Immunity
Article

Type I Interferon Signaling in Dendritic Cells Stimulates the Development of Lymph-Node-Resident T Follicular Helper Cells

Helena Cucak,[1] Ulf Yrlid,[2] Boris Reizis,[3] Ulrich Kalinke,[4,5] and Bengt Johansson-Lindbom[1,*]
[1]Immunology Section, BMC D14, Lund University, Lund 22184, Sweden
[2]Department of Microbiology and Immunology, Institute of Biomedicine, The Mucosal Immunobiology and Vaccine Center (MIVAC), University of Gothenburg and Swedish Foundation for Strategic Research, Box 435, Göteborg 40530, Sweden
[3]Department of Microbiology, Columbia University Medical Center, New York, NY 10032, USA
[4]TWINCORE, Centre for Experimental and Clinical Infection Research, 30625 Hanover, Germany
[5]Division of Immunology, Paul-Ehrlich-Institut, D-63225 Langen, Germany
*Correspondence: bengt.johansson_lindbom@med.lu.se
DOI 10.1016/j.immuni.2009.07.005

SUMMARY

T follicular helper (Tfh) cells represent a recently defined CD4$^+$ T cell subset characterized by the expression of the chemokine receptor CXCR5 and an enhanced ability to support B cells to mount antibody responses. Here, we demonstrate that lymph-node-resident CXCR5$^+$ Tfh cells and gut-homing integrin $\alpha_4\beta_7$-expressing T helper cells are generated as separate subsets in the gut-draining mesenteric lymph nodes. Type I interferon signaling in dendritic cells and in nonhematopoietic cells selectively stimulates Tfh cell development in response to antigen in conjunction with Toll-like receptor (TLR)3 or TLR4 agonists. Consistent with this, the ability of dendritic cells to produce the cytokine IL-6, required for in vivo Tfh differentiation, and antibody affinity maturation are both reduced in absence of type I interferon signaling. Thus, our results identify type I interferon as a natural adjuvant that selectively supports the generation of lymph node resident Tfh cells.

INTRODUCTION

Antibody (Ab) affinity maturation and generation of B cell memory occur within the specialized microenvironment of germinal centers (GC) that develop within B cell follicles of secondary lymphoid organs (Berek et al., 1991; Blink et al., 2005; Jacob et al., 1991; Takahashi et al., 1998). Whereas these processes are mandatory for production of long-lived IgG responses, conferring protection following natural infection, or in settings of vaccination, they may also lead to the generation of autoantibodies and autoimmune disease (Shlomchik et al., 1987). GC B cell differentiation is dependent on help provided by CD4$^+$ T helper (Th) cells (MacLennan et al., 1997). Accumulating evidence indicates that expression of the chemokine receptor CXCR5, whose ligand CXCL13 is strongly produced within B cell follicles (Gunn et al., 1998), distinguishes a subset of T follicular helper (Tfh) cells with an enhanced ability to provide B cell help (Ansel et al., 1999; Breitfeld et al., 2000; Campbell et al., 2001; Schaerli et al., 2000) and that appear to be developmentally distinct from Th1, Th2, and Th17 cells (Nurieva et al., 2008). In addition to CXCR5, Tfh cells have high IL-21 and Bcl-6 mRNA expression (Chtanova et al., 2004), and IL-21 was recently shown to regulate their generation, possibly in an autocrine way (Nurieva et al., 2008; Vogelzang et al., 2008). Expression of IL-21 is in turn induced by IL-6 that is required for Tfh differentiation in vivo (Nurieva et al., 2008; Suto et al., 2008).

CXCR5$^+$ Tfh cells develop preferentially in the draining lymph node (LN) from precursors that express high-affinity T cell receptors (TCRs), and resting memory Tfh cells persist in the draining, but not in nondraining, LN after contraction of the adaptive immune response (Fazilleau et al., 2007). Activation of CD4$^+$ T cells in skin-associated LNs leads to the concurrent generation of peripheral tissue-tropic CXCR5$^-$ effector Th cells, expressing high amounts of the skin-homing molecule P-selectin ligand (P-lig) and P-lig$^-$CD62L$^-$CXCR5$^+$ Tfh cells, and these subsets appear to be functionally specialized in supporting cutaneous inflammation and B cell Ab production, respectively (Campbell et al., 2001). How this spatial dichotomy is established is currently not entirely clear. Whether distinct subsets of Tfh cells and peripheral effector CD4$^+$ T cells develop in lymphoid tissues other than cutaneous LNs also remains to be determined.

Type I interferons (IFN I) represent a family of cytokines with antiviral and immunomodulatory activities (Theofilopoulos et al., 2005). IFN-β and multiple IFN-α proteins are produced at high amounts in response to viral and bacterial infections and signal through a common IFN I receptor (IFNAR) (Theofilopoulos et al., 2005). IFN I enhances T cell-dependent Ab responses in vivo and seems to mediate this effect at least partially through signaling in dendritic cells (DCs) (Le Bon et al., 2001). Consistent with this, IFN-α and -β underlie the ability of the TLR3 and TLR4 agonists pI:C and LPS, respectively, to induce upregulation of costimulatory molecules on antigen-presenting cells (APC) (Hoebe et al., 2003). Collectively, these observations led us to hypothesize that IFN I enhances T cell-dependent Ab responses by acting as a natural adjuvant for the generation of CXCR5$^+$ Tfh cells.

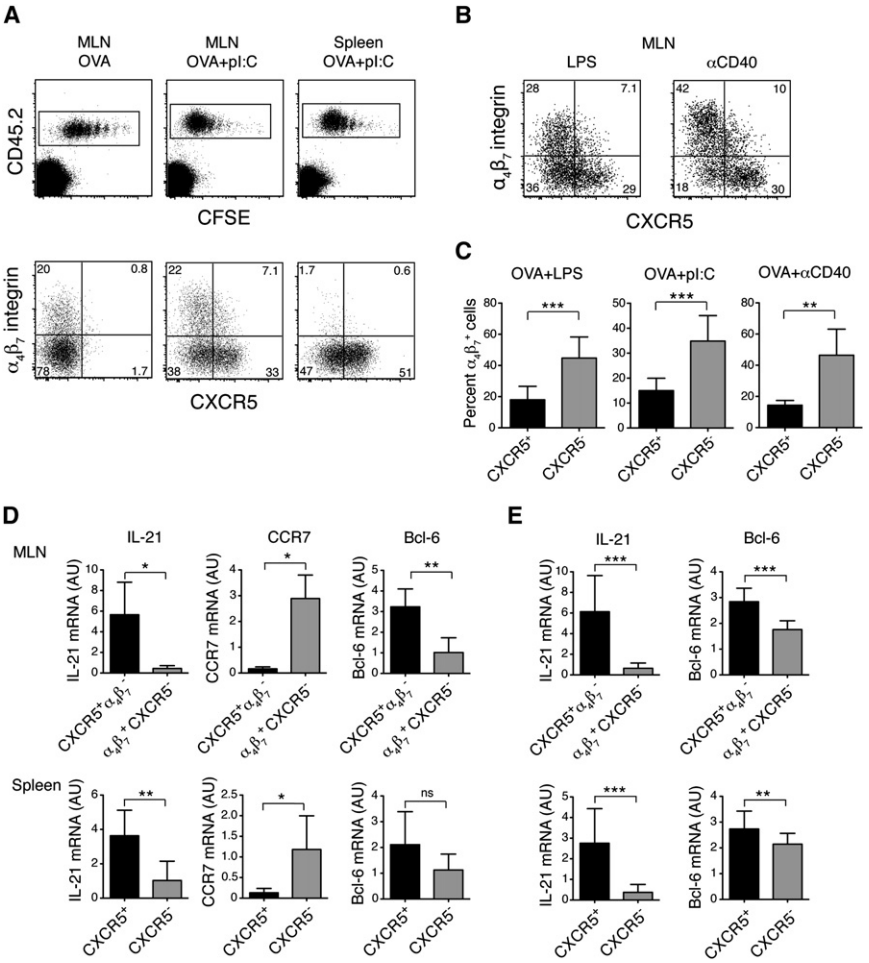

Figure 1. Generation of Separate Subsets of CXCR5$^+$ Tfh Cells and $\alpha_4\beta_7^+$ Th Cells in the MLN

CFSE-labeled CD45.2$^+$ OT-II cells were transferred into C57Bl/6-CD45.1 mice and analyzed 3 days after i.p. immunization.

(A and B) Representative flow cytometry analyses of OT-II cells in indicated organs after immunization with OVA in presence or absence of pI:C (A) or together with LPS or anti-CD40 (B). Numbers indicate percentages of cells within the quadrants. Cell division dependent dilution of CFSE and gating of CD45.2$^+$ donor cells among PI$^-$CD4$^+$ B220$^-$ cells are shown in top panel of (A).

(C) Percentages of MLN CXCR5$^+$ and CXCR5$^-$ OT-II cells coexpressing $\alpha_4\beta_7$. Pooled results (mean ± SD) from six experiments with pI:C, nine with LPS, and two with anti-CD40 (3 to 4 mice in each experiment).

(D and E) Expression of IL-21, CCR7, and Bcl-6 mRNA in MLN and spleen OT-II cells with indicated phenotypes at day 3 (n = 4–10) (D) and day 5 (n = 10) (E) postimmunization (mean ± SD). Expression is normalized to β-actin. ***p < 0.001; **p < 0.01; *p < 0.05.

