Regulation of $T_H2$ development by CXCR5$^+$ dendritic cells and lymphotoxin-expressing B cells

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Although cognate encounters between antigen-bearing dendritic cells (DCs) that express the chemokine receptor CCR7 and CCR7$^+$ naive T cells take place in the T cell zone of lymph nodes, it is unknown whether the colocalization of DCs and T cells in the T cell area is required for the generation of effector cells. Here we found that after infection with an intestinal nematode, antigen-bearing DCs and CD4$^+$ T cells upregulated the chemokine receptor CXCR5 and localized together outside the T cell zone by a mechanism dependent on the chemokine CXCL13, B cells and lymphotoxin. Notably, lymphotoxin-expressing B cells, CXCR5-expressing DCs and T cells, and CXCL13 were also necessary for development of interleukin 4 (IL-4)-producing type 2 helper T cells ($T_H2$ cells), which suggests that $T_H2$ differentiation can initiate outside the T cell zone.

According to the present paradigm$^{1,2}$, naive T cells that express the chemokine receptor CCR7 and antigen-bearing mature CCR7$^+$ dendritic cells (DCs) enter lymph nodes and migrate by a mechanism dependent on the chemokine CCL19 to the T cell zone, where antigen-specific encounters and T cell priming takes place. However, mice of the plt (paucity of lymph node T cells) strain$^3$, which lack the CCR7 ligands CCL19 and CCL21a, can have normal or even enhanced CD4$^+$ T cell responses$^{4,5}$, which suggests that DC-dependent priming of some CD4$^+$ T cell responses may occur outside the T cell zone in the lymph node. Indeed, emerging evidence suggests that T cells and DCs may also have the opportunity to engage each other near the B cell area. For example, follicular helper T cells ($T_{FH}$ cells)$^{6-8}$ and some DCs in the marginal zone of the spleen$^9$ and the dermis of the skin$^10$ express the chemokine receptor CXCR5 and localize near CXCR5$^+$ B cells, stroma-derived follicular dendritic cells$^{11,12}$ and marginal reticular cells$^{13}$. Those stromal-cell subsets, which are located below the subcapsular sinus, in the B cell follicles and in the inter- and perifollicular regions between the B cell follicles, express the chemokine CXCL13 and can attract or retain CXCR5-expressing cells. Although it makes sense that the development of $T_{FH}$ cells, which is dependent on antigen-presenting DCs and B cells$^{14-16}$, might take place near B cell follicles, it is less obvious whether other types of CD4$^+$ effector-cell responses can be initiated in the B cell area of the lymph node.

Here we found that a population of CXCR5-expressing DCs that migrated to the lymph node and localized adjacent to B cell follicles was induced in mice infected with the intestinal nematode *Heligmosomoides polygyrus*. Deletion of CXCR5 in either DCs or CD4$^+$ T cells prevented the colocalization of DCs and CD4$^+$ T cells near the B cell area and impaired the development of both $T_{FH}$ cell and type 2 helper T cell ($T_H2$ cell) effectors. Notably, lymphotoxin-expressing B cells controlled Cxcl13 transcription and regulated the CXCL13-dependent positioning of DCs and T cells in the lymph node. Most notably, cytokine production by $T_H2$ effector cells in the lymph nodes and peripheral tissues was substantially impaired in mice treated with therapeutics that either caused transient depletion of B cells or blocked signaling via membrane lymphotoxin or CXCL13. Thus, *H. polygyrus*-induced, IL-4-expressing $T_{FH}$ and $T_H2$ effector-cell responses were initiated in a CXCL13--, lymphotoxin- and B cell–dependent way in a specialized microenvironment outside the T cell zone.

**RESULTS**

*H. polygyrus* infection alters DC chemokine-receptor expression

Mature DCs typically localize in the T cell zone of the lymph node$^{17,18}$. However, CXCR5$^+$ DC populations have been identified and found to localize near B cell follicles$^9,10$. To assess whether we were able to detect DCs that ‘preferentially’ localized near B cells after various types of infections, we determined the localization of the DCs in the mediastinal lymph nodes (medLNs) of influenza virus–infected mice (Fig. 1a). As expected, CD11c$^+$ DCs were present mainly in the T cell areas of uninfected mice (Fig. 1a). Similarly, medLN CD11c$^+$ DCs from influenza virus–infected mice were also present mainly in the T cell area (Fig. 1a). In contrast, mesLN CD11c$^+$ DCs from *H. polygyrus*-infected mice were located below the subcapsular sinus, in the interfollicular areas and at the T cell–B cell border (Fig. 1a). Thus, DCs accumulated in different regions of the lymph node after infection with influenza virus or *H. polygyrus*.

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mesLN DCs after *H. polygyrus* infection (Supplementary Fig. 1a). Notably, we identified similar migratory DC subsets in the mesLNs of influenza virus–infected mice and CXCR5 by immature DCs (Fig. 2a,b). As expected, we found eight- to tenfold more mature

Figure 1 DCs migrate to the peri- and interfollicular areas of lymph nodes after infection with *H. polygyrus*. (a) Cryosections of medLNs or mesLNs from uninfected B6 mice (UI) or B6 mice infected with *H. polygyrus* at larval stage 3 (200 larvae; HP, day 8) or infected intranasally with influenza virus strain X31 (0.1 half-maximal lethal dose; X31, day 8), obtained on day 8 after infection and stained with anti-CD11c, anti-B220 and anti-CX21. (b) Enlargement of the region outlined in (a) (bottom right image). Scale bars, 400 μm. Data are representative of three or more independent experiments.

Given the unexpected positioning of the CD11c+ cells in the mesLNs of *H. polygyrus*–infected mice, we phenotypically and functionally characterized the CD11c+ DCs in the lymph nodes on day 8, at the peak of the DC response (Supplementary Fig. 1a). We identified two major populations of DCs: MHCII+CD40+CD11c+DC-205+ mature DCs and MHCII+CD40+CD11c+DC-205+ immature DCs (Fig. 2a,b). As expected, we found eight- to tenfold more mature

