that plasma cells are only specialized for antibody secretion. We demonstrate that plasma cells can participate in the cognate regulation of ongoing adaptive immunity. Isotype-switched plasma cells process and present antigen *in vivo* and can function as highly competent APCs for naïve T_H cells. However, we propose that antigen-specific plasma cells act at a later phase in the developing immune response to negatively regulate antigen-specific T_{FH} cell function. The impact of negative regulation may extend into the GC reaction with influence on distal immune response sites

and plasma cell survival niches such as the BM. We propose a novel layer of negative regulation during adaptive immunity that senses antigen-specific plasma cell production to refine the ongoing development of high affinity B cell memory.

Plasma cells are clearly specialized to secrete antigen-specific antibody. Blimp-1 orchestrates plasma cell development by repressing existing B cell programs (Aicda, Bcl-6 and Pax-5), blocking cell cycle entry (c-Myc and E2f1) and initiating major changes in the secretory apparatus (J-chain) and unfolded protein response (XBP-1)31. In the current study, we provide clear evidence for the maintenance of proteins associated with MHCII antigen processing and presentation, despite a low abundance of mRNAs. How the MHCII machinery is maintained in the presence of low mRNA abundance and beyond the acute phase of the initial primary response remains unclear42,43. Long-term protein stability is possible, but more likely the low mRNA amounts are sufficient for production of protein in plasma cells highly specialized for protein secretion31. There have been previous reports of MHCII and co-stimulatory molecules on plasma cells39,40 and transformed myelomas41. However, the capacity of plasma cells to function as competent APCs was completely unexpected.

Post-mitotic cells that are highly-specialized for secretion still need to sense and respond to environmental cues to exert effective regulatory function *in vivo*. We provide clear evidence for continued expression of BCR on newly formed and long-lived plasma cells. The T_{FH} compartment is already sub-specialized for cognate pairing with antigen-experienced B cells3–5,44. Multiple co-stimulatory and adhesion molecule pairs are expressed across these two cell types to create strong, long duration interactions upon cognate contact17,18. First contact between T_{FH} and B cells leads to increased IL-21 and Bcl-6 expression10–12. Clear evidence also exists for cognate contact between GC T_{FH} cells and GC B cells by real time imaging45 and the sorting of T_{FH}-GC B cell conjugates46. However, the developmental consequences of these cognate interactions for the GC T_{FH} cells remain unclear. IL-21 production by T_{FH} cells is central to affinity maturation, GC persistence, function and B cell fate determination10,11. In the current study, we demonstrate the capacity of plasma cells to dampen effector T_{FH} cell production of IL-21 in a negative feedback loop. We propose that these actions comprise an acute regulatory activity to contract T_{FH} function, thereby constraining ongoing B cell immunity. Further, with evidence for continued BCR expression and MHCII presentation machinery in long-lived plasma cells, it is tempting to speculate a further role for these interactions in the long-term survival of plasma cells.

Commitment to the plasma cell fate requires T_{FH} contact. In the spleen, non-GC plasma cells first appear in T cell areas, migrate out through the bridging channels into the red pulp1,2. In the lymph nodes, plasma cells can accumulate at the T-B borders and the medullary cords47. The post-GC plasma cell commitment is also considered to be dependent on GC T_{FH} signals. Evidence for PD-1 involvement13, a direct role for IL-2110,11 and expression of Blimp-1 within the GC itself48 further support this position. Under normal physiological conditions, it is difficult to see plasma cell differentiation within the GC compartment. However, in the absence of the chemokine EBI-2, selected GC B cells are incapable of leaving the GC and produce more discernible compartments of putative plasma cells *in situ*49. The rapid production of plasma cells upon antigen rechallenge is dependent

on $CD4^+$ T_{FH} cells, however the distribution of memory response plasma cells in lymph nodes has not been well characterized. Nevertheless, these trends indicate that plasma cells or their immediate precursors reside within the same microenvironments as T_{FH} subtypes to enable their selective control of the T_{FH} program *in vivo*.

Elevation of T_{FH} cells in the absence of plasma cells provides support for the existence of this negative regulatory loop *in vivo*. There were exaggerated numbers of all $CXCR5^+PD\text{-}1^+$ T_{FH} cells in these animals across multiple time-points after antigen priming. The GC T_{FH} cell compartment expresses the highest amounts of PD-1 and CXCR5 (ref. 5) and accumulates to higher numbers in the absence of plasma cells *in vivo*. It has also been suggested that these cells express the highest ICOS expression and more recently GL-7 expression50 as a GC subset-specific marker. While B cell memory develops in the absence of plasma cells30, its capacity for long-term immune protection is limited in the absence of plasma cells. These animals are also devoid of secreted antibodies that may influence the contraction of adaptive immunity. However, in the absence of FcγR on T_H cells these effects are unlikely to directly influence the T_{FH} program. Furthermore, elevated GC T_{FH} cells correlates with dysregulated GC selection and autoantibody production5. Thus, in the absence of plasma cells, we would predict that increased GC T_{FH} cells results in elevated IL-21 production that could alter normal GC physiology and memory B cell development and function.

Our studies into the mechanism of negative regulation by plasma cells indicate the requirement for antigen to dampen antigen-specific T_{FH} activity. These *in vitro* studies indicate cognate contact is necessary but do not report on the effector mechanism. Recently, PD-1 expression by T_{FH} cells and PD-L2 on GC B cells was shown to play a role in regulating selection in the GC and the numbers of long-lived plasma cells13. IL-21 itself plays in important role in these dynamic activities and the generation of high affinity memory B cells10,11. Thus, following cognate contact, the delivery of secondary modifying signals may be cell-associated and secreted. It is also plausible that different sets of GC T_{FH} cells may impact different types of GC B cells. For example, pairing of IL-4-secreting GC T_{FH} cells with IgG1-expressing GC B cells46 introduced the possibility of T_{FH}-B cell subset dedication. Similarly, the rules governing antigen-specific regulation by plasma cells *in vivo* focus around specific cognate TCR-pMHCII interactions and may be subsequently modified by cell-associated and secreted molecular determinants of cell fate.