RESULTS

Generation of Separate CXCR5$^+$ Tfh Cell and $\alpha_4\beta_7^+$ Gut-Homing CD4$^+$ T Cell Subsets in the Mesenteric LNs

T cells homing to the gut express the integrin $\alpha_4\beta_7$, whose ligand MadCAM-1 is present on intestinal postcapillary endothelial cells (Berlin et al., 1993; Lefrancois et al., 1999). Expression of $\alpha_4\beta_7$ is selectively induced on T cells activated in intestinal inductive sites, including the gut-draining mesenteric LN (MLN) (Campbell and Butcher, 2002). To determine if the MLN supports generation of CXCR5$^+$ Tfh cells that are distinct from $\alpha_4\beta_7^+$ gut-homing T cells, we transferred CFSE-labeled OVA-specific TCR transgenic CD4$^+$ T (OT-II) cells to wild-type (WT) recipients and determined the phenotype of donor cells 3 days after intraperitoneal (i.p.) immunization with OVA. In the MLN, but not in the spleen, a substantial proportion of divided OT-II cells expressed $\alpha_4\beta_7$ regardless if adjuvant was coinjected or not (Figure 1A). Whereas expression of CXCR5 on T cells requires adjuvant (Ansel et al., 1999), the large majority of MLN OT-II cells that acquired CXCR5 in the presence of LPS, pI:C, or an agonistic CD40 mAb were $\alpha_4\beta_7^-$ (Figures 1A–1C). Consistent with prior characterizations of Tfh cells (Chtanova et al., 2004; Haynes et al., 2007), the MLN CXCR5$^+\alpha_4\beta_7^-$ OT-II cells expressed higher amounts of IL-21 and Bcl-6 mRNA and contained less CCR7 mRNA, as compared to their $\alpha_4\beta_7^+$CXCR5$^-$ counterparts (Figure 1D). This differential expression in IL-21 and Bcl-6 mRNA was also evident 5 days after immunization (Figure 1E), and similar results were obtained with CXCR5$^+$ and CXCR5$^-$ OT-II cells sorted from the spleen (Figures 1D and 1E). These results collectively show that Tfh cells are generated in the MLN and spleen after i.p. immunization with a protein in adjuvant and that CXCR5$^+\alpha_4\beta_7^-$ Tfh cells and $\alpha_4\beta_7^+$ gut-homing Th cells develop as two distinct subsets in the MLN.

The Large Majority of $\alpha_4\beta_7^+$ Th Cells, but Not CXCR5$^+$ Tfh Cells, Generated in MLN Rapidly Exit the Lymph Node

Although the majority of OT-II cells homing to the gut expressed $\alpha_4\beta_7$, few CXCR5$^+$ OT-II cells were detected in this site and their proportion was also lower in the liver as compared to MLN and spleen (Figures 2A and 2B). It hence appears as CXCR5$^+$ and $\alpha_4\beta_7^+$ OT-II cells differ in their ability to exit LNs and to localize to peripheral tissues. To determine their LN egress efficiencies, we treated recipients with the sphingosine 1-phosphate receptor-1 ($S1P_1$) agonist FTY720 that shuts down lymphocyte egress from lymphoid tissues (Matloubian et al., 2004). Three days after immunization, the number of OT-II cells was ~3 times higher in the MLN of FTY720 treated mice as compared to controls, and consistent with an FTY720-mediated LN retention, they were essentially absent from peripheral blood of treated mice (Figure 2C). This was accompanied by a large proportional increase in $\alpha_4\beta_7^+$CXCR5$^-$ OT-II cells in the MLN of the FTY720-treated mice (Figure 2D), corresponding to an almost 10-fold increased number of these cells, but only ~1.7-fold higher number of the CXCR5$^+\alpha_4\beta_7^-$ OT-II cells

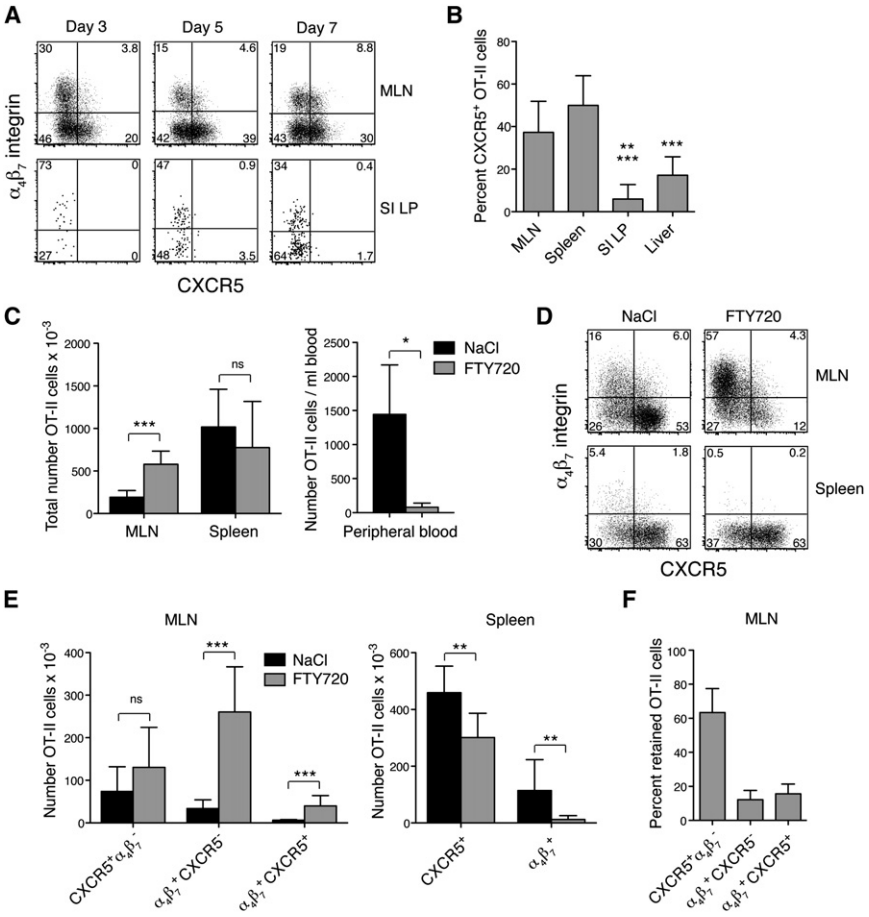

Figure 2. CXCR5$^+$ Tfh Cells Are Selectively Retained in the MLN

(A) Expression of CXCR5 versus $\alpha_4\beta_7$ on OT-II cells in MLN and small intestinal lamina propria (SI LP) 3–7 days after immunization with OVA and pI:C. Results are representative of three mice for each time point, and similar results were obtained in a second experiment with OVA plus LPS.

(B) Percentages of CXCR5$^+$ OT-II cells in indicated organs 3 days after immunization with OVA and adjuvant (mean ± SD, n = 12). Pooled data from four separate experiments (two with LPS and two with pI:C).

(C–F) Recipients received FTY720 or saline i.p. 18–24 hr after immunization, and the number and phenotype of OT-II cells in indicated organs were determined 3 days postimmunization. Pooled results (mean ± SD, n = 9) from three separate experiments (two with LPS and one with pI:C) are shown in (C) and (E). (C) Total number of OT-II cells in indicated tissues. (D) Representative flow cytometry results for expression of CXCR5 versus $\alpha_4\beta_7$. Numbers indicate percentages of cells within quadrants. (E) Number of OT-II cells with indicated phenotype. (F) Percentages (mean ± SD) of OT-II cells with indicated phenotype retained within the MLN. Results were obtained by calculating the ratio between saline- and FTY720-treated mice for each subset and are based on the mean of ratios obtained in the separate experiments in (E). ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

(Figure 2E). The small fraction of cells that coexpressed $\alpha_4\beta_7$ and CXCR5 also displayed a 10-fold higher number following FTY720 treatment (Figure 2E), indicating that all $\alpha_4\beta_7^+$ Th cells exit the LN with similar high efficiency. In keeping with the efficient egress of these cells from the MLN, a large number of $\alpha_4\beta_7^+$ OT-II cells was present in spleen of saline-treated, but not FTY720-treated, mice (Figures 2D and 2E). While we could only detect a tendency of reduced $S1P_1$ mRNA in CXCR5$^+\alpha_4\beta_7^-$ compared with $\alpha_4\beta_7^+$CXCR5$^-$ OT-II cells, expression of CD69 was confined to the former subset at this time point (Figure S1 available online). Maintained CD69 expression on Tfh cells may hence partially underlie their LN sequestration, as previously suggested (Fazilleau et al., 2007; Shiow et al., 2006). Finally, there was no difference in the number of OT-II cells lacking both CXCR5 and $\alpha_4\beta_7$ between FTY720- and saline-treated mice (Figure S2A). These double-negative cells failed to efficiently downregulate CD62L (Figure S2B) and seem to represent a second subset of LN-resident Th cells that, by virtue of their CXCR5$^-$ phenotype, are excluded from the follicles. We have not characterized this subset any further. These results collectively demonstrate that the majority of donor cells expanded in the MLN, and more than 90% of the numerous $\alpha_4\beta_7^+$ OT-II cells generated at this site, rapidly exit the LN. In marked contrast, most CXCR5$^+\alpha_4\beta_7^-$ OT-II cells are retained within the LN (Figure 2F).

CXCR5$^+$ Tfh Cells Display Lymphoid-Tissue-Specific Cytokine Production and Are Preferentially Generated under Strong Immunogenic Conditions

When restimulated in vitro, MLN $\alpha_4\beta_7^+$CXCR5$^-$ OT-II cells from mice immunized with OVA plus LPS produced IL-4, IL-10, and IL-17, but not IFN-γ (Figure 3A). In the spleen, the CXCR5$^-$ OT-II cells produced negligible amounts of IL-4 and IL-17, moderate amounts of IL-10, and relatively large quantities of IFN-γ (Figure 3A). Although this indicates that the MLN and spleen imprint different Th cell cytokine responses, MLN CXCR5$^+\alpha_4\beta_7^-$ and spleen CXCR5$^+$ OT-II cells produced relatively low amounts of IL-10, IL-17, and IFN-γ. In the MLN, but not in the spleen, the CXCR5$^+$ Tfh cell subset, however, secreted substantial amounts of IL-4 (Figure 3A), suggesting that the nature of Tfh responses also differs between these lymphoid tissues.

Both in the absence and presence of FTY720, CXCR5$^+$ OT-II cells had downregulated CD62L more efficiently than the CXCR5$^-$ fraction (Figure 3B), and whereas only the most divided cells expressed CXCR5, $\alpha_4\beta_7$ was detectable also on less-cycled cells (Figure 3C). These observations collectively suggest that CXCR5$^+$ Tfh cells represent robustly activated effector cells (Lanzavecchia and Sallusto, 2000). To determine if strong immunogenic conditions enhance their generation, we immunized mice with OVA plus pI:C, an agonistic CD40 mAb, or both together.