Figure 2 Mature DCs bearing *H. polygyrus* antigens express CXCR5 and show more responsiveness to CXCL13 and less responsiveness to CCL19. (a) Flow cytometry of mesLN cells from B6 mice at day 8 after infection with *H. polygyrus*, with gating of MHCII+CD11c+ mature DCs (Mat DCs) and MHCII+CD11c+ immature DCs (Imm DCs). (b) Expression of DEC-205 and CD40 (filled histograms) by the cells gated in (a). Open histograms, nonspecific control staining. (c) Expression of IL-4 mRNA (eGFP) by CD4+ T cells from the mesLNs of IL-4 reporter mice infected 5 d earlier with *H. polygyrus* (far left), and after 72 h in vitro culture with total (Tot) DCs purified from uninfected UI mice (far right) or sort-purified MHCII+CD11c+ mature DCs or MHCII+CD11c+ immature DCs isolated from the mesLNs of mice on day 8 after infection with *H. polygyrus* (middle left and middle right, respectively). Numbers below outlined areas indicate percent eGFP-expressing CD4+ T cells. (d) Quantification of the eGFP+ CD4+ T cells in (c) (n = 5 independent cultures per condition), presented as frequency at 72 h relative to that at the initiation of coculture. (e–g) Expression of CCR7 and CXCR5 by immature DCs (e) and mature DCs (f,g) from mice at day 8 after infection with influenza virus strain X31 (e,f) or *H. polygyrus* (e,g) or uninfected control mice (f,g), analyzed by flow cytometry. Numbers above bracketed lines indicate percent CCR7+ cells (top row) or CXCR5+ cells (bottom row). (h) Quantification of the results in (e–g) (n = 4–5 mice per group). (i,j) Chemotaxis of immature DCs (i) and mature DCs (j) from mice infected with influenza virus strain X31 or *H. polygyrus*, assessed in Transwells with CCL19 (top row) or CXCL13 (bottom row) as the chemoattractant (n = 3 independent Transwells per group); results are presented as the chemotaxis index (CI). *P < 0.001 (unpaired Student’s t-test). Data are representative of at least three independent experiments (mean and s.d. in a,c–j).
of culture, we measured the in vitro population expansion of the IL-4 mRNA–expressing T cell population that is ‘reported’ in these mice by expression of enhanced green fluorescent protein (eGFP). Approximately 10% of the input CD4+ T cells expressed eGFP before culture with DCs (Fig. 2c, far left). The eGFP+ T cell population expanded tenfold when cultured together with mature DCs from H. polygyrus–infected mice (Fig. 2c,d), which suggested that these phenotypically mature DCs were able to present H. polygyrus antigen and expand H. polygyrus–specific CD4+ T cell populations.

Next we assessed the expression of CCR7 and CXCR5 on immature (MHCII+CD11c+CD40lo) DCs and mature (MHCII+CD11cintCD40hi) DCs from the mesLN of B6plt mice at day 8 after infection with H. polygyrus. (c) Cryosections of mesLNs from the mice in a,b, stained with anti-CD11c and anti-B220. Scale bars, 400 μm. (d,e) Frequency (adjacent to outlined areas) and number (g) of IL-4-producing CD4+ T cells among cells obtained from the mesLNs of the mice in a,b, followed by restimulation in vitro for 4 h with anti-CD3 and brefeldin A (BFA), assessed by intracellular cytokine staining (ICCS). (f) Quantification of total cells and immature and mature DCs in the mesLNs of B6 mice treated with 200 μg control antibody (Ctrl) or anti-CXCL13 (α-CXCL13) at the time of infection with H. polygyrus, assessed by flow cytometry 8 d after infection. (g) Cryosections of mesLNs from the mice in f, stained with anti-CD11c and anti-B220. Scale bars, 400 μm. (h,i) Frequency (h) and number (i) of IL-4-producing CD4+ T cells among cells obtained on day 8 after infection with H. polygyrus from the mesLNs of anti-CXCL13-treated B6 mice (as in f) or Cxcr5–/– or Cxcr5+/+ mice, analyzed by ICCS after restimulation for 4 h with anti-CD3 and BFA. (j,k) Frequency (j) and number (k) of IL-4–producing CD4+ T cells among cells from the peritoneal cavity of anti-CXCL13-treated mice (as in f), assessed by ICCS after restimulation as in h,i. (l) Frequency (l) and number (m) of IFN-γ-producing CD4+ T cells among cells from the mesLNs of B6 mice treated with control antibody or anti-CXCL13 at the time of infection with influenza virus, obtained on day 8 after infection, then restimulated for 4 h with anti-CD3 and BFA and assessed by ICCS. *P < 0.01, versus control (unpaired Student’s t-test). Data are representative of two or more independent experiments (mean and s.d. of five mice per group).

**T<sub>h</sub>2 responses to H. polygyrus regulated by CXCL13 but not CCL19**

Given the altered responsiveness of mature DCs from H. polygyrus–infected mice to CCL19 and CXCL13, we postulated that the development of T<sub>h</sub>2 responses to H. polygyrus might be less reliant on CCR7 ligands and more dependent on CXCR5 ligands. To test our hypothesis, we first evaluated T cell responses in H. polygyrus–infected CCL19-deficient plt mice. As expected, the mesLNs of naive plt mice had fewer mature lymph node DCs from uninfected or influenza virus–infected mice responded to CCL19 but responded only marginally to CXCL13 (Fig. 2j). Conversely, mature DCs from H. polygyrus–infected mice showed more migration toward CXCL13 and less migration toward CCL19 (Fig. 2f). Indeed, the frequency of mature DCs that migrated toward CCL19 decreased from 22% before infection to 8% after infection with H. polygyrus, whereas the frequency of DCs that migrated toward CXCL13 more than doubled, from 3% to 8% (Supplementary Fig. 2f). Unexpectedly, given the higher CXCR5 expression in B cells than in mature DCs (Supplementary Fig. 2c), B cells from H. polygyrus–infected mesLNs migrated more effectively to CXCL13 than did mature DCs from the same lymph nodes (Supplementary Fig. 2d,e). However, antigen-bearing migratory mesLN DCs from H. polygyrus–infected mice showed altered responsiveness to chemokines such as CXCL13 and CCL19 that control the positioning of cells in the lymph node.
DCs than did those of control naive B6 mice (Supplementary Fig. 3a,b). However, by 8 days after infection with *H. polygyrus*, B6 and plt mice had a similar frequency and number of immature and mature mesLN DCs (Fig. 3a,b). Furthermore, DCs of each group of mice were present below the subcapsular sinus and in the interfollicular areas (Fig. 3c). More notably, both the frequency and number of CD4+ T cells from *H. polygyrus*-infected mice that produced IL-4 after restimulation *in vitro* with antibody to CD3 (anti-CD3) were almost identical for plt and B6 mice (Fig. 3d,e), which indicated that CCL19 was not required for the *H. polygyrus*-dependent development of T<sub>H2</sub> cells.