Under the normal dynamics of protein vaccination, constraining T_{FH} numbers and reduced IL-21 production may progressively increase competitive pressure critical for GC survival signals. In this manner, we predict that the negative regulation of GC T_{FH} function could enhance affinity maturation within the GC and promote memory B cells with higher overall affinities. This cellular `product to inducer' negative regulatory mechanism may also increase GC selection thresholds to help guard against potential self-reactivity in the GC. In the memory response, a negative regulatory loop would act as an antigen-specific plasma cell sensing mechanism to limit the accelerated and explosive plasma cell production that accompanies antigen re-challenge. We suggest that sensing the antigen-specific plasma cell product of the T_{FH} regulatory pathway may also provide an efficient means to constrain autoreactivity and fine-tune ongoing antigen-specific B cell immunity *in vivo*.

Cognate control of B cells by T_{FH} cells is an important and currently active arena of research. We believe that the impact of B cell-controlled antigen presentation on antigen-specific T_{FH} cell development and function remains poorly understood. This cascade of bi-directional regulation is central to effective adaptive immunity and regulates the emergence of antigen-specific immune memory. The new mechanism of antigen-specific plasma cell control that we uncover in the current study is one important facet of this largely unexplored cognate immune regulatory mechanism.

METHODS

Mice

C57BL/6, B10.BR, B10.BR-Thy1.1, 5C.C7-TCRαβ, 3A9-TCRαβ and $Prdm1^{flox/flox}CD19^{cre/+}$ mice were maintained in specific pathogen-free conditions at The Scripps Research Institute (TSRI). $Prdm1^{flox/flox}CD19^{cre/+}$ mice were obtained from K. Calame (Columbia University) and backcrossed 9 generations onto C57BL/6. The Scripps Research Institutional Animal Care and Use Committee approved all experiments.

Immunization

Mice were immunized subcutaneously with 400 μg NP-KLH, PCC protein (Sigma) or HEL protein (Sigma) in monophosphoryl lipid A based adjuvant7 for primary immunization. Secondary challenge was done ≥ 6 weeks after primary immunization and was either identical to primary or 40 μg soluble HEL in PBS.

Flow cytometry

Cell suspensions as previously described7. For surface staining, 4 × 108 cells per mL for 45 min at 4°C with labeled mAbs: CD3 (145.2C11), CD8 (53-6.7), CD11c (HL3), CD62L (MEL-14), CD80 (16-10A1), CD86 (GL1), CD138 (281-2), B220 (RA3-6B2), IgM (II/41), GL7 (GL7), Ly6C (AL21), CXCR5 (2G8) and $I\text{-}A^k$ (10-3.6; all from BD Pharmingen); CD4 (GK1.5), CD11c (N418), CD278 (ICOS, C398.4A), Gr1 (RB6-8C5) and $I\text{-}A^b$ (KH74; all from BioLegend); CD19 (6D5), CD38 (90), CD44 (IM7), PD1 (J43) and F4/80 (BM8; all from eBioscience); CD11b (M1/70.15), $V_\beta 3$ (KJ25), IgD (11.26), Aw3.18 NP (4-hydroxy 3-nitrophenylacetyl, Biosearch) and HEL (Sigma) were conjugated in the laboratory. For double biotinylated mAbs, cross reactivity was reduced by blocking with excess free biotin (Perbio).

For intracellular staining, surface labeled cells were then permeabilized and fixed using Cytofix/Cytoperm (BD Pharmingen) then labeled with mAbs: IgG1 (A85-1), IgG2a (R19-15), IgG2b (R12-3), IgG3 (R40-82), IgA (R5-140), IgM (R6-60.2), CD74 (In-1; all from BD Pharmingen); H2M (2C3A), H2O (Ob1); and $I\text{-}A^b$-CLIP (15G4). Samples analyzed on FACS Vantage SE or FACSAria III flow cytometers (BD Biosciences) and data processed using FlowJo software (Tree Star). Exclusion criteria used are referred to as “Dump” in the figure legend and included antibodies to CD4, CD8, Gr1 and F4/80 (for PC, naive B cells); B220, CD8, CD11b (for $CD4^+$ T_H cells) and CD19, CD3 (for $CD11c^+$ DC).

BrdU Analysis

Mice received a single injection of 800μg BrdU (BD Biosciences) IP and cells prepared using BrdU flow kit (BD Pharmingen).

Ag-presentation assays

After *in vivo* priming with protein antigen PCC or adjuvant only, plasma cells were sorted from lymph nodes 10 days after immunization using $PI^{-}Dump^{-}IgD^{-}CD138^{+}IgM^{-}$ and cultured for 4 days at 5×10^3 cells per well with 5 × 104 naïve PCC-specific T_H cells ($PI^{-}Dump^{-}CD4^{+}CD44^{-}$H $CD62L^{+}$) sorted from 5C.C7-TCRαβ transgenic mice and labeled with 5 μM CFSE (Molecular Probes) for 5 min at 24°C. For *in vivo* antigen-presentation, 9.0 × 105 isotype-switched plasma cells were sorted from lymph nodes 5 days after immunization with adjuvant only, pulsed for 1 h at 24°C with 10 μM HEL peptide and transferred subcutaneously into B10.BR mice that had previously received the equivalent of 5 × 106 CFSE-labeled 3A9-TCR transgenic T_H cells from donor 3A9 spleens. T cell proliferation was analyzed 3 days later. For *in vitro* antigen-presentation assay to naïve T_H cells, 5 × 104 CFSE-labeled naïve PCC-specific T_H cells were cultured with 5×10^3 plasma cells or DC ($PI^{-}Dump^{-}CD11c^{+}$), sorted from lymph nodes 5 days after immunization with adjuvant only, and with 10 μM $MCC_{88\text{-}103}$ peptide (Anaspec) or 10 μM PCC protein as indicated.