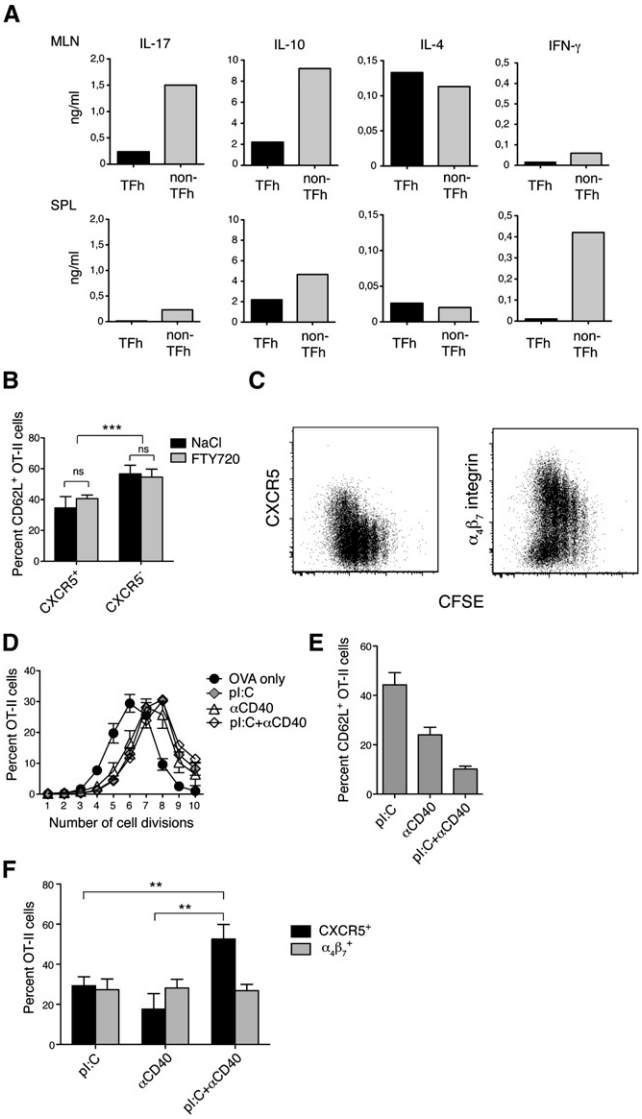

Figure 3. Tfh Cells Display Lymphoid-Tissue-Specific Cytokine Production and Are Preferentially Generated under Strong Immunogenic Conditions

(A) OT-II cell recipients received FTY720 24 hr after immunization with OVA and LPS, and donor cells with a Tfh cell phenotype ($CXCR5^+\alpha_4\beta_7^-$ in MLN; $CXCR5^+$ in spleen) or a non-Tfh cell phenotype ($CXCR5^-\alpha_4\beta_7^+$ in MLN; $CXCR5^-$ in spleen) were sorted from six pooled mice 3 days after immunization. Cells were restimulated at 10^6 cells/ml with anti-CD3 and anti-CD28 for 24 hr, and supernatants were analyzed for indicated cytokines by ELISA. One representative experiment of two performed is shown.

(B) Recipients of CFSE-labeled OT-II cells received saline or FTY720 24 hr after i.p. immunization with OVA and LPS, and percentages of $CXCR5^+$ or $CXCR5^-$ MLN OT-II cells expressing CD62L were determined 3 days after immunization by flow cytometry. Pooled results (mean ± SD; n = 6) from two separate experiments.

(C) Representative flow cytometry results of CXCR5 and $\alpha_4\beta_7$ expression by MLN OT-II in relation to CFSE dilution.

(D–F) Recipients were immunized with OVA alone or together with pI:C, anti-CD40, or both. OT-II cells in MLN were analyzed 3 days later by flow cytometry. (D) Percentages of total OT-II cells as a function of cell division. (E) Percentages of OT-II cells expressing CD62L. (F) Percentages of OT-II cells expressing CXCR5 or $\alpha_4\beta_7$. Results from one representative experiment (mean ± SD; n = 3) of two performed are shown. ***p < 0.001; **p < 0.01.

Although the combination did not further enhance the adjuvant-dependent increase in OT-II cell proliferation (Figure 3D), it more efficiently downregulated CD62L than when pI:C or anti-CD40 were injected separately (Figure 3E). This was accompanied by a 2-fold greater fraction of $CXCR5^+$ MLN OT-II cells (Figure 3F). The proportional number of $\alpha_4\beta_7^+$ donor cells did, however, not differ between the different groups. The ability of pI:C and anti-CD40 to additively enhance CXCR5 expression did not merely reflect a more efficient egress of $CXCR5^-$ OT-II cell under these conditions, because the same results were obtained with FTY720-treated mice (data not shown). Taken together, these results indicate that Tfh differentiation is selectively augmented when a relatively high threshold in T cell activation signal strength is exceeded.

Tfh Cell Generation Is Selectively Impaired in the Absence of Type I Interferon Signaling

Given the ability of IFN-α and -β to enhance the T cell stimulatory capacity of APC, we assessed the role of IFN I in Tfh cell differentiation. Following immunization with OVA and LPS, the percentage and number of $CXCR5^+$ OT-II cells were reduced in MLNs and spleens of *Ifnar1*$^{-/-}$ mice as compared to WT recipients (Figures 4A and 4C). Divided OT-II cells re-sorted from the *Ifnar1*$^{-/-}$ recipient's spleens expressed less Bcl-6 and IL-21 mRNA than their counterparts sorted from WT mice (Figure 4B). However, the *Ifnar1*$^{-/-}$ recipients contained an overall reduced number of MLN and spleen OT-II cells (Figure 4C), and although they had a similar percentage of MLN $\alpha_4\beta_7^+$ OT-II cells as WT recipients (Figure 4A), the number of $\alpha_4\beta_7^+$ OT-II cells was consequently also reduced in these mice (Figure 4C). OT-II cells activated in *Ifnar1*$^{-/-}$ mice had, however, proliferated only slightly less than those activated in WT mice (Figure 4D), and to determine if an increased egress accounted for their reduced number in *Ifnar1*$^{-/-}$ animals (Shiow et al., 2006), we repeated the experiments but now treated the immunized recipients with FTY720. When donor cells were trapped in lymphoid tissues, a similar percentage and number of $\alpha_4\beta_7^+$ OT-II cells were generated in the MLN of WT and *Ifnar1*$^{-/-}$ mice (Figures 5A and 5B). Likewise, the total number of OT-II cells was similar in the spleens and MLNs of the two groups (Figure 5B). In marked contrast, the percentage and number of $CXCR5^+$ OT-II cells were still greatly reduced in *Ifnar1*$^{-/-}$ mice (Figures 5A and 5B). Finally, we also observed a similar selective loss of $CXCR5^+$ OT-II cells in FTY720-treated *Ifnar1*$^{-/-}$ recipients when pI:C was used as adjuvant (data not shown). These results collectively demonstrate that IFN I stimulates the generation of LN resident $CXCR5^+$ Tfh cells but is not required for the generation of the peripheral $\alpha_4\beta_7^+$ effector Th cells.

Impaired Affinity Maturation and Follicular T Cell Homing in *Ifnar1*$^{-/-}$ Mice

Next, we compared serum titers of 4-Hydroxy-3-nitrophenylacetyl (NP)-specific Abs in WT and *Ifnar1*$^{-/-}$ mice after i.p. immunization with NP-conjugated OVA and LPS. Two weeks after a secondary challenge with NP-OVA, titers of specific IgM and IgG were similar in both groups of mice (Figure 6A). The predominant IgG subisotype produced was IgG1, and titers of specific IgG1 were also comparable in WT and *Ifnar1*$^{-/-}$ mice. In keeping with previous reports (Le Bon et al., 2001; Proietti et al., 2002),

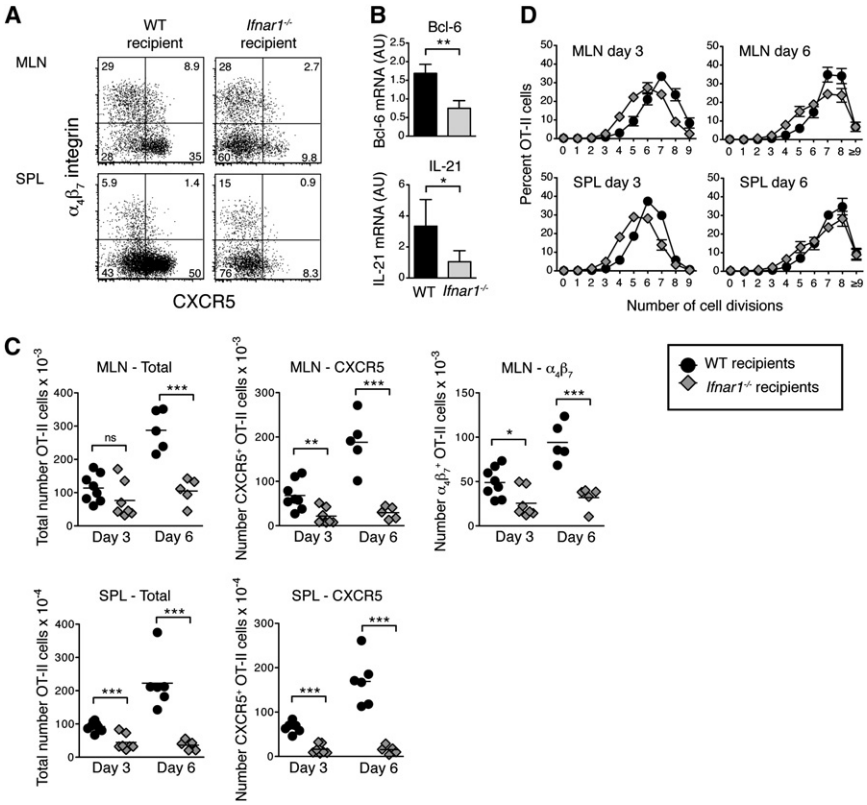

Figure 4. Reduced Number of CXCR5$^+$ Tfh Cells in *Ifnar1*$^{-/-}$ Mice

CFSE-labeled OT-II cells were transferred into WT or *Ifnar1*$^{-/-}$ mice that were immunized i.p. with OVA and LPS.

(A) Representative flow cytometry results for CXCR5 versus $\alpha_4\beta_7$ expression 3 days after immunization.

(B) Expression of IL-21 and Bcl-6 mRNA normalized to β-actin in sorted spleen OT-II cells (mean ± SD; n = 4).

(C) Number of total, CXCR5$^+$, or $\alpha_4\beta_7^+$ OT-II cells in the MLN and spleen 3 and 6 days after immunization. Numbers from individual mice and mean values from two pooled experiments are shown.