Next we treated mice with control antibody or CXCL13-specific antibody at the time of *H. polygyrus* infection and examined the DC and T cell responses 8 days later. Blockade of CXCL13 had no effect on lymph node cellularity or the number of immature and mature DCs in the reactive lymph node (Fig. 3f). However, DCs from mice treated with anti-CXCL13 localized in the T cell area rather than near the B cell follicles (Fig. 3g). Despite the presence of the DCs in the T cell zone of infected mice treated with anti-CXCL13, the frequency and number of mesLN CD4<sup>+</sup> T cells that produced IL-4 after restimulation with anti-CD3 were significantly lower for mice treated with anti-CXCL13 than for mice treated with control antibody (Fig. 3h,i). We obtained similar results with *H. polygyrus*-infected Cxcl13<sup>−/−</sup> mice<sup>21</sup> and Cxcr5<sup>−/−</sup> mice<sup>22</sup> (Fig. 3i). Notably, the frequency and number of IL-4-producing T<sub>H2</sub> cells present in a peripheral site, the peritoneal cavity, were also significantly lower for mice treated with anti-CXCL13 than for mice treated with control antibody (Fig. 3j,k). In contrast, treatment with anti-CXCL13 did not impair the development of interferon-γ (IFN-γ)-producing type 1 helper T cells (T<sub>H1</sub> cells) after infection with influenza virus (Fig. 3l,m). Therefore, although CXCL13 was important for the development of an *H. polygyrus*-specific T<sub>H2</sub> response in the lymph nodes and periphery, it was dispensable for the development of a T<sub>H1</sub> response to influenza virus.

CXCR5<sup>+</sup> DCs regulate *H. polygyrus*-induced T<sub>H2</sub> development

To determine whether the T<sub>H2</sub> responses to *H. polygyrus* were dependent on CXCR5-expressing DCs, we reconstituted irradiated wild-type B6 recipients with an 80:20 mixture of bone marrow from CD11c-DTR mice, in which DCs could be ablated after the administration of diphtheria toxin<sup>23</sup>, and Cxcr5<sup>−/−</sup> mice, respectively (to generate DC-Cxcr5<sup>−/−</sup> chimeras; Supplementary Fig. 4), or with an 80:20 mixture of bone marrow from CD11c-DTR mice and wild-type B6 mice, respectively (to generate DC-WT chimeras; Supplementary Fig. 4). After reconstitution, we treated both groups with diphtheria toxin to ablate the CD11c<sup>+</sup> cells derived from the CD11c-DTR bone marrow. We then transferred purified naive CD4<sup>+</sup> T cells from congenic B6 (CD45.1<sup>+</sup>) mice into chimeras of each group and infected the mice with *H. polygyrus*. Although the frequency (Fig. 4a) and number (data not shown) of mesLN DCs were similar for both groups of mice, most responding DCs present in the diphtheria toxin–treated *H. polygyrus*-infected DC-WT chimeras expressed CXCR5<sup>+</sup>, whereas the responding DCs from DC-Cxcr5<sup>−/−</sup> chimeras were largely CXCR5<sup>−/−</sup> (Fig. 4b). DCs from *H. polygyrus*-infected DC-WT chimeras were positioned in the interfollicular regions (Fig. 4c), whereas DCs from *H. polygyrus*-infected DC-Cxcr5<sup>−/−</sup> chimeras accumulated mostly in the T cell zone (Fig. 4c). Notably, the DC-Cxcr5<sup>−/−</sup> chimeras had a significantly lower frequency and number of IL-4-producing wild-type (CD45.1<sup>+</sup>) CD4<sup>+</sup> T cells in the mesLNs (Fig. 4d,e) and peritoneal cavity (Fig. 4f,g) than did DC-WT chimeras. Thus, CXCR5 expression by CD11c<sup>+</sup> cells controlled the positioning of DCs in the infected lymph nodes and was also necessary for the development of maximal T<sub>H2</sub> responses in lymph nodes and peripheral sites.