For antigen-presentation assay to activated T cells, PCC-specific naïve T cells were activated either *in vitro* for 4 days with DC in the presence of PCC, or *in vivo* for 7 days after transfer of 5 × 105 5CC7αβ transgenic T_H cells into B10BR immediately immunized with PCC-adjuvant or sorted antigen-activated $V_\alpha 11^{+}V_\beta 3^{+}$ T_H cells from B10BR mice without transfer as previously described8. Subsequent activated T_H cells ($PI^{-}Dump^{-}CD4^{+}CD44^{hi}CD62L^{lo}$) were sorted and cultured at a 10:1 ratio with plasma cells or DCs with or without PCC for 3 days or 24 h. All cultures were done in RPMI medium supplemented with 10% (V/V) FCS, 1× Penicillin-streptomycin-Lglutamine, 50 μM 2-mercaptoethanol (Invitrogen). For adoptive transfer into Blimp-1 cKO and littermate controls 1.0 × 108 unfractionated spleen and LN mixtures from day 4 secondary NP-KLH in Ribi immunized C57BL/6 mice. Cells were distributed evenly IP and base of tail into recipient animals at day 9 after initial priming of both groups of mice. For antigen-presentation assay to antigen-specific T_{FH} cells *in vivo*, 3.0 × 105 isotype-switched plasma cells were sorted from lymph nodes 5 days after immunization with adjuvant only, pulsed for 1 h at RT with 10 μM MCC peptide and transferred subcutaneously into B10.BR mice that had been immunized 5 days earlier with PCC in adjuvant. T_{FH} were sorted 2 days after for gene expression analysis.

ELISPOT assays.

Plasma cell function was measured by ELISPOT as described previously14 using NPBSA or anti-mouse IgM and IgG (Southern Biotechnology Associates).

mRNA estimation using RT-qPCR

For qPCR directly *ex vivo*, 1 × 106 T_H cells ($PI^-Dump^-CD4^+$), DCs ($PI^-Dump^-CD11c^+$), naïve B cells ($PI^-Dump^-B220^+IgD^+IgM^+$), or isotype-switched PC ($PI^-Dump^-IgD^-CD138^+IgM^-$) were sorted either from lymph nodes of HEL-adjuvant-immunized B10.BR mice 1 day post-immunization (T cells, DCs) or from spleen of HEL-adjuvant-immunized B10.BR mice 5 days post-secondary immunization (B cells, PCs). For gene expression analysis following antigen-presentation assay, activated T_H cells ($PI^-Dump^-CD4^+CD44^{hi}CFSE^{lo}$) were sorted from individual wells after culture. For gene expression analysis following plasma cell transfer *in vivo*, antigen-specific T_{FH} cells ($PI^-Dump^-CD4^+V\alpha11^+V\beta3^+CD44^{hi}CD62L^{lo}CXCR5^+$) were sorted from B10.BR mice 7 days after PCC immunization. All samples were sorted for RNA extraction, cDNA synthesis and qPCR as previously described8. mRNA purified after *in vitro* Ag-presentation assay to naïve T_H cells (Fig. 4b & 4c) was amplified with two rounds of *in vitro* transcription as previously described8. The primers used are included in Supplementary Table 1. The results were analyzed with Rotor-Gene 6.0 software or StepOne software v2.0.

Confocal microscopy

Inguinal lymph nodes were embedded in Tissue-Tek OCT compound (Sakura) and snap-frozen on dry ice. 6-8 μm sections were fixed in cold acetone. Staining were performed in PBS containing 10% FCS, 0.1% sodium azide with labeled Abs: IgD (11.26), $V_\beta3$ (KJ25 labeled in the laboratory); CD90.2 (53-2.1), CD138 (281.2, both from BD biosciences), anti-rat IgG (vector) and rhodamin red-streptavidin (invitrogen). Confocal images were analyzed with an Olympus Fluoview 500 confocal microscope.

Statistical analysis

Mean values, standard error of the mean (s.e.m.) values, Student's *t*-test (unpaired) and Mann-Whitney test were calculated with GraphPad Prism (GraphPad Software).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank L. Denzin for providing 2C3A and Ob1, A. Rudensky for 15G4 monoclonal antibodies (Memorial Sloan Kettering Cancer Center, New York, NY and E. Unanue (Washington University, St Louis, MO) for providing the Aw3.18 cell line. We are grateful to Kathy Spencer for her help with confocal imaging E.U received a DAAD German Academic Exchange Service Award and is German Research Foundation Fellowship recipient. K.A.W is a Canadian Institutes of Health Research Fellowship recipient (MFE-98574). The work was supported by the National Institutes of Health (AI047231, AI040215 and AI071182 to M.G.M-W.). This is TSRI manuscript 20113.