(D) Percentages (mean ± SD; n = 3) of total OT-II cells as a function of cell-cycle number as judged from CFSE dilution. One representative experiment of two performed is shown. ***p < 0.001; **p < 0.01; *p < 0.05.

the low titer of specific IgG2a produced was, however, reduced in *Ifnar1*$^{-/-}$ mice (Figure 6A). Because these results were obtained by measuring serum anti-NP reactivity against densely NP-conjugated BSA (NP_{23}-BSA), which binds both low- and high-affinity NP Abs, we next analyzed the same sera for high-affinity NP-specific IgG in the ELISA by using a sparsely NP-conjugated protein (NP_2-PSA) (Roes and Rajewsky, 1993). By this method, serum titers of high-affinity anti-NP IgG and IgG1 were found to be significantly (p < 0.001) reduced in *Ifnar1*$^{-/-}$ mice compared with WT controls (Figure 6B).

Efficient generation of GCs requires CXCR5-dependent follicular homing of primed CD4$^+$ T cells. Thus, we compared the numbers of OT-II cells that entered B cell follicles in MLN of immunized WT and *Ifnar1*$^{-/-}$ recipients. In agreement with the impaired generation of CXCR5$^+$ OT-II cells in *Ifnar1*$^{-/-}$ mice, the density of OT-II cells within the MLN B cell area was significantly (p < 0.05) reduced compared with WT mice (Figures 6C and 6D). These results show that the failure of *Ifnar1*$^{-/-}$ mice to efficiently support Tfh cell differentiation is accompanied by reduced follicular T cell homing and impaired Ab affinity maturation.

IFNAR Signaling in CD11c$^+$ DCs and Nonhematopoietic Cells Underlies IFN I-Dependent Tfh Cell Differentiation In Vivo

Robust IFN I production in response to LPS or pI:C depends on IFNAR feedback signaling. The generation of Tfh cells could, therefore, involve direct IFN I signaling in WT OT-II cells, and reduced amounts of IFN-α and -β in *Ifnar1*$^{-/-}$ recipients underlie their impaired development in these mice. To address this, we

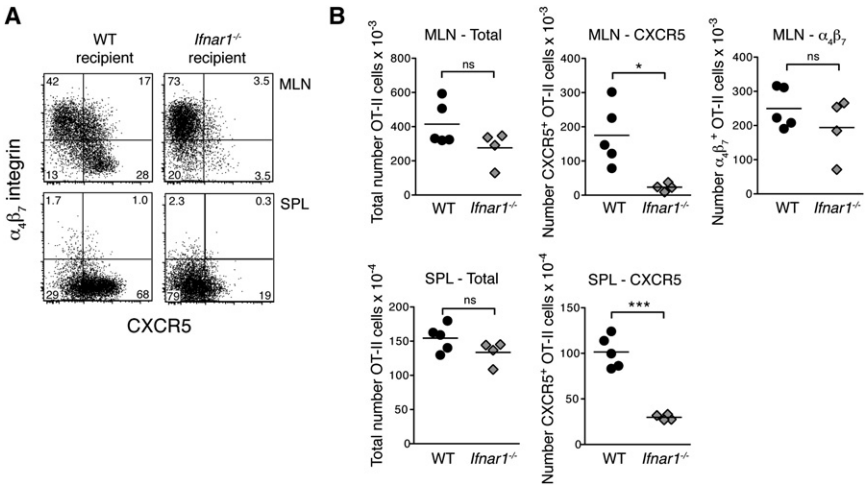

Figure 5. Impaired Generation of CXCR5$^+$ Tfh Cells in *Ifnar1*$^{-/-}$ Mice

WT and *Ifnar1*$^{-/-}$ mice were transferred with OT-II cells and immunized as described in Figure 4. Twenty hours later, the recipients were treated with FTY720, and 3 days after immunization, the total cells in MLN and spleen were counted and the phenotype of donor cells was determined by flow cytometry.

(A) Representative results for CXCR5 and $\alpha_4\beta_7$ expression.

(B) Number of total, CXCR5$^+$, or $\alpha_4\beta_7^+$ OT-II cells in the MLN and spleen. Numbers from individual mice and mean values are shown. ***p < 0.001; *p < 0.05.

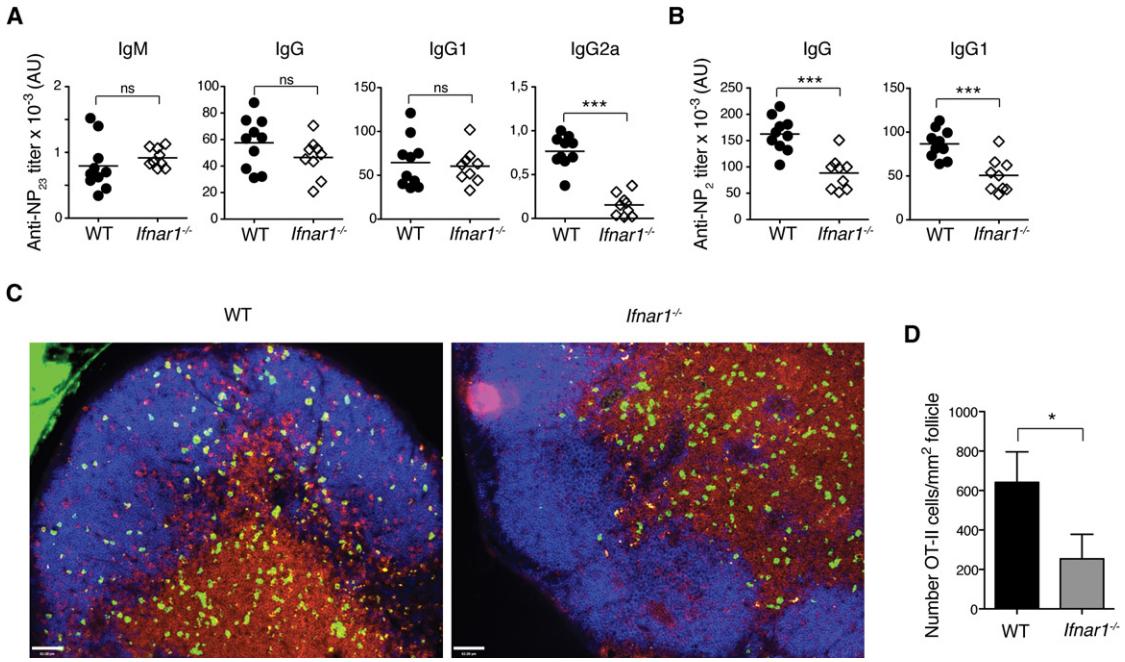

Figure 6. Impaired Affinity Maturation and Follicular T Cell Homing in *Ifnar1*$^{-/-}$ Mice

(A and B) WT and *Ifnar1*$^{-/-}$ mice were immunized i.p. with NP-OVA and LPS. Three weeks later, mice received NP-OVA i.p., and after 2 additional weeks, serum was analyzed for NP-specific Abs by ELISA. Results obtained from individual mice and mean values are shown and are expressed as arbitrary units. (A) Serum IgM, IgG, IgG1, and IgG2a reactivity against NP_{23}-BSA. (B) Serum IgG and IgG1 reactivity against NP_2-PSA.

(C) CD45.1$^+$ OT-II cells were transferred into WT or *Ifnar1*$^{-/-}$ recipients that were immunized i.p. with OVA and LPS. The density of OT-II cells within the MLN B cell follicles was determined 3 days later by immunofluorescence analysis using mAbs to CD45.1 (green), B220 (blue), and TCRVβ (red).

(D) Numbers (mean ± SD; n = 4) of OT-II cells/mm^2 B cell follicle. ***$p < 0.001$; *$p < 0.05$.

cotransferred IFNAR-deficient and -sufficient OT-II cells into *Ifnar1*$^{-/-}$ and WT mice, respectively. Three days after immunization, the majority of OT-II cells in the MLN of WT recipients expressed CXCR5, and this occurred to a similar degree on the IFNAR-deficient and -sufficient donor cells (Figures 7A and 7B). The frequency of CXCR5$^+$ OT-II cells was again strongly reduced in the *Ifnar1*$^{-/-}$ recipients, and this affected cotransferred IFNAR-deficient and -sufficient OT-II cells equally (Figures 7A and 7B). IFN I signaling in CD4$^+$ T cells is, thus, dispensable for Tfh cell development.

Next, we transferred WT OT-II cells into bone marrow (BM) chimeras lacking the IFNAR on either hematopoietic or nonhematopoietic cells. After immunization followed by FTY720 treatment, the generation of CXCR5$^+\alpha_4\beta_7{}^-$ OT-II cells was impaired in the MLN of recipients lacking the IFNAR on hematopoietic (*Ifnar1*$^{-/-}$ → B6) or on nonhematopoietic (B6 → *Ifnar1*$^{-/-}$) cells, as compared with controls expressing the receptor on both cell types (B6 → B6) (Figures 7C and 7D). However, this reduction was most pronounced when hematopoietic cells lacked the receptor (Figure 7D), indicating that IFN I signaling in APC plays an important role. To dissect the contribution of individual subsets of APC, we assessed Tfh development in conditional (floxed) *Ifnar1* mice (*Ifnar1*$^{fl/fl}$) crossed with transgenic mouse strains expressing the Cre recombinase under control of the CD11c, CD19, or M lysozyme (LysM) promoters (Cervantes-Barragan et al., 2009; Prinz et al., 2008). The frequency and number of CXCR5$^+\alpha_4\beta_7{}^-$, but not $\alpha_4\beta_7{}^+$CXCR5$^-$, OT-II cells were significantly ($p < 0.01$) reduced in the MLN of immunized recipients lacking the IFNAR selectively on CD11c$^+$ DCs (*Ifnar1*$^{fl/fl}$ CD11cCre), as compared with *Ifnar1*$^{fl/fl}$ controls (Figures 7E and 7F). A reduced Tfh development in these mice was also evident when the total number of CXCR5$^+$ (rather than CXCR5$^+\alpha_4\beta_7{}^-$) OT-II cells was counted (reduced by 45 ± 15% compared with *Ifnar1*$^{fl/fl}$ controls (mean value ± SD; n = 6; p = 0.0170). We confirmed a selective, but incomplete, deletion of the IFNAR on DCs in the *Ifnar1*$^{fl/fl}$ CD11cCre mice by flow cytometry (Figure S3). The fact that we could detect IFNAR expression on ~13%–14% of CD11c$^+$MHCII$^+$ DCs in these mice indicates that the importance of IFN I signaling in DCs may even have been underestimated in these experiments. In contrast to this, CXCR5$^+\alpha_4\beta_7{}^-$ OT-II cells were not significantly ($p > 0.05$) reduced in mice lacking the receptor selectively on B cells (*Ifnar1*$^{fl/fl}$ CD19Cre) or on granulocytes and macrophages (*Ifnar1*$^{fl/fl}$ LysMCre) (Figures 7E and 7F). Yet, we detected a complete and selective deletion of the IFNAR on B cells in *Ifnar1*$^{fl/fl}$ CD19Cre mice (Figure S3). The cell-specific IFNAR deletion in the *Ifnar1*$^{fl/fl}$ LysMCre mice has recently been shown to give substantial deletion on monocytes and macrophages and a partial deletion on granulocytes (Prinz et al., 2008; Stockinger et al., 2009). Collectively, these results demonstrate that IFNAR signaling in DCs and in cells of nonhematopoietic origin underlies the IFN I-dependent generation of CXCR5$^+$ Tfh cells.

