Cutting Edge: Dendritic Cell-Restricted Antigen Presentation Initiates the Follicular Helper T Cell Program but Cannot Complete Ultimate Effector Differentiation


*J Immunol* 2011; 187:1091-1095; Prepublished online 29 June 2011; doi: 10.4049/jimmunol.1100853
http://www.jimmunol.org/content/187/3/1091

Supplementary Material http://www.jimmunol.org/content/suppl/2011/06/29/jimmunol.1100853.DC1

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Follicular helper T (TFH) cells are critical for germinal center (GC) formation. The processes that drive their generation and effector potential remain unclear. In this study, we define requirements for MHC class II APCs in murine TFH cell formation by either transiently ablating or restricting Ag presentation to dendritic cells (DCs). We find that cognate interactions with DCs are necessary and sufficient to prime CD4+ T cells toward a CXCR5+ICOS+Bcl6+ TFH cell intermediate. However, in the absence of additional APCs, these TFH cells fail to produce IL-21. Furthermore, in vitro priming of naive T cells by B cells engenders optimal production of IL-21, which induces a GC B cell transcriptional profile. These results support a multistep model for effector TFH cell priming and GC initiation, in which DCs are necessary and sufficient to induce a TFH cell intermediate that requires additional interactions with distinct APCs for full effector function. The Journal of Immunology. 2011, 187: 1091–1095.

Germline centers (GCs) are transient structures formed during thymus-dependent immune responses, where expanding B cells undergo affinity maturation, yielding memory B and plasma cells that mediate protective humoral immunity. The exact interactions dictating whether B and helper T cells assume GC status versus alternative fates remain unclear. A key event in GC formation is the Bcl6-dependent differentiation of follicular helper T (TFH) cells (1, 2). TFH cells express ICOS and CXCR5, enabling their migration to the B cell follicle (3–5) and their continued differentiation into GC TFH cells (6). Moreover, TFH cells produce IL-21 and express costimulatory molecules that are essential for GC B cell survival and differentiation (7, 8).

The sources of MHC class II (MHC II)-restricted Ag presentation required for TFH cell differentiation are controversial. Several lines of evidence suggest that B cells provide unique signal(s) required for complete TFH cell differentiation. Thus, the absence of B cells, the presence of B cells with irrelevant specificity, or an inability of B cells to express ICOSL impairs TFH cell generation (2, 9–11). Moreover, T cells unable to conjugate stably with B cells display defective TFH cell differentiation (12). The notion of a unique B cell signal has been challenged by findings that suggest instead that only prolonged, high-avidity Ag presentation is needed for GC TFH cell differentiation that can be mediated by dendritic cells (DCs) (13). Indeed, costimulation via DCs promotes T cell migration into the B cell follicle (14), suggesting that DC Ag presentation contributes to the TFH cell program.

We have dissected the steps of TFH cell differentiation by examining the requirement for MHC II-expressing DCs. We find that cognate interactions with conventional DCs are both necessary and sufficient to prime CD4+ T cells toward a previously unappreciated TFH cell intermediate that expresses Bcl6, CXCR5, and ICOS. However, DC priming alone is insufficient to generate programmed death ligand 1 (PD1)hi GC TFH cells and IL-21 production, indicating the need for subsequent, distinct APC interactions for commitment to the TFH cell program. Furthermore, we show that naive T cells primed in vitro by B cells express more IL-21 than those primed by DCs. Moreover, addition of IL-21 to in vitro B cell activation cultures that mimic cognate T cell help induces a GC B transcriptional profile. Thus, we conclude that TFH cell differentiation is a multistep process in which conventional DCs are critical for the initial priming events; however, they are insufficient for imparting complete effector potential.

Materials and Methods

Mice and immunizations

C57BL/6J, OTII, and CD11c-DTR mice were purchased from The Jackson Laboratory. CD11c/Agb mice were bred in-house (15). Six- to fourteen-week-old mice were used throughout experiments. CD11c-DTR mice were bred as described (15). CD11c-dTR mice were purchased from The Jackson Laboratory. OTII mice were bred in-house (15). Six- to fourteen-week-old mice were used throughout experiments. CD11c-dTR mice were purchased from The Jackson Laboratory. OTII mice were bred in-house (15). Six- to fourteen-week-old mice were used throughout experiments.