REFERENCES

1. MacLennan IC. Germinal centers. Annu Rev Immunol. 1994; 12:117–139. [PubMed: 8011279]
2. McHeyzer-Williams LJ, McHeyzer-Williams MG. Antigen-specific memory B cell development. Annu Rev Immunol. 2005; 23:487–513. [PubMed: 15771579]
3. Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG. Follicular helper T cells: lineage and location. Immunity. 2009; 30:324–335. [PubMed: 19303387]

4. King C, Tangye SG, Mackay CR. T follicular helper (TFH) cells in normal and dysregulated immune responses. Annu Rev Immunol. 2008; 26:741–766. [PubMed: 18173374]
5. Vinuesa CG, Sanz I, Cook MC. Dysregulation of germinal centres in autoimmune disease. Nat Rev Immunol. 2009; 9:845–857. [PubMed: 19935804]
6. Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG, Cyster JG. In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. J Exp Med. 1999; 190:1123–1134. [PubMed: 10523610]
7. Fazilleau N, et al. Lymphoid reservoirs of antigen-specific memory T helper cells. Nat Immunol. 2007; 8:753–761. [PubMed: 17529982]
8. Fazilleau N, McHeyzer-Williams LJ, Rosen H, McHeyzer-Williams MG. The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. Nat Immunol. 2009; 10:375–384. [PubMed: 19252493]
9. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. Nat Rev Immunol. 2005; 5:853–865. [PubMed: 16261173]
10. Linterman MA, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010; 207:353–363. [PubMed: 20142429]
11. Zotos D, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010; 207:365–378. [PubMed: 20142430]
12. Poholek AC, et al. In vivo regulation of Bcl6 and T follicular helper cell development. J Immunol. 2010; 185:313–326. [PubMed: 20519643]
13. Good-Jacobson KL, et al. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. Nat Immunol. 2010; 11:535–542. [PubMed: 20453843]
14. McHeyzer-Williams LJ, Cool M, McHeyzer-Williams MG. Antigen-specific B cell memory: expression and replenishment of a novel b220(−) memory b cell compartment. J Exp Med. 2000; 191:1149–1166. [PubMed: 10748233]
15. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998; 392:245–52. PAGES? [PubMed: 9521319]
16. Garside P, et al. Visualization of specific B and T lymphocyte interactions in the lymph node. Science. 1998; 281:96–99. [PubMed: 9651253]
17. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. Nature. 2008; 455:764–769. [PubMed: 18843362]
18. Okada T, et al. Antigen-engaged B cells undergo chemotaxis toward the T zone and form motile conjugates with helper T cells. PLoS Biol. 2005; 3 doi: 10.1371/journal.pbio.0030150. Pages or DOI?
19. Johnston RJ, et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science. 2009; 325:1006–1010. [PubMed: 19608860]
20. Nurieva RI, et al. Bcl6 mediates the development of T follicular helper cells. Science. 2009; 325:1001–1005. [PubMed: 19628815]
21. Yu D, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity. 2009; 31:457–468. [PubMed: 19631565]
22. Nurieva RI, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity. 2008; 29:138–149. [PubMed: 18599325]
23. Vogelzang A, et al. A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity. 2008; 29:127–137. [PubMed: 18602282]
24. Ozaki K, et al. A critical role for IL-21 in regulating immunoglobulin production. Science. 2002; 298:1630–4. PAGES? [PubMed: 12446913]
25. Spolski R, Leonard WJ. The Yin and Yang of interleukin-21 in allergy, autoimmunity and cancer. Curr Opin Immunol. 2008; 20:295–301. [PubMed: 18554883]
26. Jacob J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. II. A common clonal origin for periarteriolar lymphoid sheath-associated foci and germinal centers. J Exp Med. 1992; 176:679–687. [PubMed: 1512536]

27. McHeyzer-Williams MG, McLean MJ, Lalor PA, Nossal GJ. Antigen-driven B cell differentiation in vivo. J Exp Med. 1993; 178:295–307. [PubMed: 8315385]
28. Ho F, Lortan JE, MacLennan IC, Khan M. Distinct short-lived and long-lived antibody-producing cell populations. Eur J Immunol. 1986; 16:1297–1301. [PubMed: 3490389]
29. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. Immunity. 1998; 8:363–372. [PubMed: 9529153]
30. Shapiro-Shelef M, et al. Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells. Immunity. 2003; 19:607–620. [PubMed: 14563324]
31. Martins G, Calame K. Regulation and functions of Blimp-1 in T and B lymphocytes. Annu Rev Immunol. 2008; 26:133–169. [PubMed: 18370921]
32. Reimold AM, et al. Plasma cell differentiation requires the transcription factor XBP-1. Nature. 2001; 412:300–307. [PubMed: 11460154]
33. Todd DJ, et al. XBP1 governs late events in plasma cell differentiation and is not required for antigen-specific memory B cell development. J Exp Med. 2009; 206:2151–9. PAGES? [PubMed: 19752183]
34. Ozaki K, et al. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol. 2004; 173:5361–5371. [PubMed: 15494482]
35. Kwon H, et al. Analysis of interleukin-21-induced Prdm1 gene regulation reveals functional cooperation of STAT3 and IRF4 transcription factors. Immunity. 2009; 31:941–52. PAGES? [PubMed: 20064451]
36. Piskurich JF, et al. BLIMP-I mediates extinction of major histocompatibility class II transactivator expression in plasma cells. Nat Immunol. 2000; 1:526–532. [PubMed: 11101876]
37. McHeyzer-Williams MG, Nossal GJ, Lalor PA. Molecular characterization of single memory B cells. Nature. 1991; 350:502–505. [PubMed: 2014051]
38. Dadaglio G, Nelson CA, Deck MB, Petzold SJ, Unanue ER. Characterization and quantitation of peptide-MHC complexes produced from hen egg lysozyme using a monoclonal antibody. Immunity. 1997; 6:727–738. [PubMed: 9208845]
39. Ellyard JI, et al. Antigen-selected, immunoglobulin-secreting cells persist in human spleen and bone marrow. Blood. 2004; 103:3805–12. PAGES? [PubMed: 14701691]
40. Gonzalez-Garcia I, Ocana E, Jimenez-Gomez G, Campos-Caro A, Brieva JA. Immunization-induced perturbation of human blood plasma cell pool: progressive maturation, IL-6 responsiveness, and high PRDI-BF1/BLIMP1 expression are critical distinctions between antigen-specific and nonspecific plasma cells. J Immunol. 2006; 176:4042–50. PAGES? [PubMed: 16547239]
41. Yi Q, Dabadghao S, Osterborg A, Bergenbrant S, Holm G. Myeloma bone marrow plasma cells: evidence for their capacity as antigen-presenting cells. Blood. 1997; 90:1960–1967. [PubMed: 9292530]
42. Denzin LK, Fallas JL, Prendes M, Yi W. Right place, right time, right peptide: DO keeps DM focused. Immunol Rev. 2005; 207:279–292. [PubMed: 16181343]
43. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. Annu Rev Immunol. 2005; 23:975–1028. [PubMed: 15771591]
44. Crotty S, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nat Immunol. 2010; 11:114–20. PAGES? [PubMed: 20084069]
45. Allen CD, Okada T, Tang HL, Cyster JG. Imaging of germinal center selection events during affinity maturation. Science. 2007; 315:528–531. [PubMed: 17185562]
46. Reinhardt RL, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. Nat Immunol. 2009; 10:385–393. [PubMed: 19252490]
47. Mohr E, et al. Dendritic cells and monocyte/macrophages that create the IL-6/APRIL-rich lymph node microenvironments where plasmablasts mature. J Immunol. 2009; 182:2113–23. PAGES? [PubMed: 19201864]
48. Angelin-Duclos C, Cattoretti G, Lin KI, Calame K. Commitment of B lymphocytes to a plasma cell fate is associated with Blimp-1 expression in vivo. J Immunol. 2000; 165:5462–5471. [PubMed: 11067898]