IFN I Enhances IL-6 Production from CD11c$^+$ DCs

The ICOSL, in particular ICOSL expressed by B cells, CD28 ligands, and IL-6 are all required for efficient Tfh differentiation

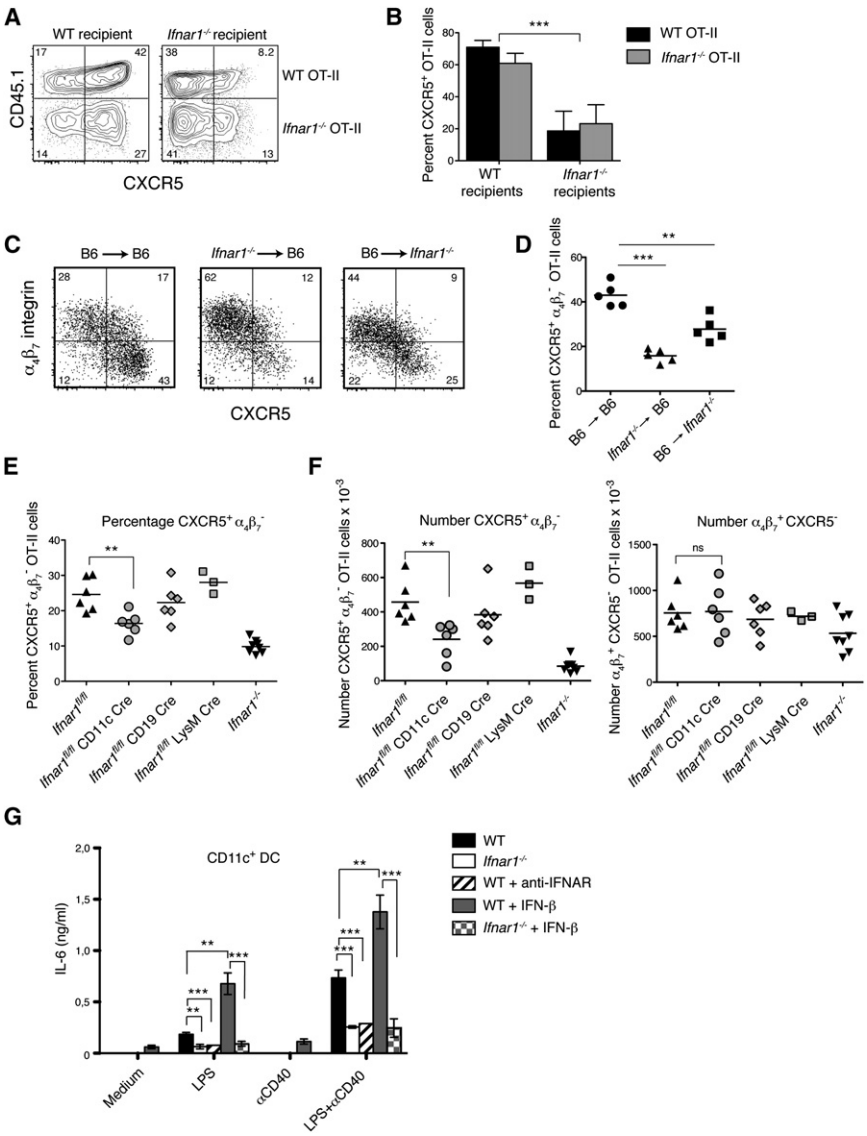

Figure 7. IFNAR Signaling in CD11c$^+$ DCs Stimulates Tfh Cell Differentiation and IL-6 Production

(A and B) CFSE-labeled *Ifnar1*$^{-/-}$ (CD45.2$^+$) and WT (CD45.1$^+$CD45.2$^+$) OT-II cells were cotransferred into *Ifnar1*$^{-/-}$ and WT mice (both CD45.2$^+$), respectively. Expression of CXCR5 on donor cells in the MLN was determined by flow cytometry 3 days after immunization with OVA and LPS. Total donor cell population was identified as CFSE$^+$TCRVα2$^+$TCRVβ5.1$^+$ cells among PI$^-$CD4$^+$ B220$^-$ cells. (A) Representative flow cytometry results. Numbers indicate percentages of cells within quadrants. (B) Percentages (mean ± SD; n = 4) of cotransferred WT and *Ifnar1*$^{-/-}$ OT-II cells expressing CXCR5 in WT and *Ifnar1*$^{-/-}$ recipients. (C–F) OT-II cells were transferred into indicated cell-specific IFNAR-deficient or control mice. Recipients were immunized with OVA and LPS, and treated with FTY720 1 day later. The percentage and number of indicated OT-II cell subsets in the MLN were determined 3 days after immunization by flow cytometry analysis. (C and D) Results obtained with IFNAR-sufficient control BM chimeras (B6 → B6) and BM chimeras lacking the IFNAR selectively on hematopoietic (*Ifnar1*$^{-/-}$ → B6) or nonhematopoietic (B6 → *Ifnar1*$^{-/-}$) cells. (C) Representative results. Numbers indicate percentages of cells within quadrants. (D) Percentages of CXCR5$^+\alpha_4\beta_7^-$ OT-II cells. Individual mice and mean values are shown (n = 5). (E and F) Percentages CXCR5$^+\alpha_4\beta_7^-$ OT-II cells (E) and number CXCR5$^+\alpha_4\beta_7^-$ and $\alpha_4\beta_7^+$CXCR5$^-$ OT-II cells (F) in indicated mouse strains. Pooled results from two separate experiments are shown (n = 6, except for *Ifnar1*$^{fl/fl}$ LysM Cre, where n = 3). (G) IL-6 secretion from WT or *Ifnar1*$^{-/-}$ CD11c$^+$ DCs stimulated with LPS, anti-CD40, or both, in the absence or presence of neutralizing anti-IFNAR or recombinant IFN-β. Supernatants were harvested after 20 hr, and IL-6 concentrations were determined by ELISA. One representative experiment (mean values of triplicate wells ± SD) of four performed is shown. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

in vivo (Akiba et al., 2005; Haynes et al., 2007; Nurieva et al., 2008; Walker et al., 1999). Wheras IFN I upregulates CD86 on DCs and B cells (Montoya et al., 2002; Zhu et al., 2007b), it is not required for expression of the ICOSL on these cells and, instead, appears to inhibit TLR-mediated ICOSL induction (Zhou et al., 2005). We confirmed that IFN I enhances expression of CD86, but not ICOSL, on DCs and B cells in vivo (Figure S4), suggesting that IFN I regulates Tfh cell development by mechanisms distinct from ICOS and ICOSL interaction but possibly involving an enhanced CD86 expression (Vogelzang et al., 2008; Walker et al., 1999). Next, we measured IL-6 production from CD11c$^+$ DCs purified from WT and *Ifnar1*$^{-/-}$ mice (Figure 7G). LPS and anti-CD40 synergistically triggered abundant IL-6 production by DCs from WT, but not from *Ifnar1*$^{-/-}$, mice, and this production was further enhanced by addition of recombinant IFN-β, which also induced substantial production from DCs stimulated with LPS in the absence of anti-CD40. As expected, added IFN-β did not augment IL-6 secretion from *Ifnar1*$^{-/-}$ cells. A neutralizing Ab against the IFNAR reduced IL-6 production from WT DCs to the degree obtained with *Ifnar1*$^{-/-}$ cells. These results collectively show that LPS-induced IL-6 production by DCs is regulated by autocrine and paracrine IFN I signaling.

DISCUSSION

In the current study, we have shown that separate subsets of CXCR5$^+$ Tfh cells and $\alpha_4\beta_7^+$ gut tropic effector CD4$^+$ T cells are generated in the gut-draining MLN. This dichotomy in the mucosa-associated Th cell response is, thus, analogous to the findings of Campbell et al. (2001) that separable effector CD4$^+$ T cell subsets specialized for B cell help or cutaneous inflammation develop in skin-draining LNs. We have focused our studies on the MLNs because these LNs support the generation of a large number of peripheral tissue tropic effector T cells that are easily identified by their robust expression of the gut-homing molecule $\alpha_4\beta_7$. These $\alpha_4\beta_7^+$ Th cells represent a subset of effector cells clearly distinct from CXCR5-expressing Tfh cells, and we have

herein shown that essentially all of them (~90%) exit the MLN with a rapid kinetics. In marked contrast to this, we provide evidence for a highly efficient retention of the CXCR5$^+$ Tfh cells within the LN, which is consistent with their role as B cell-helping effector T cells. Thus, these experiments reveal a fundamental difference in the ability of recently generated effector Th cell subsets to exit the lymphoid organs. During the preparation of our revised manuscript, Fazilleau et al. (2009) reported similar observations from studies on nonmucosal LNs. Except from CXCR5$^+$CD62L$^-$ Tfh cell and CXCR5$^-$CD62L$^-$ LN emigrant effector Th cells, the authors identified a third population of LN-resident and T cell-zone-localized CXCR5$^-$CD62L$^+$ Th cells with high expression of ICOS, T-bet, and in particular, Blimp-1 mRNA. We observed a MLN-resident subset that was enriched for CD62L$^+$ cells and also failed to acquire either CXCR5 or $\alpha_4\beta_7$ expression. We do not currently know if this subset is equivalent to the CXCR5$^-$CD62L$^+$ LN resident T_H cells identified in the nonmucosal LN or, alternatively, if the failure to downregulate CD62L reflects a relatively poor T cell activation status of some of these double-negative cells (Lanzavecchia and Sallusto, 2000).

