Expression of αvβ8 integrin on dendritic cells regulates Th17 cell development and experimental autoimmune encephalomyelitis in mice

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Th17 cells promote a variety of autoimmune diseases, including psoriasis, multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease. TGF-β is required for conversion of naive T cells to Th17 cells, but the mechanisms regulating this process are unknown. Integrin αvβ8 on DCs can activate TGF-β, and this process contributes to the development of induced Tregs. Here, we have now shown that integrin αvβ8 expression on DCs plays a critical role in the differentiation of Th17 cells. Th17 cells were nearly absent in the colons of mice lacking αvβ8 expression on DCs. In addition, these mice and the DCs harvested from them had an impaired ability to convert naive T cells into Th17 cells in vivo and in vitro, respectively. Importantly, mice lacking αvβ8 on DCs showed near-complete protection from experimental autoimmune encephalomyelitis. Our results therefore suggest that the integrin αvβ8 pathway is biologically important and that αvβ8 expression on DCs could be a therapeutic target for the treatment of Th17-driven autoimmune disease.

Introduction
For the adaptive immune system to elicit an effective response to different types of pathogens, naive T cells must undergo a series of epigenetic changes that specialize them for specific responses. Th17 cells develop from naive T cells and aid in the destruction of certain bacteria and fungi. The cytokines IL-17A, IL-17F, and IL-22, produced by these cells, support the recruitment and activation of neutrophils and drive the production of antimicrobial peptides by epithelial cells (1). Because of the prominent role these cells play in host defense and autoimmune disease, the mechanisms that regulate Th17 development are a topic of intensive investigation.

IL-6 and TGF-β are sufficient to differentiate Th17 cells from naive CD4+ T cells (2–4). Other cytokines, including IL-21 and IL-23, augment Th17 conversion and expand the population (5, 6). TGF-β directs the transcriptional program of Th17 cells by inducing expression of the Th17 lineage-specific transcription factor RORγt (7). TGF-β also stimulates expression of Foxp3, a transcription factor that is required for Treg function, and IL-6 is required to mediate the stabilization of RORγt and repression of Foxp3 (8). TGF-β is also necessary for expression of the IL-23 receptor on T cells, which allows the Th17 population to expand in response to IL-23 (4). While TGF-β is known to be a critical factor for Th17 differentiation and function, the mechanisms that regulate TGF-β activity in Th17 development have not been described.

TGF-β is produced in a latent form and must be activated to exert a biological effect. Each of the 3 TGF-β mammalian genes encode a translation product consisting of an N-terminal propeptide (termed “latency-associated peptide” [LAP]) and bioactive TGF-β. This product is cleaved intracellularly by furin, and LAP associates with TGF-β in a noncovalent fashion to form the small latent complex. In most cells, LAP is covalently attached to 1 of 3 latent TGF-β binding proteins prior to secretion to form a large latent complex (9). TGF-β is generally inactive when it is incorporated into a small latent complex or large latent complex and is unable to bind TGF-β receptors. Latent TGF-β has been reported to be activated by proteases, thrombospondin-1, reactive oxygen species, and, at least for TGF-β1 and -β3, the integrins αvβ6 and αvβ8 (10, 11). A prominent role for integrin-mediated TGF-β activation is suggested by recent evidence that mice expressing a mutant form of TGF-β1 that cannot be activated by integrins phenocopy mice completely lacking TGF-β1 (12), and mice lacking αvβ8 treated at birth with antibodies blocking αvβ6 express all of the phenotypic features of mice lacking TGF-β1 and TGF-β3 (13).

We have recently shown that mice lacking αvβ8 on DCs develop colitis and autoimmunity and have a quantitative defect in development of CD4+ Foxp3+ Tregs in the colon (14), demonstrating a critical role for this pathway in maintenance of immune homeostasis. However, the importance of this pathway in the induction of effector Th17-mediated immune responses is completely unknown. Because of the prominent role of TGF-β in Th17 cell induction and the absence of any data describing how TGF-β gets activated to induce these cells, we sought to determine what role integrin αvβ8 on DCs plays in the development of Th17 cells.