T<sub>FH</sub> and T<sub>H2</sub> development requires CXCR5<sup>+</sup> T cells

Our data suggested that *H. polygyrus*-induced T<sub>H2</sub> responses might also be dependent on a CXCR5-expressing T cell population. To address this possibility, we used flow cytometry to assess CXCR5 expression on...
CXCR5 is expressed by lymph node T<sub>FH</sub> and Th2 cells and is required for the development of T<sub>FH</sub> and Th2 cells after infection with *H. polygyrus*. (a) Flow cytometry of CD4<sup>+</sup> T cells among mesLN cells from IL-4 reporter mice at day 8 after infection with *H. polygyrus*, separated into naive (CD4<sup>+</sup>eGFP<sup>+</sup>) and antigen-experienced (CD4<sup>+</sup>eGFP<sup>-</sup>) populations (left), followed by analysis of the expression of ICOS and PD-1 (right) for subdivision into three subsets: naive cells (CD4<sup>+</sup>eGFP<sup>-</sup>ICOS<sup>-</sup>PD-1<sup>-</sup>), T<sub>FH</sub> cells (CD4<sup>+</sup>eGFP<sup>-</sup>ICOS<sup>+</sup>PD-1<sup>-</sup>), and effector T cells (CD4<sup>+</sup>eGFP<sup>-</sup>ICOS<sup>+</sup>PD-1<sup>+</sup>). (b) Frequency of IL-4-producing naive, ICOS<sup>-</sup>PD-1<sup>-</sup> and ICOS<sup>+</sup>PD-1<sup>-</sup> and CD4<sup>+</sup> T cells among cells from the mesLNs of *H. polygyrus*-infected B6 mice, obtained on day 8 after infection, then restimulated for 4 h with anti-CD3 and BFA and assessed by iICS. (c, d) Bcl-6 expression in naive, ICOS<sup>-</sup>PD-1<sup>-</sup> and ICOS<sup>+</sup>PD-1<sup>-</sup> gated CD4<sup>+</sup> T cells from the mesLNs of IL-4 reporter mice infected for 8 d with *H. polygyrus*, assessed by intracellular staining (c), and expression of CXCR5 and PD-1 on gated naive, eGFP<sup>+</sup>Bcl-6<sup>-</sup> and eGFP<sup>-</sup>Bcl-6<sup>+</sup> CD4<sup>+</sup> T cells from those same mice, assessed by iICS (d; gating strategy, Supplementary Fig. 5d). (e) Serial cryosections of mesLNs from uninfected B6 mice or B6 mice at day 8 after infection with *H. polygyrus*, stained with anti-CD8, anti-B220, anti-CD11c or anti-CD4. Scale bars, 400 μm. (f) Cryosections of mesLNs from B6 mice treated with control antibody or anti-CXCL13 and infected with *H. polygyrus*, analyzed on day 8 after infection by staining with anti-CD4 and anti-B220. Scale bars, 400 μm. (g, h) iICS of ICOS<sup>-</sup>PD-1<sup>-</sup>CXCR5<sup>-</sup> T<sub>FH</sub> cells (g) and IL-4<sup>-</sup>ICOS<sup>-</sup>PD-1<sup>-</sup> Th2 cells (h) among mesLN cells from B6 mice treated as in f, analyzed by flow cytometry immediately after isolation (g) or after 4 h of restimulation with anti-CD3 and BFA (h). (i, j) Frequency of CD45<sup>1+</sup> and CD45<sup>2+</sup> cells among total cells (i) and cells in the gated naive (CD44<sup>hi</sup>) T<sub>FH</sub> (ICOS<sup>-</sup>PD-1<sup>-</sup>) and effector (ICOS<sup>-</sup>PD-1<sup>-</sup>) CD4<sup>+</sup> populations (j) from the mesLNs of chimeric mice reconstituted with 50% wild-type (WT) B6 (CD45.1<sup>+</sup>) bone marrow and 50% *Cxcr5<sup>−/−</sup>* (CD45.2<sup>+</sup>) bone marrow, assessed by flow cytometry before infection (day 0; i) or on day 8 after infection with *H. polygyrus* (j). (k, l) Frequency of CD45<sup>1+</sup> and CD45<sup>2+</sup> cells in the IL-4<sup>+</sup> CD4<sup>+</sup> T cell population of the mesLNs (k) and peritoneal cavities (l) of chimeric mice as described in j, obtained on day 8 after infection and restimulated for 4 h with anti-CD3 and BFA, then assessed by iICS. *P < 0.01 and **P < 0.001 (unpaired Student’s t-test). Data are representative of three independent experiments (mean and s.d. of five mice per group).
After treatment with anti-CXCL13, CD4+ T cells no longer accumulated in the perifollicular region and instead accumulated in the T cell area (Fig. 5f). Despite the finding that treatment with anti-CXCL13 facilitated the colocalization of CD4+ T cells and DCs in the T cell zone, there were significantly fewer ICOS+PD-1hi TFH cells (Fig. 5g) and IL-4-producing ICOS+PD-1hi CD4+ T cells, among cells from the mesLN of B6 mice treated as in a, b, obtained on day 8 after infection and analyzed by flow cytometry either immediately after isolation (c) or after 4 h of restimulation with anti-CD3 and BFA (d, e). (f-h) Quantification of influenza virus nucleoprotein-specific (NP+) ICOS+PD-1hi TFH cells (f), and frequency (g) and number (h) of IFN-γ-producing NP+ICOS+PD-1hi CD4+ T cells, among CD4+ T cells from the mesLNs of B6 mice treated with 250 μg control antibody or anti-CD20 4 d before infection with influenza virus strain X31, obtained on day 8 after infection and analyzed by flow cytometry either immediately after isolation (f) or after 4 h of restimulation with anti-CD3 and BFA (g, h). *P < 0.001 (unpaired Student's t-test). Data are representative of three independent experiments (mean and s.d. of five mice per group in c–h).

**B cell depletion impairs TFH and T\(_{\mu 2}\) development**

Given that CXCR5 expression by both CD4+ T cells and DCs was required for maximal TFH and T\(_{\mu 2}\) responses to *H. polygyrus*, we reasoned that localization of B cells together with DCs or CD4+ T cells may be necessary for the optimal generation of T\(_{\mu 2}\) cells. To assess this, we treated wild-type B6 mice with control antibody or B cell–depleting anti-CD20 4 d before infecting the mice with *H. polygyrus* and, on day 8 after infection, we evaluated the architecture of the mesLNs and the CD4+ T cell response. In mice treated with control antibody, DCs and CD4+ T cells were positioned in close proximity to B cells (Fig. 6a and Supplementary Fig. 7). In contrast, in mice treated with anti-CD20, B cell follicles were ablated (Fig. 6b) and DCs and CD4+ T cells were disorganized and present throughout the lymph node (Fig. 6a, b and Supplementary Fig. 7). As expected, mice depleted of B cells had significantly fewer ICOS+PD-1hi TFH cells than did mice treated with control antibody (Fig. 6c). However, mice depleted of B cells had a significantly lower frequency and number of ICOS+PD-1hi IL-4+ T\(_{\mu 2}\) cells (Fig. 6d, e) and IL-13-producing T\(_{\mu 2}\) cells (Supplementary Fig. 8a, b) in the lymph nodes (Fig. 6d, e) and IL-4+ CD4+ T cells in the peritoneal cavity (Supplementary Fig. 8c, d) than did mice treated with control antibody. We obtained similar results with *H. polygyrus*-infected MD4μMT mice, which have a monoclonal repertoire of B cells that express a transgene encoding a B cell antigen receptor specific for an irrelevant antigen (Supplementary Fig. 8e–g). As a control, we also depleted mice of B cells, treated them with anti-CD20 and infected them with influenza virus, then determined the number of influenza virus nucleoprotein–specific ICOS+PD-1hi T\(_{\mu 2}\) cells and ICOS+PD-1hi effector T cells in the mesLNs on day 8. Although depleting mice of B cells suppressed the nucleoprotein-specific T\(_{\mu 2}\) cell response (Fig. 6f), it had no effect on the nucleoprotein-specific IFN-γ-producing CD4+ T\(_{\mu 1}\) response at day 8 after infection (Fig. 6g, h). Thus, B cells and CXCL13 were dispensable for the development of a primary T\(_{\mu 1}\) response to influenza virus but were necessary for the generation of an optimal T\(_{\mu 2}\) response to *H. polygyrus*.