The selective egress of $\alpha_4\beta_7^+$ T_H cells from the MLN indicates that molecules differentially expressed by the $\alpha_4\beta_7^+$ and CXCR5$^+$ subsets regulate LN exit. When activated, T cells acquire transient expression of CD69, which downmodulates surface expression of $S1P_1$, leading to their transient sequestration within the lymphoid tissues (Shiow et al., 2006). In agreement with previous results (Fazilleau et al., 2007), we found that CD69 was maintained selectively on a subset of CXCR5$^+$ Tfh cells. Downregulation of CD69 may, thus, underlie the rapid and vigorous egress of $\alpha_4\beta_7^+$ T_H cells from the LN by conferring regained sensitivity to S1P. The molecular mechanism accounting for retention of LN-resident Th cells lacking detectable CD69 remains to be determined.

The cellular interactions underlying Tfh differentiation are currently not fully elucidated but appear to involve sequential priming on DCs and B cells because constitutive expression of the OX40 ligand by DCs leads to increased T cell accumulation within B cell follicles (Brocker et al., 1999) and T_{FH} cell differentiation is impaired in B cell-deficient mice or in mice where the ICOS ligand is selectively absent on B cells (Haynes et al., 2007; Nurieva et al., 2008). Our study demonstrates that IFN I stimulates Tfh cell differentiation and that signaling in DCs is important for this effect. This is consistent with the ability of IFN-β to enhance Ab responses and induce isotype switching when only DCs express the IFNAR (Le Bon et al., 2001). Whereas IFN I clearly enhances T cell-dependent Ab responses by direct signaling in T and B cells (Le Bon et al., 2006), we show that Tfh differentiation is not affected when these cells, or macrophages and granulocytes, are unable to respond to IFN I. Instead, we show that IFNAR signaling in nonhematopoietic cells also augments this differentiation process. As IFN-α and -β expression in, for example, fibroblasts is reinforced by an early IFN-β-dependent induction of interferon regulatory factor (IRF)-7 (Erlandsson et al., 1998; Sato et al., 2000), impaired Tfh cell generation in mice lacking IFNAR selectively on nonhematopoietic cells could reflect DCs sensing reduced amounts of IFN-α and -β in these mice. Currently, however, we cannot exclude the possibility that LN stromal cells interact physically or via soluble mediators with CD4$^+$ T cells and that such molecular interactions occur downstream of IFN I signaling.

It is clear from the literature that IFN I strengthens the T cell stimulatory capacity of DCs by mechanisms involving increased expression of MHC and costimulatory molecules, including CD86, but not the ICOSL (Le Bon and Tough, 2002; Zhou et al., 2005). A strong TCR signal strength (Fazilleau et al., 2009) and robust costimulation (Vogelzang et al., 2008; Walker et al., 1999) appear to be essential for Tfh cell differentiation. This is not the least reflected by the preferential selection of high-affinity clones into the Tfh cell compartment (Fazilleau et al., 2007) and by our results herein, showing that CXCR5 is selectively expressed by the most divided effector Th cells and that coinjection of a CD40 mAb and pI:C leads to an enhanced generation of Tfh cells, but not $\alpha_4\beta_7^+$ peripheral effectors. Finally, IL-6 and its direct signaling in T cells appear to be important as IL-6 deficiency, or T cell-specific deletion of the IL-6-receptor-associated signaling molecule STAT3, leads to reduced Tfh differentiation (Nurieva et al., 2008). We show here that IL-6 production from LPS-exposed DCs is regulated by autocrine and paracrine IFN I signaling, and previous work has identified a critical role for autocrine IFN I signaling in adenovirus-induced IL-6 production (Fejer et al., 2008; Zhu et al., 2007a). As the IFN I-dependent IL-6 production from DCs was further augmented by CD40 engagement on these cells, it seems possible that CD40L-expressing T_H cells elicit abundant IL-6 secretion from DCs under IFN I-permissive conditions.

As we could not detect a reduced development of emigrant $\alpha_4\beta_7^+$ T_H cells in the absence of IFN I signaling, IFNAR deficiency appears to selectively affect the LN resident Tfh cells while leaving the peripheral Th cell compartment relatively intact. However, published results have identified a deviation from a Th1 to a Th17 orientated response in IFNAR-deficient mice (Guo et al., 2008; Shinohara et al., 2008), indicating that the nature of the peripheral Th cell response under some conditions is modified by IFN I. The generation of $\alpha_4\beta_7^+$ T cells is regulated by a functionally specialized subset of CD103$^+$ DCs derived from the gut mucosa and the vitamin A metabolite retinoic acid (Iwata et al., 2004; Johansson-Lindbom et al., 2005). Because CXCR5 and $\alpha_4\beta_7$ are expressed in a mutually exclusive manner on Th cells activated in the MLN and IFNAR deficiency does not affect the generation of the $\alpha_4\beta_7^+$ subset, it seems possible that distinct subsets of DCs regulate the generation of these two effector Th cell subsets. This, however, remains to be determined by in vitro experiments with purified subsets of APCs.

Our results further reveal that the failure of *Ifnar1*$^{-/-}$ mice to efficiently support Tfh cell differentiation and accumulation within B cell follicles is linked with an impaired Ab affinity maturation. This is in agreement with previous results, showing that IFN I promotes GC formation and is critical for production of neutralizing Abs following adenoviral infection (Zhu et al., 2007b). We found that *Ifnar1*$^{-/-}$ mice however displayed normal serum titers of low affinity NP-specific IgG. This could be explained by the relatively high dose of densely NP-conjugated OVA that we used for immunization; an immunization regime likely to have provoked a strong T cell dependent extra-follicular Ab response as abundant epitopes steer B cells toward extra-follicular plasma cell differentiation (Paus et al., 2006).

An increased number of Tfh cells has been observed in different Ab-mediated autoimmune diseases and in particular in murine models for SLE, such as *sanroque* mice (Vinuesa et al., 2005) or the bicongenic *B6.Sle1yaa* mice (Subramanian et al., 2006). In addition, Tfh cells were recently shown to be required for the systemic pathology that develops in *sanroque* mice (Linterman et al., 2009). Although we are not aware of any studies on IFN I in the context of lupus-associated Tfh cell differentiation, this family of cytokines is an integral part of SLE pathology (Banchereau and Pascual, 2006). Indeed, SLE patients have increased serum titers of IFN-α that correlate positively with serum anti-DNA reactivity and disease severity (Bengtsson et al., 2000), and IFN I treatment of nonautoimmune disorders frequently gives rise to production of autoantibodies and occasionally humoral autoimmune symptoms (Belardelli and Gresser, 1996). Studies in different lupus-prone mouse strains have also revealed a critical role for IFN I in murine lupus-like pathology (Braun et al., 2003; Mathian et al., 2005; Santiago-Raber et al., 2003). IFN I-dependent Tfh cell generation in humoral autoimmunity clearly merits further investigation.

In summary, we have shown that the mucosal peripheral tissue tropic and lymph-node-resident effector $CD4^+$ T cell compartments develop independently and that IFN I acts as an adjuvant on DCs to selectively stimulate the generation of the lymph-node-resident $CXCR5^+$ Tfh cells. These results are highly relevant to the development of vaccines and may prove useful for clarification of mechanisms underlying Ab mediated autoimmune disease.

EXPERIMENTAL PROCEDURES

Mice

Ifnar1$^{-/-}$ mice on a C57Bl/6 background (provided by J. Demengeot [Instituto Gulbenkian de Ciência, Oeiras, Portugal]), C57Bl/6 (Taconic), C57Bl/6-CD45.1, and OT-II mice were bred and maintained at the Biomedical Center animal facility (Lund University, Lund, Sweden). $CD45.1^+CD45.2^+$ OT-II mice were obtained from OT-II × C57Bl/6-CD45.1 mating. *Ifnar1*$^{-/-}$ OT-II mice were obtained by crossing OT-II × *Ifnar1*$^{-/-}$ litters and screening the offspring by flow cytometry using Abs against TCRVα2, TCRVβ5.1, and the IFN I receptor (MAR1-5A3, Leinco Technologies, Inc.). *Ifnar1*$^{fl/fl}$ CD11cCre, *Ifnar1*$^{fl/fl}$ CD19Cre, *Ifnar1*$^{fl/fl}$ LysMCre, and *Ifnar1*$^{fl/fl}$ mice have been described elsewhere (Cervantes-Barragan et al., 2009; Prinz et al., 2008). Animal experiments were performed in accordance with guidelines from the Lund and Malmö Animal Ethics Committee.

Adoptive Transfers

Spleen and LN cells from OT-II mice were labeled with 5 μM carboxyfluorescein diacetate succinimidyl ester (CFSE), and total cells corresponding to 10^6 $CD4^+TCRV\alpha2^+$ cells were transferred into CD45 congenic recipients. In experiments with *Ifnar1*$^{-/-}$ recipients, $CD4^+$ donor T cells were first enriched by negative MACS selection (Miltenyi Biotech). Recipients were immunized i.p. with 500 μg OVA in the absence or presence of 50 μg LPS, 100 μg pI:C (all from Sigma-Aldrich), or 50 μg anti-CD40 mAb (IC11, Biolegend). In some experiments, mice received FTY720 (1 mg/kg body weight; Cayman) in saline i.p. 18–24 h later. Mice were euthanized 2–6 days later, and organs were collected after perfusion of the liver with ~10 ml PBS. Isolation of leukocytes from indicated tissues was performed as previously described (Stenstad et al., 2006).

Bone Marrow Chimeras

The recipients were irradiated (1000 rad) before 4 × 10^6 bone marrow cells, were prepared from femurs and tibias of donor mice, and were transferred by i.v. injection. After 7 weeks, the mice were used in adoptive transfer experiments as described above. Chimerism was confirmed by flow cytometry using biotin anti-IFNAR mAb and PE SA (BD PharMingen).

Flow Cytometry Analysis and Cell Sorting

Cells were incubated with anti-CXCR5 (2G8, provided by O. Pabst, Hanover Medical School, Hanover) followed by APC donkey $F(ab')_2$ anti-rat IgG (eBioscience). After blocking remaining anti-rat reactivity with 10% rat serum, cells were incubated with propidium iodide (PI) and fluorochrome-conjugated rat or mouse mAbs to the following markers: CD4 (RM4-5), B220 (RA3-6B2), CD62L (MEL-14) (eBioscience), $\alpha_4\beta_7$ (DATK32, BD PharMingen), and either CD45.2 (104, eBioscience) or CD45.1 (A20, BioLegend). In experiments described in Figure 7, the total population of cotransferred WT and *Ifnar1*$^{-/-}$ OT-II cells was identified by TCRVα2 mAb (B20.1, eBioscience) and biotin TCRVβ5.1, followed by QD605 SA. Acquisition of data and sorting of indicated OT-II cell subsets were done on a FACSAria (BD Biosciences). Sorting and analysis of donor cells were performed after gating on PI^- (viable) $CD4^+B220^-$ cells also staining positive for the donor-specific CD45 isoform. Data analysis was done using the FlowJo software (Tree Star, Inc.).