Results
We examined the colons of mice lacking αvβ8 on DCs for the presence of IL-17–producing CD4+ T cells. Intracellular flow cytometry analysis of cells isolated from the colonic lamina propria of β8fl/fl × CD11c-cre mice showed a near absence of IL-17–expressing CD4+ T cells.
T cells (0.6% ± 0.2%), whereas 8.5% ± 1.3% of colonic CD4⁺ T cells in control mice expressed IFN-γ (Figure 1A). Since IFN-γ impedes development of Th17 cells in vitro, we examined whether increased production of IFN-γ was responsible for impaired Th17 development in β8⁻/⁻ × CD11c-cre mice by crossing them to mice deficient in IFN-γ. The colons of Ifng⁻/⁻ β8⁻/⁻ × CD11c-cre mice also showed a dramatic reduction in the number of CD4⁺ IL-17⁺ cells, with only 3.1% ± 0.5% of the CD4⁺ cells expressing IL-17 compared with 26% ± 4.0% in control mice (Figure 1A). Interestingly, Ifng⁻/⁻ β8⁻/⁻ × CD11c-cre mice exhibited colitis and enlarged mesenteric lymph nodes that closely resembled β8⁻/⁻ × CD11c-cre mice, suggesting that the colitis seen in the absence of αβ8 on DCs occurs independently of IFN-γ (data not shown). We previously reported that β8⁻/⁻ × CD11c-cre mice have a decreased frequency of Tregs in the colon but a normal proportion in the spleen (14). Since Tregs are significant producers of TGF-β, we questioned whether a decreased frequency of Tregs in the colon could decrease the amount of total TGF-β available for Th17 development in β8⁻/⁻ × CD11c-cre mice. We found no significant difference in the amount of TGF-β in the colons from control and β8⁻/⁻ × CD11c-cre mice (417 ± 29 and 384 ± 13 pg/ml; P = 0.16). These data indicate that excess production of IFN-γ might be responsible for impaired Th17 development in β8⁻/⁻ × CD11c-cre mice. Control and β8⁻/⁻ × CD11c-cre mice developed similar EAE after passive immunization with activated MOGβ₈⁺⁺ T cells, whereas only 0.6% ± 0.1% in the draining lymph nodes of control mice, but the numbers of T cells were dramatically reduced in the CNS of β8⁻/⁻ × CD11c-cre mice (Figure 1B). Since IFN-γ–producing T cells stimulated with MOGβ₈⁺⁺ peptide derived from myelin oligodendrocyte protein) into control and β8⁻/⁻ × CD11c-cre mice. Control and β8⁻/⁻ × CD11c-cre mice developed similar EAE after passive immunization with activated 2D2 T cells (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI43786DS1). We also checked whether β8 expression on microglia, an important antigen-presenting cell in the CNS, was affected in β8⁻/⁻ × CD11c-cre mice. We found no difference in microglia β8 mRNA expression from control and β8⁻/⁻ × CD11c-cre mice (1.3 ± 0.3 ± 4.2 ± 10⁻⁴ and 1.1 ± 1.3 ± 2.5 ± 10⁻⁴; P = 0.35). These data support a critical role for induction of Th17 cells by αβ8 on DCs in periphery during the development of EAE.