**Lymphotoxin regulates CXCL13 expression and T\(_{\mu 2}\) development**

CXCL13 expression by stromal cells in the spleen is controlled by lymphotoxin–expressing B cells. To investigate whether lymphotoxin–expressing B cells regulate the CXCR5-dependent T\(_{\mu 2}\) response by controlling CXCL13 expression in the mesLNs, we first examined membrane expression of lymphotoxin-α\(_2\)β3 on mesLN cells after infection with *H. polygyrus*. Although mesLN T cells had only marginal expression of lymphotoxin (Supplementary Fig. 9a), we detected lymphotoxin on a fraction of mesLN B cells from uninfected mice (Fig. 7a) and found it was upregulated on B cells after infection with *H. polygyrus* (Fig. 7b), specifically in the activated (Fas+PNAhi) and germinal-center (PNAhiFas*) B cell populations (Supplementary Fig. 9b).
with *H. polygyrus* (b), assessed by flow cytometry with LTβR-Fc to detect lymphotoxin-α1β2. Far right (α-LTβ), control staining in the presence of blocking antibody to lymphotoxin-β. (c) Detection of membrane lymphotoxin-α1β2 on CD19+ B cells from B6 and MD4µMT mice on day 8 after infection with *H. polygyrus*, stained with LTβR-Fc (open histograms) or control antibody (filled histogram). (d) Membrane expression of lymphotoxin-α1β2 (open histograms) on CD19+ B cells from CD11c-DTR bone marrow chimeras treated with PBS (DC sufficient) or with diptheria toxin on days 0 and 3 (DC depletion), then infected with *H. polygyrus*. Filled histograms, staining with isotype-matched control antibody. (e) Quantitative PCR analysis of CXCL13 mRNA in mesLN cells from B6 mice treated intraperitoneally with 100 μg LTβR-Fc or control protein (Control) at the time of *H. polygyrus* infection or with 250 μg anti-CD20 or control antibody (data not shown) 4 d before *H. polygyrus* infection, followed by analysis on day 8 after infection, or in mesLN cells from Cxcl13−/− (far right); results are presented relative to those of B6 mice treated with control protein. ND, not detected. (f,g) Frequency (f) and number (g) of MHCIi+CD11chi mature and MHCIi+CD11cint immature mesLN DCs from B6 mice treated with LTβR-Fc or control protein and infected with *H. polygyrus*, assessed by flow cytometry on day 8 after infection. (h,i) Cyrosections of mesLN from B6 mice treated with control protein (h) or LTβR-Fc (i) at the time of infection with *H. polygyrus*, assessed on day 8 after infection by staining with anti-B220, anti-CD11c and anti-C4D. Scale bars, 400 μm. (j) Quantification of IL-4+ CD4+ T cells among cells from the mesLN of B6 mice treated with LTβR-Fc or control protein at the time of infection with *H. polygyrus*, obtained on day 8 after infection and restimulated for 4 h with anti-CD3 and BFA, then assessed by ICCS. *P < 0.001, versus control, **P < 0.05 and ***P < 0.01 (unpaired Student's t-test). Data are representative of three independent experiments with five or three mice per group (mean and s.d. (a,b,f,g,j) and mean and s.d. of three mice per group with triplicate samples (e)).

Notably, neither nonspecific B cells from MD4µMT mice (Fig. 7c) nor B cells from CD11c-DTR mice depleted of DCs (Fig. 7d) upregulated lymphotoxin after infection with *H. polygyrus*.

We next determined whether CXCL13 expression in the lymph nodes of *H. polygyrus*–infected mice was controlled by lymphotoxin or B cells. To assess this, we either depleted mice of B cells with anti-CD20 or blocked lymphotoxin signaling with a fusion protein consisting of the receptor for lymphotoxin-β and the Fc portion of immunoglobulin (LTβR-Fc). We then infected the mice with *H. polygyrus* and quantified CXCL13 mRNA in the mesLN. We found significantly lower CXCL13 expression in the mesLN of mice depleted of B cells or treated with LTβR-Fc than in infected B6 mice treated with control antibody (data not shown) or control protein (Fig. 7e), respectively.

Finally, we treated mice with LTβR-Fc at the time of infection with *H. polygyrus* and then examined the positioning of DCs and CD4+ T cells in the lymph nodes and the development of the *H. polygyrus*–induced T112 response. Neither the frequency nor the number of mature DCs was affected by treatment with LTβR-Fc (Fig. 7f–g). Although treatment with LTβR-Fc did not ablate the B cell follicles (Fig. 7h–i), DCs and CD4+ T cells in the mice treated with LTβR-Fc no longer accumulated in the interfollicular areas and were instead present mostly in the T cell areas of the lymph nodes (Fig. 7h–i and Supplementary Fig. 10). Furthermore, mice treated with LTβR-Fc and infected with *H. polygyrus* had significantly fewer IL-4–producing T cells than did infected B6 mice treated with protein (Fig. 7j). Thus, CXCL13, B cells and lymphotoxin were each required for the proper positioning of DCs and T cells near the B cell follicles after infection with *H. polygyrus* and were also required for the optimal development of T112 cells.

**B cell–derived lymphotoxin controls T112 responses to *H. polygyrus***

Collectively, our data suggested that lymphotoxin produced by B cells regulated CXCL13 expression and thereby controlled the positioning of DCs and T cells in the mesLN and the development of an *H. polygyrus*–specific T112 response. To assess that model, we reconstituted μMT mice (which are genetically deficient in B cells) with bone marrow containing 80% μMT bone marrow plus 20% B6 bone marrow or 20% Lta−/− bone marrow. In these chimeras, the B cells were either lymphotoxin deficient (B-Lta−/−) or lymphotoxin sufficient (B-WT; Supplementary Fig. 11). We infected both groups of chimeric mice with *H. polygyrus* and evaluated the DC response, CXCL13 expression and T112 response in the mesLN on day 8 after infection. The numbers of mature and immature DCs in the mesLN of *H. polygyrus*–infected B-Lta−/− chimeras were equivalent to those in the mesLN of infected B-WT chimeras (Fig. 8a,b). Although DCs from *H. polygyrus*–infected B-WT chimeras were adjacent to the B cell follicles (Fig. 8a), DCs from infected B-Lta−/− chimeras accumulated mainly in the T cell area with the CD4+ T cells (Fig. 8d). Consistent with those results, CXCL13 mRNA expression was lower in *H. polygyrus*–infected B-Lta−/− chimeras than in infected B-WT chimeras (Fig. 8e). Furthermore, the frequency and number of CD4+ T cells able to produce IL-4 after restimulation were significantly lower in the infected B-Lta−/− chimeras than in B-WT chimeras (Fig. 8f,g). Together these data indicated that
H. polygyrus–induced T~H~2~ and T~F~H~ responses developed in a CXCL13-dependent manner outside the T cell zone and were regulated by CXCR5-expressing DCs and lymphotixin-expressing B cells.

**DISCUSSION**

Although present models suggest that naive T cells are first primed by antigen-bearing DCs in the T cell zone, we have shown here that optimal development of H. polygyrus–induced T~F~H~ and T~H~2~ cells required CXCR5, CXCL13 and lymphotixin and seemed to take place outside the T cell zone, adjacent to B cell follicles. We found that treatment with anti-CXCL13 and LT~B~Fc, which impaired the development of T~H~2~ cells in response to H. polygyrus, did not prevent the colocalization of CD4~+~ T cells and DCs in the T cell zone but did prevent those cells from accumulating near the B cell follicles. Thus, T~H~2~ responses to H. polygyrus were not only dependent on DC–T cell interactions but also controlled by the microenvironment in which these interactions occurred.