Immunofluorescence Microscopy

Cryostat sections (9 μm) were fixed in 4% PFA, quenched with 3% H_2O_2 in PBS, and blocked with mouse and rat serum (5% of each in PBS) and a SA-biotin blocking kit (Vector Laboratories). Sections were incubated with biotin anti-CD45.1 (A20) and anti-TCRβ (H57-597, eBioscience), followed by horseradish peroxidase (HRP) SA (Invitrogen), Cy3 goat anti-hamster IgG (polyclonal, Jackson ImmunoReserach), and Alexa Fluor 647 anti-B220 (RA3-6B2, Biolegend). HRP was revealed using a tyramide Alexa Fluor 488 Amplification Kit (Invitrogen). Analysis was done with an Axiovert 200 microscope (Carl Zeiss, Inc.) and Volocity software (Improvision).

Quantitative Real-Time PCR

Spleen and MLN from immunized OT-II cell recipient mice (C57Bl/6 or *Ifnar1*$^{-/-}$) were first enriched for $CD4^+$ T cells by negative selection using anti-CD19-conjugated MACS beads and LS columns (Miltenyi Biotech). Total divided OT-II cell or indicated subsets were sorted on a FACSAria as described above. RNA was prepared with a RNeasy Micro Kit (QIAGEN) and converted into linearly amplified cDNA by WT-Ovation Pico RNA Amplification System (NuGEN Technologies, Inc). The cDNA was purified by QIAquick PCR Purification Kit (QIAGEN). QRT-PCR was performed using 20 ng cDNA template, SYBR Green ER qPCR SuperMix (Invitrogen), and 0.5 μM of forward and reverse primers in a final volume of 20 μl. Reactions were run and recorded on an iCycler (Bio-Rad). Primer sequences are described in Supplemental Data.

NP-Specific ELISA

Sera from C57Bl/6 and *Ifnar1*$^{-/-}$ mice immunized i.p. 5 weeks previously with 500 μg NP_{19}-OVA (Biosearch Technologies, Inc.) and 50 μg LPS, and given an i.p. boost with 200 μg NP_{19}-OVA 2 weeks before collection of the sera, were analyzed for NP-specific Abs. To this end, 96-well plates coated with NP_{23}-BSA (broad-affinity range) or NP_2-Porcrine serum albumin (PSA) (high-affinity) (both from Biosearch Technologies, Inc.) were used. Plates were blocked with 1% BSA in PBS and incubated with serial dilutions of the sera and a reference immune serum serving as an interplate standard. Serum Abs bound to NP conjugates were detected with biotin anti-mouse IgG (polyclonal γ-specific, Southern Biotech) and biotinylated isotype-specific mAbs from Biolegend: IgM (RMM-1), IgG1 (RMG1-1), and IgG2a/c (RMG2a-62). Plates were developed with HRP-SA (Biolegend) followed by 3,3′, 5,5′- tetramethyl-benzidine dihydro-chloride hydrate (TMB) substrate solution (Sigma-Aldrich). The reaction was terminated with 2 M H_2SO_4, and plates were read at 450 nm on a microplate ELISA reader (Molecular Devices). Serum anti-NP reactivity is given in arbitrary units/ml and is calculated using the reference immune serum as standard. The titer of this standard was set to the inverse dilution factor required to reach 50% of the optical maximum value.

IL-6 Production from DCs

DCs were purified from collagenase-IV-digested spleens with CD11c MACS beads as previously described. Preparations routinely contained 90%–95% $CD11c^+MHCII^+$ cells as assessed by flow cytometry. DCs were cultured in complete RPMI 1640 media at 5 × 10^5 cells/ml in the absence or presence

of titrated doses of LPS (500 ng/ml), anti-CD40 mAb (IC10; 5μg/ml), recombinant IFN-β (R&D systems; 400 U/ml), and/or anti-IFNAR (MAR1-5A3; 10 μg/ml) as indicated. Supernatants were collected after 20 hr, and the concentration of IL-6 was determined by sandwich ELISA using capture rat anti-IL-6 (MP5-20F3) and detection biotin rat anti-IL-6 (MP5-32C11) mAbs (Biolegend).

Cytokine ELISA

OT-II cell recipient mice (C57Bl/6) were immunized i.p. with 500 μg OVA and 50 μg LPS, and 3 days after, indicated OT-II cell subsets were sorted on a FACSAria as described above. Cells were restimulated in vitro with plate-bound anti-CD3 (10 μg/ml) and soluble anti-CD28 (1 μg/ml) for 24 hr. Culture supernatants were analyzed for IL-4, IL-10, IL-17 and IFN-γ by sandwich ELISA with the following rat capture and detection mAbs: anti-IL-4 (11B11), biotin anti-IL-4 (BVD6-24G2), anti-IL-10 (JES5-2A5) (all produced in the lab from hybridomas), biotin anti-IL-10 (JES5-16E3), anti-IL17A (TC11-18H10.1), biotin anti-IL17A (TC11-8H4) (all from Biolegend), anti-IFN-γ (R4-6A2), and biotin anti-IFN-γ (XMG1.2) (both from BD PharMingen).

Statistical Analysis

Statistical analyses were performed using unpaired or paired two-tailed Student's t test.

SUPPLEMENTAL DATA

Supplemental Data include four figures and can be found with this article online at http://www.cell.com/immunity/supplemental/S1074-7613(09)00365-3.

ACKNOWLEDGMENTS

The authors wish to thank J. Demengeot (Instituto Gulbenkian de Ciência, Portugal) for kindly providing *Ifnar1*$^{-/-}$ mice and O. Pabst (Hannover Medical School, Germany) for the kind gift of CXCR5 mAb. We also would like to thank A. Mowat (University of Glasgow, UK), F. Ivars (Lund University, Sweden), and W.W. Agace (Lund University) for their valuable input during the preparation of this manuscript. B.J.-L. and U.Y. are Assistant Professors with the Swedish Medical Research Council. This work was supported by grants to B.J.-L. from the Swedish Medical Research Council, the Royal Physiographic Society, the Crafoord Foundation, Åke Wiberg, and Österlunds.

Received: November 7, 2008
Revised: May 15, 2009
Accepted: July 15, 2009
Published online: September 3, 2009

REFERENCES

Akiba, H., Takeda, K., Kojima, Y., Usui, Y., Harada, N., Yamazaki, T., Ma, J., Tezuka, K., Yagita, H., and Okumura, K. (2005). The role of ICOS in the CXCR5+ follicular B helper T cell maintenance in vivo. J. Immunol. *175*, 2340–2348.

Ansel, K.M., McHeyzer-Williams, L.J., Ngo, V.N., McHeyzer-Williams, M.G., and Cyster, J.G. (1999). In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. J. Exp. Med. *190*, 1123–1134.

Banchereau, J., and Pascual, V. (2006). Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity *25*, 383–392.

Belardelli, F., and Gresser, I. (1996). The neglected role of type I interferon in the T-cell response: implications for its clinical use. Immunol. Today *17*, 369–372.

Bengtsson, A.A., Sturfelt, G., Truedsson, L., Blomberg, J., Alm, G., Vallin, H., and Ronnblom, L. (2000). Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. Lupus *9*, 664–671.

Berek, C., Berger, A., and Apel, M. (1991). Maturation of the immune response in germinal centers. Cell *67*, 1121–1129.

Berlin, C., Berg, E.L., Briskin, M.J., Andrew, D.P., Kilshaw, P.J., Holzmann, B., Weissman, I.L., Hamann, A., and Butcher, E.C. (1993). Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell *74*, 185–195.

Blink, E.J., Light, A., Kallies, A., Nutt, S.L., Hodgkin, P.D., and Tarlinton, D.M. (2005). Early appearance of germinal center-derived memory B cells and plasma cells in blood after primary immunization. J. Exp. Med. *201*, 545–554.

Braun, D., Geraldes, P., and Demengeot, J. (2003). Type I Interferon controls the onset and severity of autoimmune manifestations in lpr mice. J. Autoimmun. *20*, 15–25.

Breitfeld, D., Ohl, L., Kremmer, E., Ellwart, J., Sallusto, F., Lipp, M., and Forster, R. (2000). Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J. Exp. Med. *192*, 1545–1552.

Brocker, T., Gulbranson-Judge, A., Flynn, S., Riedinger, M., Raykundalia, C., and Lane, P. (1999). CD4 T cell traffic control: in vivo evidence that ligation of OX40 on CD4 T cells by OX40-ligand expressed on dendritic cells leads to the accumulation of CD4 T cells in B follicles. Eur. J. Immunol. *29*, 1610–1616.

Campbell, D.J., and Butcher, E.C. (2002). Rapid acquisition of tissue-specific homing phenotypes by CD4(+) T cells activated in cutaneous or mucosal lymphoid tissues. J. Exp. Med. *195*, 135–141.

Campbell, D.J., Kim, C.H., and Butcher, E.C. (2001). Separable effector T cell populations specialized for B cell help or tissue inflammation. Nat. Immunol. *2*, 876–881.

Cervantes-Barragan, L., Kalinke, U., Zust, R., Konig, M., Reizis, B., Lopez-Macias, C., Thiel, V., and Ludewig, B. (2009). Type I IFN-mediated protection of macrophages and dendritic cells secures control of murine coronavirus infection. J. Immunol. *182*, 1099–1106.

Chtanova, T., Tangye, S.G., Newton, R., Frank, N., Hodge, M.R., Rolph, M.S., and Mackay, C.R. (2004). T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. J. Immunol. *173*, 68–78.

Erlandsson, L., Blumenthal, R., Eloranta, M.L., Engel, H., Alm, G., Weiss, S., and Leanderson, T. (1998). Interferon-beta is required for interferon-alpha production in mouse fibroblasts. Curr. Biol. *8*, 223–226.

Fazilleau, N., Eisenbraun, M.D., Malherbe, L., Ebright, J.N., Pogue-Caley, R.R., McHeyzer-Williams, L.J., and McHeyzer-Williams, M.G. (2007). Lymphoid reservoirs of antigen-specific memory T helper cells. Nat. Immunol. *8*, 753–761.

Fazilleau, N., McHeyzer-Williams, L.J., Rosen, H., and McHeyzer-Williams, M.G. (2009). The function of follicular helper T cells is regulated by the strength of T cell antigen receptor binding. Nat. Immunol. *10*, 375–384.