Received for publication March 28, 2011. Accepted for publication June 7, 2011.

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The online version of this article contains supplemental material.

Abbreviations used in this article: Blimp1, B lymphocyte-induced maturation protein 1; BTLA, B- and T-lymphocyte attenuator; DC, dendritic cell; DT, diphtheria toxin; GC, germinal center; MHC II, MHC class II; NP–OVA, (4-hydroxy-3-nitrophenyl)acetyl coupled to OVA at a substitution ratio of 15; PD1, programmed death ligand 1; p.i., postimmunization; PGLa, P-selectin glycoprotein ligand 1; TFH, follicular helper T; WT, wild type.
FIGURE 1. DCs are necessary for initiating \( T_{\text{FH}} \) cell priming. WT and CD11c-DTR mice were treated with DT and infected with \( T. gondii \) as described (16). \( A \). Numbers of \( T_{\text{FH}} \) (CD4+CXCR5+ICOS+) cells were calculated at day 7 postinfection. Data are pooled from two experiments (\( n > 8 \) per group). \( B \). Representative FACS plots of CXCR5 and ICOS expression on CD4+ T cells in WT or CD11c-DTR mice treated with DT. ** \( p < 0.01 \) (two-tailed \( t \) test at \( \alpha = 0.05 \)). CD11cDTR, CD11c-DTR.

Flow cytometry

Abs were purchased from BioLegend, eBioscience, BD Pharmingen, or Invitrogen. DAPI or AQUA (Invitrogen) was used to identify live cells. Foxp3 fixation and permeabilization kit was used for intracellular Bcl6 staining (eBioscience). Cells were acquired or sorted on an LSR II cytometer or FACSAria II, respectively (BD Biosciences). Data was analyzed using FlowJo software (Tree Star).

Immunohistochemistry

Splenocytes were immunized in OCT (Tissue Tek) were flash frozen using 2-methylbutane/liquid nitrogen. Eight-micrometer sections were fixed with cold acetone and stained with peanut agglutinin and Abs to IgD and CD90.1. Sections were imaged with a Zeiss LSM510META NLO laser scanning confocal system.

In vitro stimulations

\( T_{\text{FH}} \) cells were polarized toward \( T_{\text{FH}} \) cell lineage as published (9) with CD11c\(^{hi} \) or CD23\(^{+} \) cells at a ratio of 1 APC/10 T cells and harvested on day 4. B cell stimulations were performed as described (17) with anti-CD40 (10 ng/ml; BD Biosciences) and IL-21 (100 ng/ml; R&D).

Quantitative PCR

Quantitative PCR was conducted as previously described (17). 18S or GAPDH were used as housekeeping gene for T and B cells, respectively.

ELISPOT assay

Splenocytes were incubated on 10 \( \mu \)g/ml NP33-BSA (Biosearch Technologies) coated plates and developed with biotin anti-mouse IgM or IgG1 (Southern Biotech) followed by ExtrAvidin–Alkaline Phosphatase and NBT–BCIP substrate (Sigma). Spots were enumerated on CTL-ImmuNoSpot (Cellular Technologies).

Results and Discussion

\( DCs \) are necessary for \( T_{\text{FH}} \) cell formation

Using a loss of function approach, we determined whether DC–T cell interactions are necessary for initiation of \( T_{\text{FH}} \) cell priming in vivo. We transiently ablated DCs by diphtheria toxin (DT) in CD11c-DTR mice (18) and infected the mice with \( T. gondii \) as described (16). \( T_{\text{FH}} \) cells were present in untreated mice at day 7 postinfection but were significantly reduced in DT-treated CD11c-DTR animals (Fig. 1A). Normal DC numbers were required for optimal generation of CD4+CXCR5+ cells (wild type [WT], 1.71 \( \pm \) 0.15; CD11c-DTR, 0.67 \( \pm \) 0.13; \( p < 0.01 \)) as well as induction of ICOS (Fig. 1B). To verify that these results were not limited to toxoplasmosis, we transferred \( 10^5 \) CD90.1+ OVA-specific OTII CD4+ T cells into CD90.2+ WT or CD11c-DTR recipient mice followed by DT treatment and i.p. immunization with NP–OVA/alum. Again, there was a profound decrease in the number of OTII \( T_{\text{FH}} \) cells generated at day 7 postimmunization (p.i.) (data not shown). The few CD4+CXCR5+ T cells formed after DC depletion (Fig. 1A) could potentially migrate into the follicle and initiate GC via other costimulatory molecules, including CD40L. We conclude that DCs are necessary to generate \( T_{\text{FH}} \) cells, as expected from their role in priming other helper T lineages (19).