49. Gatto D, Paus D, Basten A, Mackay CR, Brink R. Guidance of B cells by the orphan G protein-coupled receptor EBI2 shapes humoral immune responses. Immunity. 2009; 31:259–69. PAGES? [PubMed: 19615922]

50. Yusuf I, et al. Germinal center T follicular helper cell IL-4 production is dependent on signaling lymphocytic activation molecule receptor (CD150). J Immunol. 2010; 185:190–202. [PubMed: 20525889]

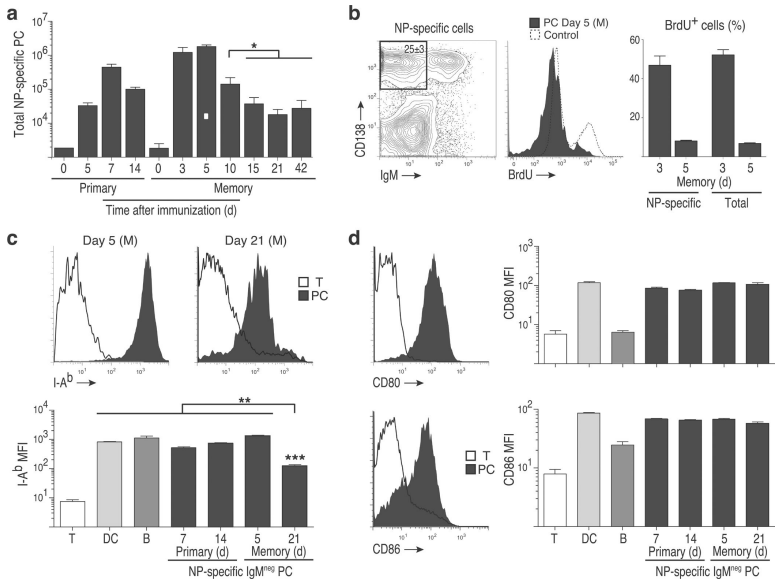

Figure 1. Isotype-switched plasma cells retain expression of MHCII and co-stimulation molecules

a, NP-specific plasma cell numbers over time ($PI^{-}Dump^{-}IgD^{-}$ and $NP^{+}CD138^{+}$) in the spleen of C57BL/6 mice after primary and secondary immunization with NP-KLH (adjuvant only for day 5 memory white square)(mean±sem; n≥4, * $p<0.05$).

b, CD138 versus IgM expression on NP-specific cells (gated as $PI^{-}Dump^{-}IgD^{-}$ and NP^{+}) at day 5 after secondary challenge (left). BrdU expression on NP-specific $IgM^{-}CD138^{+}$ plasma cells at day 5 memory (middle). Frequency of $BrdU^{+}$ NP-specific $IgM^{-}CD138^{+}$ plasma cells at day 3 and day 5 memory compared to total $IgM^{-}CD138^{+}$ plasma cell compartment (right), 20h after single IP injection with BrdU (mean±sem, n≥3).

c–d, $I\text{-}A^{b}$ (MHCII) **(c)**, CD80 and CD86 **(d)** expression with summary mean fluorescence intensity (MFI) on NP-specific $IgM^{-}CD138^{+}$ plasma cells compared to $CD4^{+}$ T_H cells (T), $CD11c^{+}$ DC and naive B cells (B) (mean±sem, n≥3, ** $p<0.01$, *** $p<0.001$).