To assess whether integrin αβ8 on DCs participates in the conversion of naïve CD4⁺ T cells to Th17 cells, we transferred Thy1.1-labeled naïve CD4⁺ 2D2 TCR transgenic T cells into control and β8⁻/⁻ × CD11c-cre mice immunized with an emulsion of MOGβ₈⁺⁺ and CFA. The Thy1.1 label allowed us to identify the transferred cells among endogenous CD4⁺ T cells upon re-isolation from the draining lymph nodes and spleen. When transferred Thy1.1 2D2 T cells were re-stimulated and analyzed 8 days after immunization, 60% ± 1.1% of the cells produced IL-17 (Figure 3, A and B). However, only 22% ± 1.4% Thy1.1 2D2 T cells produced IL-17 after transfer into β8⁻/⁻ × CD11c-cre mice (Figure 3, A and B). Additionally, IL-17–expressing 2D2 T cells recovered from β8⁻/⁻ × CD11c-cre mice produced significantly less IL-17 as measured by MFI of IL-17 staining (Figure 3C). We also determined
the role of integrin αβ8 on DCs in the conversion of naive CD4+ T cells to Th17 cells by transferring polyclonal naive CD4+ T cells into β8fl/fl × CD11c-cre mice crossed onto the SCID background. SCID mice lack functional T and B cells and therefore allowed us to study the role of integrin αβ8 on DCs in Th17 differentiation in the absence of endogenous lymphocytes. When naive CD4+ T cells were transferred into SCID control mice and recovered from the colonic lamina propria and spleen 12 days later, 10% ± 1.2% and 16.1% ± 2.4%, respectively, produced IL-17 upon restimulation (Figure 3, D and E). However, only 1.2% ± 0.3% and 2.8% ± 0.6% of CD4+ T cells recovered from the colonic lamina propria and spleen, respectively, of SCID β8fl/fl × CD11c-cre mice produced IL-17. Furthermore, the few IL-17–expressing CD4+ T cells isolated from SCID β8fl/fl × CD11c-cre mice produced significantly less IL-17 as measured by MFI of IL-17 staining (Figure 3F). These data indicate that integrin αβ8 on DCs is critical for the development of Th17 cells in response to antigen challenge in vivo.

To examine the mechanism by which loss of αβ8 on DCs protected mice from EAE and impaired Th17 differentiation, we characterized DCs from the draining lymph nodes of β8fl/fl × CD11c-cre mice. DCs have been categorized into the lymphoid, myeloid, and plasmacytoid groups based on their developmental origin and function. The presence of these DC populations in the draining lymph nodes of control or β8fl/fl × CD11c-cre mice was determined 7 days after immunization for EAE. We observed 31.4% ± 1.7% lymphoid, 66.8% ± 1.7% myeloid, and 3.9% ± 0.6% plasmacytoid DCs in control mice and 35.1% ± 3.4% lymphoid, 62.6% ± 3.4% myeloid, and 4.5% ± 0.6% plasmacytoid DCs in β8fl/fl × CD11c-cre mice (Figure 4, A and B). Next, we checked the ability of DCs from β8fl/fl × CD11c-cre mice to stimulate the proliferation of naive T cells after immunization for EAE. At day 7 after immunization, there were 6.0% ± 1.5% myelin oligodendrocyte glycoprotein–specific (MOG-specific) T cells after enrichment for tetramer-labeled cells in control mice and 8.1% ± 0.8% in β8fl/fl × CD11c-cre mice (Figure 4, C and D). Furthermore, we saw that MOG-specific TCR transgenic T cells (clone 2D2) adoptively transferred into control and β8fl/fl × CD11c-cre mice underwent a similar number of cell divisions following EAE immunization (Figure 4, E and F). We then analyzed the expression of costimulatory and coinhibitory molecules on DCs from β8fl/fl × CD11c-cre mice. As shown in Table 1, no significant differences were found in the expression of CD40, CD80, CD86, PD-L1, PD-L2, ICAM-1, OX40L, ICOSL, or MHCII. Finally, we examined the ability of DCs from β8fl/fl × CD11c-cre mice to produce cytokines related to the development of Th17 cells. As shown in Table 2, we found no significant differences in the levels of IL-17, IL-23, or IL-6 (P = 0.037). Collectively, these data suggest that DCs from β8fl/fl × CD11c-cre mice are protected from EAE.