Fejer, G., Drechsel, L., Liese, J., Schleicher, U., Ruzsics, Z., Imelli, N., Greber, U.F., Keck, S., Hildenbrand, B., Krug, A., et al. (2008). Key role of splenic myeloid DCs in the IFN-alphabeta response to adenoviruses in vivo. PLoS Pathog. *4*, e1000208.

Gunn, M.D., Ngo, V.N., Ansel, K.M., Ekland, E.H., Cyster, J.G., and Williams, L.T. (1998). A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. Nature *391*, 799–803.

Guo, B., Chang, E.Y., and Cheng, G. (2008). The type I IFN induction pathway constrains Th17-mediated autoimmune inflammation in mice. J. Clin. Invest. *118*, 1680–1690.

Haynes, N.M., Allen, C.D., Lesley, R., Ansel, K.M., Killeen, N., and Cyster, J.G. (2007). Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. J. Immunol. *179*, 5099–5108.

Hoebe, K., Janssen, E.M., Kim, S.O., Alexopoulou, L., Flavell, R.A., Han, J., and Beutler, B. (2003). Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by Trif-dependent and Trif-independent pathways. Nat. Immunol. *4*, 1223–1229.

Iwata, M., Hirakiyama, A., Eshima, Y., Kagechika, H., Kato, C., and Song, S.Y. (2004). Retinoic acid imprints gut-homing specificity on T cells. Immunity *21*, 527–538.

Jacob, J., Kelsoe, G., Rajewsky, K., and Weiss, U. (1991). Intraclonal generation of antibody mutants in germinal centres. Nature *354*, 389–392.

Johansson-Lindbom, B., Svensson, M., Pabst, O., Palmqvist, C., Marquez, G., Forster, R., and Agace, W.W. (2005). Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. J. Exp. Med. *202*, 1063–1073.

Lanzavecchia, A., and Sallusto, F. (2000). Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science *290*, 92–97.

Le Bon, A., and Tough, D.F. (2002). Links between innate and adaptive immunity via type I interferon. Curr. Opin. Immunol. *14*, 432–436.

Le Bon, A., Schiavoni, G., D'Agostino, G., Gresser, I., Belardelli, F., and Tough, D.F. (2001). Type i interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity *14*, 461–470.

Le Bon, A., Thompson, C., Kamphuis, E., Durand, V., Rossmann, C., Kalinke, U., and Tough, D.F. (2006). Cutting edge: enhancement of antibody responses through direct stimulation of B and T cells by type I IFN. J. Immunol. *176*, 2074–2078.

Lefrancois, L., Parker, C.M., Olson, S., Muller, W., Wagner, N., Schon, M.P., and Puddington, L. (1999). The role of beta7 integrins in CD8 T cell trafficking during an antiviral immune response. J. Exp. Med. *189*, 1631–1638.

Linterman, M.A., Rigby, R.J., Wong, R.K., Yu, D., Brink, R., Cannons, J.L., Schwartzberg, P.L., Cook, M.C., Walters, G.D., and Vinuesa, C.G. (2009). Follicular helper T cells are required for systemic autoimmunity. J. Exp. Med. *206*, 561–576.

MacLennan, I.C., Gulbranson-Judge, A., Toellner, K.M., Casamayor-Palleja, M., Chan, E., Sze, D.M., Luther, S.A., and Orbea, H.A. (1997). The changing preference of T and B cells for partners as T-dependent antibody responses develop. Immunol. Rev. *156*, 53–66.

Mathian, A., Weinberg, A., Gallegos, M., Banchereau, J., and Koutouzov, S. (2005). IFN-alpha induces early lethal lupus in preautoimmune (New Zealand Black x New Zealand White) F1 but not in BALB/c mice. J. Immunol. *174*, 2499–2506.

Matloubian, M., Lo, C.G., Cinamon, G., Lesneski, M.J., Xu, Y., Brinkmann, V., Allende, M.L., Proia, R.L., and Cyster, J.G. (2004). Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature *427*, 355–360.

Montoya, M., Schiavoni, G., Mattei, F., Gresser, I., Belardelli, F., Borrow, P., and Tough, D.F. (2002). Type I interferons produced by dendritic cells promote their phenotypic and functional activation. Blood *99*, 3263–3271.

Nurieva, R.I., Chung, Y., Hwang, D., Yang, X.O., Kang, H.S., Ma, L., Wang, Y.H., Watowich, S.S., Jetten, A.M., Tian, Q., and Dong, C. (2008). Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity *29*, 138–149.

Paus, D., Phan, T.G., Chan, T.D., Gardam, S., Basten, A., and Brink, R. (2006). Antigen recognition strength regulates the choice between extrafollicular plasma cell and germinal center B cell differentiation. J. Exp. Med. *203*, 1081–1091.

Prinz, M., Schmidt, H., Mildner, A., Knobeloch, K.P., Hanisch, U.K., Raasch, J., Merkler, D., Detje, C., Gutcher, I., Mages, J., et al. (2008). Distinct and nonredundant in vivo functions of IFNAR on myeloid cells limit autoimmunity in the central nervous system. Immunity *28*, 675–686.

Proietti, E., Bracci, L., Puzelli, S., Di Pucchio, T., Sestili, P., De Vincenzi, E., Venditti, M., Capone, I., Seif, I., De Maeyer, E., et al. (2002). Type I IFN as a natural adjuvant for a protective immune response: lessons from the influenza vaccine model. J. Immunol. *169*, 375–383.

Roes, J., and Rajewsky, K. (1993). Immunoglobulin D (IgD)-deficient mice reveal an auxiliary receptor function for IgD in antigen-mediated recruitment of B cells. J. Exp. Med. *177*, 45–55.

Santiago-Raber, M.L., Baccala, R., Haraldsson, K.M., Choubey, D., Stewart, T.A., Kono, D.H., and Theofilopoulos, A.N. (2003). Type-I interferon receptor deficiency reduces lupus-like disease in NZB mice. J. Exp. Med. *197*, 777–788.

Sato, M., Suemori, H., Hata, N., Asagiri, M., Ogasawara, K., Nakao, K., Nakaya, T., Katsuki, M., Noguchi, S., Tanaka, N., and Taniguchi, T. (2000). Distinct and essential roles of transcription factors IRF-3 and IRF-7 in response to viruses for IFN-alpha/beta gene induction. Immunity *13*, 539–548.

Schaerli, P., Willimann, K., Lang, A.B., Lipp, M., Loetscher, P., and Moser, B. (2000). CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J. Exp. Med. *192*, 1553–1562.

Shinohara, M.L., Kim, J.H., Garcia, V.A., and Cantor, H. (2008). Engagement of the type I interferon receptor on dendritic cells inhibits T helper 17 cell development: role of intracellular osteopontin. Immunity *29*, 68–78.

Shiow, L.R., Rosen, D.B., Brdickova, N., Xu, Y., An, J., Lanier, L.L., Cyster, J.G., and Matloubian, M. (2006). CD69 acts downstream of interferon-alpha/beta to inhibit S1P1 and lymphocyte egress from lymphoid organs. Nature *440*, 540–544.

Shlomchik, M.J., Marshak-Rothstein, A., Wolfowicz, C.B., Rothstein, T.L., and Weigert, M.G. (1987). The role of clonal selection and somatic mutation in autoimmunity. Nature *328*, 805–811.

Stenstad, H., Ericsson, A., Johansson-Lindbom, B., Svensson, M., Marsal, J., Mack, M., Picarella, D., Soler, D., Marquez, G., Briskin, M., and Agace, W.W. (2006). Gut-associated lymphoid tissue-primed CD4+ T cells display CCR9-dependent and -independent homing to the small intestine. Blood *107*, 3447–3454.

Stockinger, S., Kastner, R., Kernbauer, E., Pilz, A., Westermayer, S., Reutterer, B., Soulat, D., Stengl, G., Vogl, C., Frenz, T., et al. (2009). Characterization of the interferon-producing cell in mice infected with Listeria monocytogenes. PLoS Pathog. *5*, e1000355.

Subramanian, S., Tus, K., Li, Q.Z., Wang, A., Tian, X.H., Zhou, J., Liang, C., Bartov, G., McDaniel, L.D., Zhou, X.J., et al. (2006). A Tlr7 translocation accelerates systemic autoimmunity in murine lupus. Proc. Natl. Acad. Sci. USA *103*, 9970–9975.

Suto, A., Kashiwakuma, D., Kagami, S., Hirose, K., Watanabe, N., Yokote, K., Saito, Y., Nakayama, T., Grusby, M.J., Iwamoto, I., and Nakajima, H. (2008). Development and characterization of IL-21-producing CD4+ T cells. J. Exp. Med. *205*, 1369–1379.

Takahashi, Y., Dutta, P.R., Cerasoli, D.M., and Kelsoe, G. (1998). In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. V. Affinity maturation develops in two stages of clonal selection. J. Exp. Med. *187*, 885–895.

Theofilopoulos, A.N., Baccala, R., Beutler, B., and Kono, D.H. (2005). Type I interferons (alpha/beta) in immunity and autoimmunity. Annu. Rev. Immunol. *23*, 307–336.

Vinuesa, C.G., Cook, M.C., Angelucci, C., Athanasopoulos, V., Rui, L., Hill, K.M., Yu, D., Domaschenz, H., Whittle, B., Lambe, T., et al. (2005). A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature *435*, 452–458.

Vogelzang, A., McGuire, H.M., Yu, D., Sprent, J., Mackay, C.R., and King, C. (2008). A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity *29*, 127–137.

Walker, L.S., Gulbranson-Judge, A., Flynn, S., Brocker, T., Raykundalia, C., Goodall, M., Forster, R., Lipp, M., and Lane, P. (1999). Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. J. Exp. Med. *190*, 1115–1122.

Zhou, Z., Hoebe, K., Du, X., Jiang, Z., Shamel, L., and Beutler, B. (2005). Antagonism between MyD88- and TRIF-dependent signals in B7RP-1 up-regulation. Eur. J. Immunol. *35*, 1918–1927.

Zhu, J., Huang, X., and Yang, Y. (2007a). Innate immune response to adenoviral vectors is mediated by both Toll-like receptor-dependent and -independent pathways. J. Virol. *81*, 3170–3180.

Zhu, J., Huang, X., and Yang, Y. (2007b). Type I IFN signaling on both B and CD4 T cells is required for protective antibody response to adenovirus. J. Immunol. *178*, 3505–3510.