\( DCs \) are sufficient for the generation of a Bcl6+ \( T_{\text{FH}} \) intermediate

Using a gain of function approach, we asked if cognate DC interactions are sufficient to generate \( T_{\text{FH}} \) cells. We used the transgenic CD11c\(^{hi} \) \textbf{b} mouse model, in which MHC II Ag presentation is limited to conventional CD11c\(^{hi} \) DCs (15). As
CD11c/A<sub>b</sub> mice lack endogenous CD4<sup>+</sup> T cells, we transferred 10<sup>6</sup> CFSE-labeled OTII cells to WT and CD11c/A<sub>b</sub> recipients and immunized the mice with NP-OVA/alum. Splenic OTII cells proliferated similarly in both WT and CD11c/A<sub>b</sub> recipients at day 7 p.i. (Fig. 2A, 2B). Further, the numbers of OTII-derived CD62L<sup>+</sup>CXCR5<sup>+</sup>T<sub>FH</sub> cells generated in WT (WT-T<sub>FH</sub>) or CD11c/A<sub>b</sub> (CD11c-T<sub>FH</sub>) mice were not significantly different, and both subsets displayed equivalent expression of ICOS (Fig. 2B, 2C). Moreover, transfer of 10<sup>5</sup> OTII cells yielded similar results (Supplemental Fig. 1A). Furthermore, OTII cells migrated into the follicle in both mouse strains (Fig. 2E). Because Bcl6 is necessary and sufficient for differentiation of CXCR5<sup>+</sup>T<sub>FH</sub> cells (1, 2), we asked if Ag presentation restricted to DCs effectively drove Bcl6 induction. Compared with naive OTII T cells, WT-T<sub>FH</sub> or CD11c-T<sub>FH</sub> cells expressed higher but equivalent levels of Bcl6 message (Fig. 2D) and protein (Fig. 2C). Thus, cognate interactions with DCs are sufficient to skew naive CD4<sup>+</sup> T cells toward Bcl6<sup>+</sup> T<sub>FH</sub> cells.

**Cognate DC–T cell interactions cannot induce GC status in T cells**

MHC II expression by B cells is necessary for Ab class switching (15, 20) and presumably for GC formation. Consistent with this view, GC B cells were absent both by flow cytometry (Supplemental Fig. 2A, 2B) and histology (Fig. 2E) in immunized CD11c/A<sub>b</sub> mice. Further, CD11c/A<sub>b</sub> mice generated few NP-specific IgG1 ASCs, despite an intact IgM ASC response (Supplemental Fig. 2C), as reported before (15, 20). PD1 expression is thought be an indicator of chronic Ag exposure in the GC, as correlative evidence links the presence of GC B cells with PD1 expression on T<sub>FH</sub> cells (4). Thus, we examined the expression of PD1 on T<sub>FH</sub> cells. Among the CXCR5<sup>+</sup> cells, a small subset of the WT-T<sub>FH</sub> cells expressed high levels of PD1 as well as GL7, which were absent within the CD11c-T<sub>FH</sub> cells (Fig. 3A, 3B) consistent with GC T<sub>FH</sub> phenotype (Fig. 3C) (6). Moreover, the transfer of 10<sup>3</sup> polyclonal WT CD4<sup>+</sup> T cells did not alter the observed phenotype (data not shown), indicating that the absence of endogenous helper T cells does not impact the evolution of this response in the CD11c/A<sub>b</sub> mice. Because T<sub>FH</sub> cells downregulate P-selectin glycoprotein ligand 1 (PSGL1) (11) and upregulate B- and T-lymphocyte attenuator (BTLA) (6), we examined their expression on CD11c-T<sub>FH</sub> and WT-T<sub>FH</sub> cells. Compared with naive WT T cells, both CD11c-T<sub>FH</sub> and WT-T<sub>FH</sub> cells had significantly upregulated BTLA and downregulated PSGL1 (data not shown). However, CD11c-T<sub>FH</sub> cells expressed more PSGL1 than that of WT-T<sub>FH</sub> cells, whereas BTLA expression was comparable (data not shown). Collectively, we conclude that not only do GC B cells require T<sub>FH</sub> cells for their induction, but GC T<sub>FH</sub> cells also rely on interactions with other APCs, including activated B cells, to complete their phenotype.