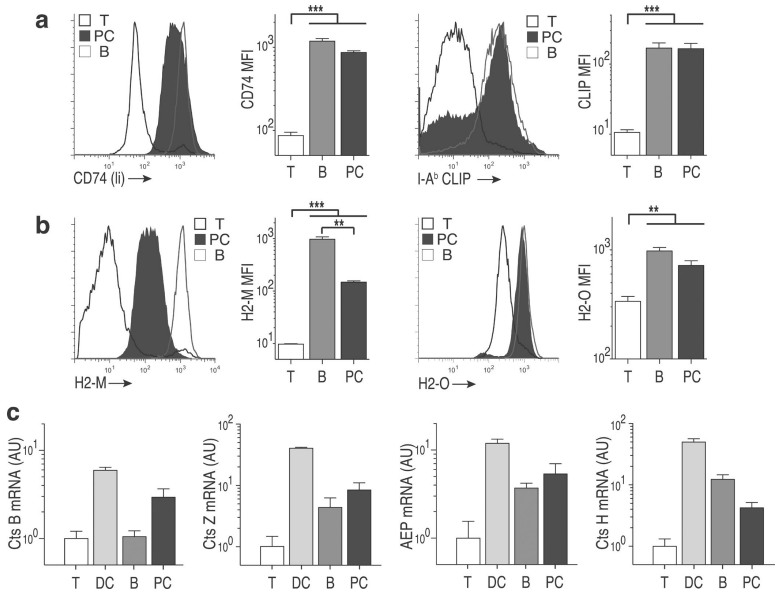

Figure 2. Plasma cells express intracellular machinery for MHCII antigen processing and presentation

a–b, Intracellular expression of CD74 (Ii, Invariant chain) and I-A^b-CLIP **(a)**, H2-M and H2-O **(b)** with summary MFI on NP-specific IgM^-CD138^+ plasma cells (PC) in C57Bl/6 spleens at day 5 post secondary immunization with NP-KLH compared to naive B cells (B) and $CD4^+$ T_H cells (T)(mean±sem; n=3, ** p<0.01, *** p<0.001).

c, mRNA expression analysis by quantitative PCR for the indicated proteases in $CD4^+$ T_H cells, $CD11c^+$ DC, naïve B cells and IgM^-CD138^+ plasma cells (PC). Estimates are presented in arbitrary units (AU) relative to naïve T cells set as 1 (mean±sem, n≥3).

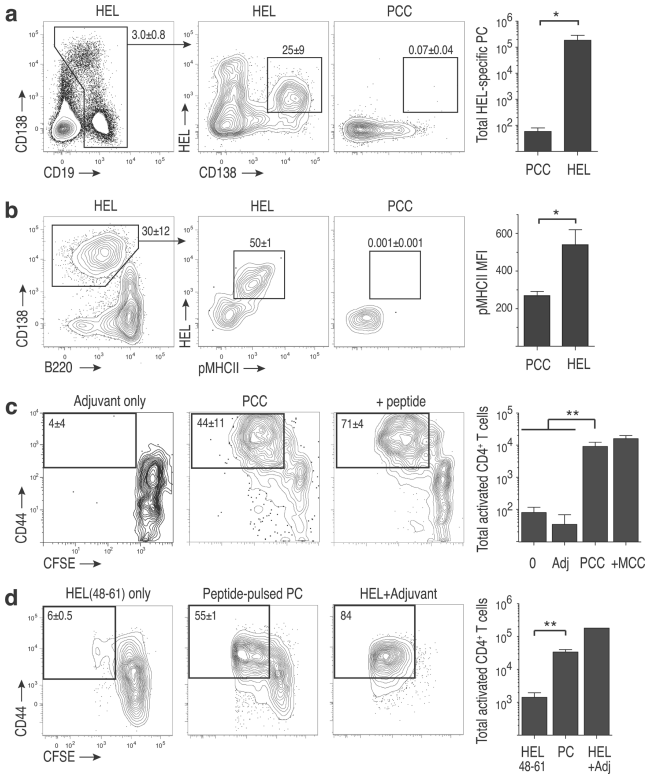

Figure 3. Antigen-specific plasma cells process and present antigen in vivo

a, Day 5 after secondary challenge with soluble HEL in B10.BR mice primary immunized with HEL (left and middle) or PCC (right). Isotype-switched B cell compartment ($Dump^{-}IgD^{-}IgM^{-}$ and $CD19^{+}/CD138^{+}$) (left) displaying HEL-binding and CD138 expression (middle), frequencies (insert), total cell numbers (right) for switched HEL-specific plasma cells (mean±sem; n=3, * $p<0.05$).

b, HEL binding versus peptide-MHCII (pMHCII) expression (left panels) with summary MFI for pMHCII (right) on isotype-switched plasma cells (gated as $Dump^{-}IgD^{-}IgM^{-}$ and $B220^{-}CD138^{+}$ as described in **a** (mean±sem; n=3, * $p<0.05$).

c, CD44 and CFSE expression on CFSE-labeled 5C.C7αβ TCR transgenic T_H cells cultured for 4 days with isotype-switched plasma cells isolated 10 days after adjuvant only or PCC protein vaccination compared to addition of MCC peptide. Frequency of responders (inserts), total $CD44^{hi}CFSE^{lo}$ T_H cells (right) (mean±sem, n>3, ** $p<0.01$).

d, CD44 and CFSE expression (left) on CFSE-labeled 3A9αβ HEL-specific TCR transgenic T_H cells 24h after transfer into B10.BR: immunization with peptide alone (left), transfer of $IgM^{-}CD138^{+}$ plasma cells pulsed with 10μM HEL peptide (middle) (mean±sem; n=3), immunization with HEL in adjuvant (n=1) (right). Frequencies of responders (inserts), total $CD44^{hi}CFSE^{lo}$ T_H cells (right) (mean±sem; n=3, ** $p<0.01$).