Our data indicated that expression of CXCR5 by DCs was required for optimal T~F~H~ and T~H~2~ responses to H. polygyrus. Although activated DCs typically upregulate CCR7 (ref. 18), we found very few CCR7~+~CXCR5~−~ DCs after infection with H. polygyrus. Instead, we identified populations of CXCR5~−~CCR7~−~ and CXCR5~+~CCR7~+~ mature DCs. Some of these DCs were responsive, at least in vitro, to CCL19 and CXCL13. Although these H. polygyrus–induced DCs were less responsive to CXCL13 than were B cells, the DCs were also less responsive to CCL19 than were the ‘prototypical’ mature DCs from influenza virus–infected mice. This dual, but modest, responsiveness to ligands of CCR7 and CXCR5 seemed sufficient for the migratory DCs to reach the lymph node. However, once the DCs entered the subcapsular sinus region of the lymph node through the lymphatics, the DCs would encounter CXCL13. We speculate that CXCL13 probably provided a retention signal and prevented the DCs, which were only modestly responsive to CCL19, from migrating to the T cell area. CXCR5–expressing DCs have been identified in the marginal zone of the spleen and the dermis of the skin. Although the function of these DCs was not evaluated, the CXCR5~+~ skin-derived DCs responded to CXCL13 and migrated to the B cell area of the lymph node after adoptive transfer. Furthermore, the marginal-zone CXCR5~+~ DCs disappeared in mice lacking B cells or lymphotixin.

Thus, these splenic and skin-derived populations of DCs may be similar to the CXCR5–expressing DC population induced after infection with H. polygyrus.

CXCR5 expression by T cells was also required for optimal T~F~H~ and T~H~2~ responses to H. polygyrus. Although T~F~H~ cells express CXCR5 (refs. 6–8), a role for CXCR5 in T~H~2~ development has not been evaluated. We found a population of CXCR5~−~ and Bcl-6–expressing mesLN T cells that also had the phenotypic and functional hallmarks of true T~H~2~ cells that are committed to the production of IL-4 and IL-13. Given that both T~F~H~ cells and T~H~2~ cells in the mesLNs of H. polygyrus–infected mice expressed IL-4 mRNA and Bcl-6 and required B cells and CXCL13 for their optimal development, it seems that the H. polygyrus–induced differentiation of T~F~H~ cells and T~H~2~ cells is linked. In support of that conclusion, T~F~H~ and T~H~2~ cells responding to Schistosoma mansoni egg antigens are reported to arise from a common precursor (ref. 29). Similarly, it has been reported that T~F~H~1~ differentiation is marked by a T~F~H~ cell–like transition (ref. 30). Our data further indicate that the development of this common T~F~H~1~ precursor is controlled in part by the unique microenvironment in which the cells are located. We speculate that naive H. polygyrus–specific T cells that enter the lymph node near B cell follicles are detained at this site by CXCR5~+~ antigen–presenting DCs and are directed to activate the transcription networks that control both T~F~H~ differentiation (ref. 31) and T~F~H~2~ differentiation. T cells that receive reinforcing signals from nearby antigen–presenting B cells can maintain expression of Bcl-6 and CXCR5 and become fully committed T~F~H~ cells that retain their ability to produce IL-4 (refs. 29,31). In contrast, those T cells that do not maintain extended interactions with antigen–presenting B cells lose Bcl-6 and CXCR5 expression and become fully committed T~H~2~ cells that can leave the lymph node and migrate to peripheral effector sites.

One unique feature of the microenvironment in which T~F~H~ and T~H~2~ cells develop is the presence of B cells. Our data showing that B cells regulated T~H~2~ and T~F~H~ development after infection with H. polygyrus are consistent with results showing that B cells maintain
\[ T_{FH} \text{ responses}^{14-16}, \text{regulate the magnitude of primary } T_{H2} \text{ responses}^{32-35} \text{ and facilitate the development and maintenance of memory } T_{H2} \text{ cells}^{32,34}. \text{ Our data have shown that one way } B \text{ cells modulate the development of } T_{FH} \text{ and } T_{H2} \text{ effector cells is by ensuring abundant expression of CXCL13 in the B cell area of the reactive lymph node, thereby enhancing the retention of CXCR5* DCs and CD4* } T \text{ cells in this location. Given our data showing that } B \text{ cells controlled the abundance of CXCL13 in a lymphotoxin-dependent way in the lymph node, we speculate that lymphotoxin-expressing } B \text{ cells communicate with nearby LTβR-expressing stromal cells}^{24,36-38} \text{ and induce those cells to produce more CXCL13, which allows more effective retention of CXCR5-expressing DCs. This infection-induced response in the lymph node seems similar to the homeostatic feedback loop by which lymphotoxin-expressing } B \text{ cells organize the } T \text{ cell area and CXCL13-expressing follicular dendritic cells in the spleen}^{31,39}.\]

It is not immediately obvious why \( T_{FH} \) and \( T_{H2} \)-dependent responses to \( H. \text{polygyrus} \) were more effective when \( T \) cells and DCs localized together in the perifollicular area than when they localized together in the \( T \) cell zone. One possibility is that \( B \) cells that present antigen and express cytokines or costimulatory molecules may be needed to drive the differentiation of \( T_{H2} \) and \( T_{FH} \) cells. Alternatively, other cells, such as macrophages that line the subcapsular sinus and transfer antigens to \( B \) cells, also populate the perifollicular area\(^{40,41}\) and may contribute to the process. It is also possible that \( IL-4 \)-producing cells such as mast cells\(^{39}\), which can express CXCR5 (ref. 42), may be able to interact with \( T \) cells in the \( B \) cell area. Finally, CXCR5* DCs may receive additional conditioning signals from nearby \( B \) cells, follicular dendritic cells or marginal reticular cells that would direct the DCs to induce expression of \( IL-4 \) and \( Bcl-6 \) in the responding \( T \) cells. Each of these possibilities will need to be addressed.