**IL-21 expression is profoundly reduced in T<sub>FH</sub> cells primed by DCs alone**

IL-21 is a key T<sub>FH</sub> effector cytokine that mediates differentiation of GC B cells (6–8). Strikingly, CD11c-T<sub>FH</sub> cells primed solely by DCs expressed minimal IL-21 compared with that of WT-T<sub>FH</sub> cells (Fig. 4A). To determine if B cells were intrinsically more adept at inducing IL-21–producing T<sub>FH</sub> cells, we polarized naive OTII T cells in vitro toward the T<sub>FH</sub> cell lineage with splenic CD11c<sup>+</sup> DCs or CD23<sup>+</sup> B cells as APCs as described (9). Whereas both cells and DCs induced expression of Bcl6, B cells induced greater IL-21 transcript in polarized T<sub>FH</sub> cells (Fig. 4B). Together, these data suggest that B cells provide qualitatively different signals than DCs, perhaps via expression of costimulatory molecules that facilitate optimal IL-21 production by T<sub>FH</sub> cells.

Consistent with a previous study (9), in vitro primed T<sub>FH</sub> cells expressed little to no IL-17 transcript (Supplemental Fig. 1E), further confirming their T<sub>FH</sub> character. Moreover, the superior ability of DCs to induce IL-17 compared with that of...
B cells was recapitulated in vivo, as OTII Tfh and OTII non-Tfh effector cells primed solely via conventional DCs in CD11c/Aβ mice expressed more IL-17 compared with their WT counterparts (Supplemental Fig. 1 F). Together, these data highlight the critical role played by non-DC APCs, presumably B cells, in enforcing progression to Tfh status while inhibiting other lineage potential.

**Sustained Ag presentation on conventional DCs is insufficient to rescue GC Tfh cells or production of IL-21**

Recent findings suggest that sustained Ag presentation restores the formation of PD1b/CXCR5b Tfh cells in the absence of B cells (13). We performed a similar experiment in the CD11c/Aβ mice. We transferred 10^5 OTII cells, immunized with NP-OVA the next day, and then administered 10 μg OVA323–339 peptide i.v. on day 3 p.i. Consistent with Deenick et al. (13), we observed greater expansion of OTII cells in the CD11c/Aβ mice given exogenous peptide (Supplemental Fig. 1 A) on day 7 p.i. However, neither PD1b GC Tfh cells nor IL-21 production were observed in CD11c/Aβ mice that received extra peptide (Supplemental Fig. 1 B–D). Whether these differences arise because of the use of irradiation chimeras by Deenick et al. (13) or because of the lack of other MHC II’ APCs in the CD11c/Aβ mice is unclear. Thus, sustained Ag presentation by conventional DCs is insufficient to induce GC Tfh cells, and a second, distinct APC population is required.

**In conjunction with BCR and CD40 ligation, IL-21 induces a GC B transcription profile**

Recent studies suggest that IL-21R ligation yields maximal Bcl6 expression in B cells, thus fostering GC formation in vivo (7, 8). However, in the presence of anti-CD40 Ab in vitro, IL-21 induces B lymphocyte-induced maturation protein 1 (Blimp1) (21) skewing B cells toward a plasma cell fate. To simulate Ag-driven, T-dependent activation of follicular B cells in vitro, we ligated both the BCR and CD40 with anti-IgM and anti-CD40 Abs, respectively, then assayed for the induction of Blimp1 and Bcl6 transcripts. Compared with B cells stimulated with only anti-IgM or anti-IgM plus anti-CD40, IL-21 markedly augmented the transcriptional profile associated with the adoption of the GC fate. Both activation-induced cytidine deaminase (data not shown) and Bcl6 were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated, whereas Blimp1 transcripts, which were elevated after BCR and CD40 ligation alone, were substantially upregulated.