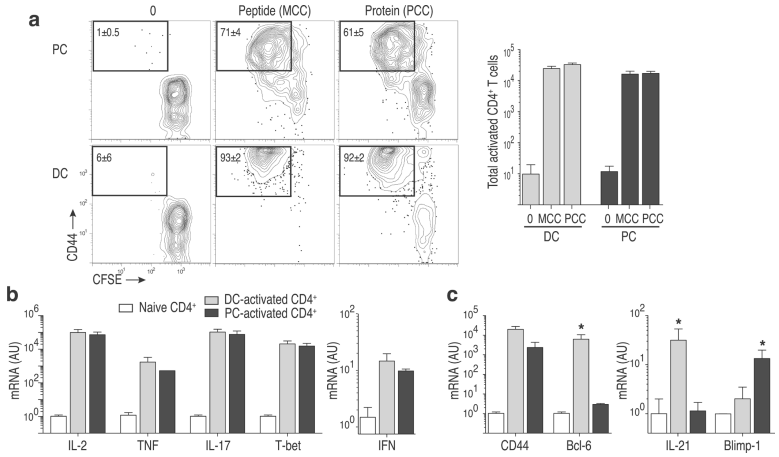

Figure 4. Plasma cells induce proliferation, multiple T_H cell functions and Blimp-1 but not Bcl-6 or IL-21 expression

a, CD44 and CFSE expression on CFSE-labeled 5C.C7αβ TCR transgenic T_H cells activated for 4 days in vitro with IgM^-CD138^+ plasma cells or $CD11c^+$ DC alone (0), MCC (peptide) or PCC (protein) (mean±sem frequency, n≥10). Frequencies of responders are displayed within inserts (left panels) and summary for total activated $CD44^{hi}CFSE^{lo}$ T_H cell numbers is presented for each condition (right) (mean±sem, n≥10).

b–c, mRNA expression by RT-qPCR for the indicated molecules in $CD4^+$ T_H cells activated in cultures using $CD11c^+$ DC or IgM^-CD138^+ plasma cell as antigen-presenting cells in the presence of PCC protein. Levels of expression are compared to levels in naive $CD4^+$ T_H cells directly ex vivo. Signals from all samples are normalized to β2m and compared to naive T_H cell values set at 1 (mean±sem, n≥3, * $p<0.05$).

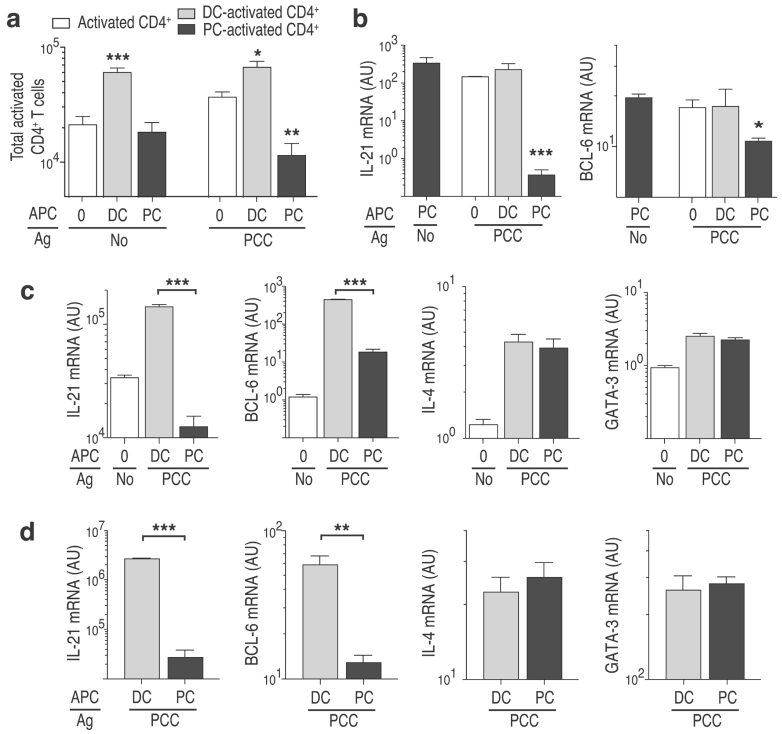

Figure 5. Switched plasma cells selectively inhibit IL-21 and Bcl-6 expression in T_{FH} cells

a, Total $CD44^{hi}CD62L^{lo}$ T_H cells after secondary culture of in vitro-activated CFSE-labeled 5C.C7αβ TCR transgenic T_H cells in medium alone (0) or with $CD11c^+$ DC or IgM^-CD138^+ plasma cells (PC) as APC with or without PCC antigen (mean±sem, n≥3, statistics to "0" condition, *** $p<0.001$).

b, IL-21 and Bcl-6 mRNA expression by RT-qPCR of in vitro-activated T_H cells reactivated in the indicated secondary culture conditions. Signals are normalized to β2m and compared to naive T_H cell values set at 1 (mean±sem, n≥3, statistics to "0" condition, * $p<0.05$, *** $p<0.001$).

c, mRNA expression for the indicated molecules by RT-qPCR in 5C.C7αβ transgenic T_H cells activated in vivo, sorted and reactivated in the indicated culture conditions. The white bar indicates the mRNA level of in vivo-activated T_H cells before culture. Signals as in b (mean±sem, n≥3, statistics to "0" condition, ** $p<0.01$, *** $p<0.001$).

d, mRNA expression for the indicated molecules by RT-qPCR from non-transgenic, in vivo antigen-activated $Vα11^+Vβ3^+$ T_H cells day 7 post PCC immunization and reactivated in the indicated culture conditions. Signals as in c (mean±sem, n=4 from two separate experiments, ** $p<0.01$, *** $p<0.001$).