Collectively, our results support a model of DC-driven \( CD4^+ \) \( T \) cell differentiation in which pathogen-derived signals instruct DCs to upregulate CXCR5, migrate to a unique microenvironment in the lymph node and initiate the development of \( T_{FH} \) and \( T_{H2} \) cells. Although we do not know whether this model is applicable to all \( T_{FH} \) responses, we have found that \( T_{FH} \) responses in \( L. \text{ major} \)-infected BALB/c mice were controlled in a similar CXCR5- and CXCL13-dependent way (B.L. and F.E.L., data not shown). Finally, and perhaps most notably, our data suggest that some \( T_{FH} \) responses may be attenuated with therapies that result in the depletion of \( B \) cells or block CXCL13 or lymphotoxin signaling.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

B.L., A.B.-T., F.E.L. and T.D.R. contributed to the design of the experiments and the writing of the manuscript; B.L. did all experiments with help from A.B.-T.; J.L.B. and R.D. provided advice, discussions and reagents; and all authors reviewed the manuscript before submission.

**COMPETING FINANCIAL INTERESTS**

The authors declare competing financial interests: details are available in the online version of the paper.

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30. Nakayamada, S. et al. Early Th1 cell differentiation is marked by a Tfh cell-like transition. *Immunity* **35**, 919–931 (2011).

31. King, I.L. & Mohrs, M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J. Exp. Med.* **206**, 1001–1007 (2009).

32. Wojciechowski, W. et al. Cytokine-producing effector B cells regulate type 2 immunity to H. polygyrus. *Immunity* **30**, 421–433 (2009).

33. Liu, Q. et al. The role of B cells in the development of CD4 effector T cells during a polarized Th2 immune response. *J. Immunol.* **179**, 3821–3830 (2007).

34. Crawford, A., Macleod, M., Schumacher, T., Corlett, L. & Gray, D. Primary T cell expansion and differentiation in vivo requires antigen presentation by B cells. *J. Immunol.* **176**, 3498–3506 (2006).

35. Linton, P.J. et al. Costimulation via OX40L expressed by B cells is sufficient to determine the extent of primary CD4 cell expansion and Th2 cytokine secretion in vivo. *J. Exp. Med.* **197**, 875–883 (2003).

36. Fu, Y.X., Huang, G., Wang, Y. & Chaplin, D.D. B lymphocytes induce the formation of follicular dendritic cell clusters in a lymphotoxin α-dependent fashion. *J. Exp. Med.* **187**, 1009–1018 (1998).

37. Gonzalez, M., Mackay, F., Browning, J.L., Kosco-Vilbois, M.H. & Noelle, R.J. The sequential role of lymphotoxin and B cells in the development of splenic follicles. *J. Exp. Med.* **187**, 997–1007 (1998).

38. Endres, R. et al. Mature follicular dendritic cell networks depend on expression of lymphotoxin β receptor by radioresistant stromal cells and of lymphotoxin beta and tumor necrosis factor by B cells. *J. Exp. Med.* **189**, 159–168 (1999).

39. Ngo, V.N., Cornall, R.J. & Cyster, J.G. Splenic T zone development is B cell dependent. *J. Exp. Med.* **194**, 1649–1660 (2001).

40. Junt, T. et al. Subcapsular sinus macrophages in lymph nodes clear lymph-borne viruses and present them to antiviral B cells. *Nature* **450**, 110–114 (2007).

41. Phan, T.G., Grigorova, I., Okada, T. & Cyster, J.G. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* **8**, 992–1000 (2007).

42. Matsuura, J., Sakanaka, M., Sato, N., Ichikawa, A. & Tanaka, S. Suppression of CXCR4 expression in mast cells upon IgE-mediated antigen stimulation. *Inflamm. Res.* **59**, 123–127 (2010).
ONLINE METHODS

Mice and infection. All mice were bred at the University of Rochester Animal Care and Use Committee. C57BL/6J (B6), B6.SL-Pepc<sup>−/−</sup>B6J (CD45.1<sup>−/−</sup> B6 congenics), B6.129S2–Ighm<sup>−/−</sup>/J (µMT), B6.FVB-Tg(lgdx-DTR/eGFP<sup>FL</sup>)57Lan/J (CD11c–DTR), B6.129X1-Cxcl13<sup>−/−</sup>/J (Cxcl13<sup>−/−</sup>), B6.129S2(Cg)–C5cr<sup>−/−</sup>/J (Ccr5<sup>−/−</sup>) and C57BL/6-Lta<sup>b38y</sup>/J (Lta<sup>−/−</sup>) mice were originally from Jackson Laboratory. B6.129–Ila<sup>−/−</sup>/J (B6 ilet 4G reporter) and B6.129–Ila<sup>−/−</sup>/J/Ighm<sup>−/−</sup>/J (µMT) mice were obtained from M. Mohrs; C57BL/6-Tg(ghdMD4)<sup>−/−</sup>/J (Lta<sup>−/−</sup>) mice were from J. Kearney; and B6NL.129–plt/MkJ (plt) mice were from J. Cyster. Adult mice were infected with gavage with *H. polygyrus* at larval stage 3 (200 maximal larvae) or intranasally with influenza virus strain H3N2 A/X31 (0.1 half-maximal lethal dose).

Bone marrow chimeras. Recipient mice were irradiated with 950 rads from a 137Cs source delivered in a split dose and were reconstituted with 1 × 10<sup>6</sup> total bone marrow cells. For the generation of CD11c–DTR bone marrow chimeras, B6 recipients were reconstituted with 100% CD11c–DTR bone marrow. For the generation of DC–Ccr5<sup>−/−</sup> and DC-WT chimeras, B6 recipients were reconstituted with either 80% CD11c–DTR bone marrow plus 20% Ccr5<sup>−/−</sup> bone marrow or 80% CD11c–DTR bone marrow plus 20% B6 bone marrow. For the generation of B-Lta<sup>−/−</sup> and B-WT chimeras, µMT recipients were reconstituted with 80% µMT bone marrow plus 20% Lta<sup>−/−</sup> bone marrow or with 80% µMT bone marrow plus 20% B6 bone marrow. For the generation of B–Ccr5<sup>−/−</sup> and 50/50 mixed bone marrow chimeras, B6 (CD45.1<sup>−/−</sup>) congenic recipients were reconstituted with 50% B6 (CD45.1<sup>−/−</sup>) bone marrow and 50% Ccr5<sup>−/−</sup> bone marrow. Experiments were initiated with the chimERIC mice 8–12 weeks after reconstitution.