Collectively, our data suggest that complete differentiation of Tfh cells is most effectively accomplished via successive, cognate interactions with distinct APCs. In this study, we demonstrate that DCs are necessary and sufficient to initiate the Tfh cell program, arguing against the notion that B cells are critical for priming (2, 9–11). Priming solely via DCs in the CD11c/Aβ system leads to accumulation and persistence of a novel intermediate or pre-Tfh cell, which expresses CXCR5, ICOS, and Bcl6 but lacks IL-21. Thus, optimal induction of IL-21 as well as further differentiation toward GC Tfh cell requires cognate interactions with other APCs, including activated B cells. Thus, we propose that as poised CXCR5b/ICOSb Tfh cells relocate from the DC-rich T cell zone to B cell-rich follicles, their interactions with activated B cells via costimulatory ligands including PDL2 and ICOSL increases the likelihood of adopting a Tfh cytokine profile, including IL-21 production (24, 25). Whether B cells provide qualitatively distinct signal(s) to Tfh cells for optimal effector cytokine production remains unclear.

Because CD4+ T cells require continued interactions with APCs for their expansion and effector functions (26), the assumption of alternate effector fates likely reflects the receipt of chronologically ordered signals during the early primary response. This suggests a stepwise model for GC Tfh cell formation. T cells primed by DCs differentiate into a novel pre-Tfh cell subset. These cells can then migrate toward the T–B border, where cognate interactions with Ag-primed B cells induce optimal IL-21 production, thus acquiring effector potential characteristic of the Tfh cell subset while inhibiting other fates, such as Th17. IL-21, in turn, reinforces a GC transcriptional program in the activated B cells. Thus, fully differentiated Tfh cells are only generated when a humoral response is appropriate and Ag-specific T cell help is very specifically directed toward Ag-experienced B cells.

**Disclosures**

The authors have no financial conflicts of interest.
Supplemental Figure 1. Sustained antigen presentation by conventional DCs is not sufficient to induce GC T<sub>FH</sub> formation. 10<sup>5</sup> CD90.1<sup>+</sup> OTII T cells were transferred into either WT or CD11c<sub>A<sup>b</sup></sub>/A<sub>b</sub> mice and next day the mice were immunized with NP-OVA/alum. On day 3, some mice were administered 10 µg OVA peptide i.v. or left untreated and analyzed at day 7 p.i.. (A) Total number of OTII and OTII T<sub>FH</sub> present in immunized WT or CD11c/A<sub>b</sub> recipients (n=3-4 mice). (B) Representative FACS gating strategy to identify PD1<sup>hi</sup> CXCR5<sup>+</sup> within activated OTII T<sub>FH</sub>. (C) The numbers of GC T<sub>FH</sub> (n=3-4, 2 independent experiments). (D) Expression of IL-21 transcript in FACS sorted OT-II T<sub>FH</sub> compared to levels found in naïve T cells. Results are representative of 2 independent experiments. (E) Expression of IL-17A transcript in OTII polarized towards T<sub>FH</sub> or Th17 relative to naïve OTII using β-actin as an endogenous control. (F) Expression of IL-17A transcript in FACS sorted OT-II T<sub>FH</sub> or Teff (CD90.1<sup>+</sup>CD62L<sup>hi</sup>CXCR5<sup>lo/neg</sup>) compared to levels found in naïve T cells. * denotes statistical significance of 0.01<p<0.05 in a two-tailed t test at α=0.05

Supplemental Figure 2. Impaired GC formation in the absence of B cell antigen presentation. 10<sup>6</sup> OTII T cells were transferred into WT and CD11c<sub>A<sup>b</sup></sub>/A<sub>b</sub> mice and mice were immunized with NP-OVA/alum. (A) Representative FACS gating strategy to identify splenic GC B cells as CD19<sup>+</sup> IgD<sup>-</sup> Fas<sup>+</sup> cells. (B) The total number of GC B cells in WT or CD11c<sub>A<sup>b</sup></sub>/A<sub>b</sub> at d7 p.i. or non-immunized WT mice. (n=4-5 mice, representative of >5 Experiments) (C) Total number of IgM and IgG1 Antibody Secreting Cells (ASCs) present in immunized WT or CD11c/A<sub>b</sub> recipients on d7 p.i. or in control non-immunized WT mice (n=4-5 mice, representative of 3 Experiments), enumerated via ELISPOT.
Supplemental Figure 1.

A. 

B. 

C. 

D. 

E. 

F.
Supplemental Figure 2.