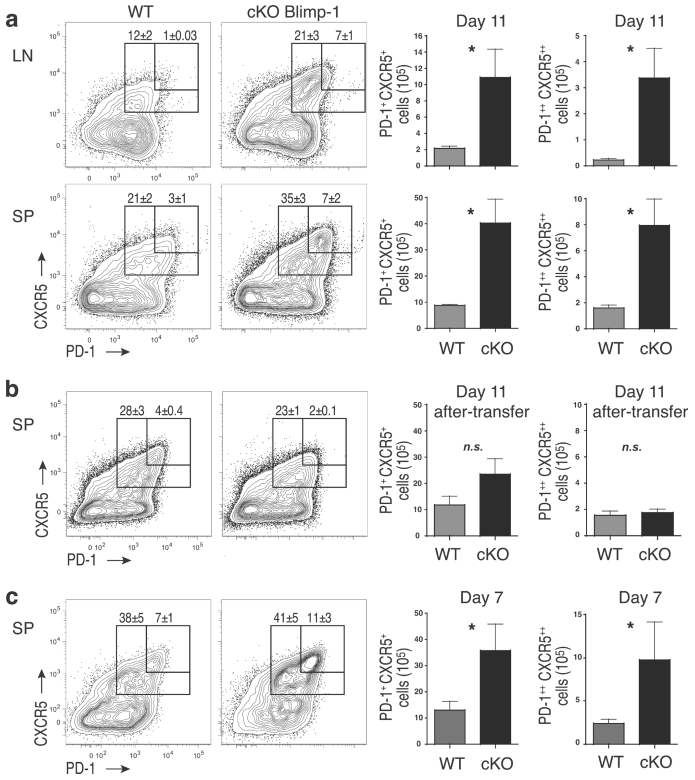

Figure 6. Elevated T_{FH} accumulation in the absence of plasma cells in vivo

a, CXCR5 versus PD-1 expression on lymph node (LN) and spleen (SP) activated CD4 T_H cells (gated as CD16/32$^-$B220$^-$CD8^{--}CD4$^+$CD62L^{lo}CD44^{hi}) from littermate (WT; left) and Prdm1$^{flox/flox}$CD19$^{Cre/+}$ (right; cKO Blimp-1) mice 11 days after vaccination with NP-KLH in adjuvant (mean±sem frequency; n=3). Summary of total PD-1$^+$CXCR5$^+$ and PD-1^{++}CXCR5^{++} activated T_H cell numbers (mean±sem, n=3, * $p<0.05$).

b, CXCR5 versus PD-1 expression on activated spleen CD4 T_H cells from littermate (WT, left) and cKO Blimp-1 (right) 11 days after vaccination with NP-KLH in adjuvant (mean ±sem frequency; n=3). Both groups of animals received 5.0 × 107 unfractionated LN and spleen cell mixtures IP and base of tail from C57BL/6 animals day 4 following secondary NP-KLH immunization. Summary of total PD1$^+$CXCR5$^+$ activated T_H cell numbers (mean ±sem, n=3, * $p<0.05$; ns, not significant).

c, CXCR5 versus PD-1 expression on activated splenic CD4 T_H cells (gated as in above) from littermate (WT; left) and Prdm1$^{flox/flox}$CD19$^{Cre/+}$ (right; cKO Blimp-1) mice 7 days after vaccination with NP-KLH in adjuvant (mean±sem frequency; n=3). Summary of total PD-1$^+$CXCR5$^+$ and PD-1^{++}CXCR5^{++} activated T_H cell numbers (mean±sem, n=3, * $p<0.05$).

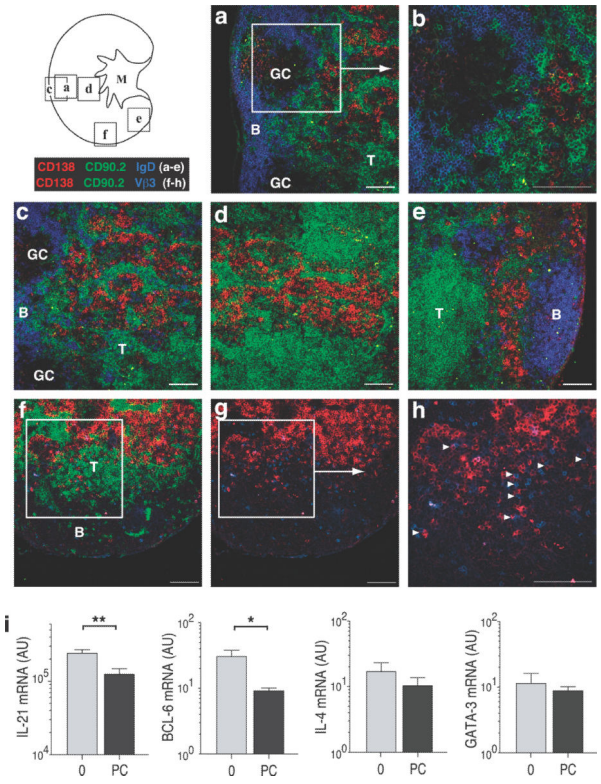

Figure 7. Plasma cells co-localize with CD4$^+$ T cells and negatively impact antigen specific T_{FH} program in vivo

a–h, Confocal microscopy of draining LNs from PCC-immunized B10.BR mice at day 10 after priming. The approximate location of images is indicated on the diagram, medulla (M), with regions indicated for B cell follicle (B), germinal center (GC), T cell area (T) indicated in panels. Labeled antibodies for antigens are based on representative color used as depicted separately for panels a–e and f–h. White arrowheads in h highlight examples of CD138$^+$ plasma cell contact with Vβ3$^+$ T cells. Representative sections from one of three similar experiments are shown. Scale bar represents 100μm.

d, mRNA expression for the indicated molecules by RT-qPCR in T_{FH} cells sorted as Vα11$^+$Vβ3$^+$CD44^{hi}CD62L^{lo} and CXCR5$^+$ from lymph nodes of B10.BR mice day 7 post PCC vaccination that had received (PC) or not (0) plasma cell transfer 2 days before analysis. Signals are normalized to β2m and compared to naive T_H cell values set at 1 (mean ±sem, n=3 across duplicate wells, * $p<0.05$, ** $p<0.01$).