In vivo depletion and blocking studies. For blockade of LTβR signaling in vivo, mice were treated intraperitoneally at the time of infection with 100 µg LTβR-Fc fusion protein<sup>43</sup> (Biogen Idec) or 100 µg control human immunoglobulin (Biogen Idec). For blockade of in vivo CXCL13 activity, mice were injected intraperitoneally at the time of infection with 200 µg anti–mouse CXCL13 (34614; R&D Systems) or control antibody (54447; R&D Systems). For depletion of CD20<sup>+</sup> B cells, 4 d before infection, mice were injected intraperitoneally with 250 µg mouse anti–mouse CD20 (immunoglobulin G2a isotype; 18B12; Biogen Idec)<sup>44</sup> or isotype-matched control antibody (2B8; Biogen Idec). For depletion of CD11c<sup>+</sup> cells in vivo, CD11c–DTR bone marrow chimeras were treated with 60 ng diphtheria toxin (Sigma–Aldrich) and then immediately infected. Mice received additional injections of diphtheria toxin on days 3 and 5.

Cell preparation and flow cytometry. Cells isolated from the lymph nodes, spleen or peritoneal cavity were pelleted with anti–mouse CD16/32 (5 µg/ml; 2.4G2; BD-Biosciences) and then stained with the following fluorochrome-conjugated antibodies: anti–CD11c (HL3), anti–B220 (RA3–6B2), anti–CD19 (1D3), anti–CD11b (M1/70), anti–CD40 (4D9) and anti–MHC class II (AF6–120.1), anti–IL-4 (11B11; BD-Biosciences), anti–IL-12 (1D11; BD-Biosciences), anti–IFN-γ (XMG1.2; BD-Biosciences). The Mouse regulatory T cell staining kit (eBioscience) and anti–Bcl-6 (K12–91; BD-Biosciences) were used for intracellular staining of Bcl-6. Determination of lymphocytes, cells were stained with LTβR-Fc or human immunoglobulin control protein, followed by conjugated F(ab')<sub>2</sub> antibody to human immunoglobulin absorbed against mouse immunoglobulin G (204.39–Southern Biotechnology Association). In some experiments, cells were preincubated with unconjugated antibody to lymphotoxin-α (BB6F6; Biogen Idec) for blockade of the binding of LTβR-Fc to membrane lymphotoxin-αβ<sub>2</sub>. Cells were analyzed with a FACScanto II (BD Biosciences) or C6 (Accuri) flow cytometer located in the University of Rochester Flow Cytometry Core Facility.

Cell purification, T cell transfer and in vitro culture. DCs from pooled lymph nodes were pelleted on LS columns with anti–CD11c MACS beads (Miltenyi Biotec) and then were stained with fluorochrome-labeled antibody to MH C class II (AF6–120.1), anti–B220 (RA3–6B2) and anti–CD11c (HL3); all from BD Biosciences. DC subsets were purified with a FACSaria (BD) in the University of Rochester Flow Cytometry Core. CD4<sup>+</sup> T cells were isolated by MACS from the spleens of naive CD45.1<sup>−/−</sup> B6 congenics or from the mesLNs of *H. polygyrus*-infected IL-4 reporter mice (day 5 after infection). All T cell preparations were >95% pure. Naive B6 (CD45.1<sup>−/−</sup>) T cells (1 × 10<sup>6</sup>/mouse) were injected intravenously into DC-WT and DC–Ccr5<sup>−/−</sup> mice (bone marrow chimeras) that were subsequently infected with *H. polygyrus*. DCs (2 × 10<sup>5</sup>) and CD4<sup>+</sup> T cells (1 × 10<sup>5</sup>/mouse) were cultured together for 72 h at 37 °C in round-bottomed 96-well plates in 200 µl of complete medium containing RPMI 1640 medium, sodium pyruvate, HEPES (pH 7.4), nonessential amino acids, penicillin, streptomycin, 2-mercaptoethanol and 10% heat-inactivated FBS (Gibco).

Migration assays. CXCL13 (1 µg/ml) or CCL19 (0.1 µg/ml) in RPMI medium with 1% FBS (R&D Systems) was added to the bottom chamber of each 24-well Transwell plate (polycarbonate filter with a 5-µm pore; Costar). DCs or B cells (1 × 10<sup>5</sup> cells per Transwell) were added to the upper chamber, followed by incubation for 90 min at 37 °C. Cells that had transmigrated were collected from the lower chamber, stained and counted on a flow cytometer, with Accuri C6 CFlow software. Results are presented as chemotaxis index or as a percentage of the input cells that migrated in the assay. The chemotaxis index represents number of cells that migrated in response to chemoattractants relative to the number of cells that spontaneously migrated in response to control medium.

Immunofluorescence. Frozen sections (5 µm in thickness) were prepared from lymph node embedded in optimum cutting temperature compound (Sakura Finetek) and were incubated with 10 µg/ml of Fc Block and 5% normal donkey serum in PBS, then were stained with fluorescein isothiocyanate–labeled anti–CD11c (HL3), biotin-labeled anti–CD4 (L3T4), biotin-labeled anti–CD8α (53-6.7) or Alexa Fluor 648–labeled anti–B220 (RA3–6B2; all from BD Biosciences). Primary antibodies were detected with Alexa Fluor 488–labeled goat antibody to fluorescein isothiocyanate (A6430; Invitrogen Life Sciences) and streptavidin–Alexa Fluor 555 (S21381; Invitrogen Life Sciences). Slides were mounted with SlowFade Gold antifade reagent (Invitrogen). Images were collected with a Zeiss Axioplan 2 microscope and recorded with a Zeiss Axiocam digital camera (Zeiss). The images were obtained with a 20× objective for a final magnification of ×200. Images were collected with Zeiss Axiosview Image software and saved as JPEG files.

Real-time PCR. RNA was extracted from mesLNs with RNeasy (Qiagen) and treated with DNase, then 2 µg RNA was reverse transcribed with random hexamers and Superscript II (Invitrogen). Quantitative PCR was done with Taqman master mix and primers and probes for Cxcl13 (Applied Biosystems). All reactions were run on a Lightcycler 480 Real-time PCR System (Roche). The abundance of CXCL13 mRNA was determined and then normalized to the abundance of GAPDH mRNA. Results are presented relative to expression in mesLN cells from *H. polygyrus*–infected B6 mice treated with control antibody or control protein.

Statistical Analyses. GraphPad Prism software (Version 5.0a) was used for data analysis. Data were analyzed with the unpaired Student’s t test. P values of 0.05 or less were considered significant.

43. Fava, R.A. et al. A role for the lymphotoxin/LIGHT axis in the pathogenesis of murine collagen-induced arthritis. *J. Immunol. 171*, 115–126 (2003).

44. Hamel, K. et al. Suppression of proteoglycan-induced arthritis by anti–CD20 B Cell depletion therapy is mediated by reduction in autoantibodies and CD4<sup>+</sup> T cell reactivity. *J. Immunol. 180*, 4994–5003 (2008).

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