Integrin αβ8 controls the activation of TGF-β1 and -β3 through interaction with an Arg-Gly-Asp sequence on the LAP of the latent TGF-β complex (11). To determine whether integrin αβ8 on DCs regulates Th17 development through activation of latent TGF-β, we used an in vitro assay to monitor

![Figure 2](image-url)
The mechanism by which integrin αvβ8 on DCs presents active TGF-β to induce IL-17 expression in T cells is unknown. One possibility is that latent TGF-β is constitutively activated by αvβ8 on DCs to create a surrounding milieu of active TGF-β (i.e., a field effect). Alternatively, αvβ8 might only induce IL-17 expression in T cells if it activates TGF-β in the context of antigen presentation. This would be a teleologically attractive mechanism to tightly regulate, in space and time, this important immunologic decision. To distinguish between these mechanisms, we cultured naive CD4+ 2D2 T cells under Th17-polarizing conditions (in the absence of exogenous TGF-β) with mixtures of αvβ8-deficient DCs and αvβ8-containing DCs that express either matched or mismatched MHCIi. Thus, MHCIi-mismatched DCs would be unable to present MOG peptides to 2D2 T cells. We first established that a 1:1 mixture of control and αvβ8-deficient DCs was able to completely rescue the defect in IL-17 induction seen with αvβ8-deficient DCs alone (Figure 6). We then cultured αvβ8-expressing, MHCIi-mismatched (H2q MHC haplotype from FVB mice) DCs at a 4:1 mixture with αvβ8-deficient DCs (H2b MHC haplotype from C57BL/6 mice) DCs. MHCIi-mismatched DCs were unable to rescue the impaired IL-17 production seen with αvβ8-deficient DCs (Figure 6). These data suggest that Th17 cell induction requires simultaneous presentation of antigen (by MHCIi) and activation of TGF-β by integrin αvβ8 on the same DCs.

Th17 development in the presence of control or αvβ8-deficient DCs. As shown in Figure 5, αvβ8-deficient DCs cultured with naive CD4+ 2D2 T cells under conditions that drive Th17 development (i.e., IL-1β and IL-6), but in the absence of exogenous TGF-β, showed a reduced ability to promote IL-17 production by T cells. However, IL-17 production after culture with αvβ8-deficient DCs was restored by the addition of exogenous TGF-β (Figure 5A, A–C). Furthermore, the addition of a TGF-β–neutralizing antibody significantly reduced IL-17 production by T cells cultured with control DCs but had a minimal effect on αvβ8-deficient DCs (Figure 5A, A–C). To confirm that IL-17 produced by T cells in this assay was from bona fide Th17 cells, we measured IL-17F, another canonical Th17 cytokine. Indeed, IL-17F was detected in the supernatant of cultures grown without the addition of exogenous TGF-β and was significantly reduced in cultures with αvβ8-deficient DCs (Figure 5D). Furthermore, we measured mRNA expression of 2 genes that have been shown previously to be regulated by TGF-β — Rorc, which encodes the Th17–specific transcription factor RORγt, and Il23r, which encodes the IL-23 receptor, a receptor required for clonal expansion of Th17 cells. We detected transcripts of these genes in T cells from cultures with control DCs but found a dramatic reduction in expression of these genes in T cells from cultures with αvβ8-deficient DCs (Figure 5E).

Figure 3
Blunted Th17 conversion of naive CD4+ T cells adoptively transferred into β8fl/fl × CD11c-cre mice. (A) Flow cytometry plots of Thy1.1 2D2 TCR transgenic T cells isolated from the spleen and draining lymph nodes 8 days after transfer into control or β8fl/fl × CD11c-cre mice immunized with MOG plus CFA. Isolated cells were activated with PMA/ionomycin and stained for IL-17 and IFN-γ. Numbers in quadrants indicate the percentage of positive cells. (B) Percentage of IL-17- and IFN-γ-producing Thy1.1+ CD4+ T cells isolated from control and β8fl/fl × CD11c-cre mice. (C) MFI of IL-17 and IFN-γ staining in Thy1.1+ CD4+ T cells isolated from control and β8fl/fl × CD11c-cre mice. (D) Flow cytometry plots of polyclonal CD4+ T cells isolated from the colonic lamina propria (top) or spleen (bottom) 12 days after transfer into SCID control or SCID β8fl/fl × CD11c-cre mice. Isolated cells were activated with PMA/ionomycin and stained for IL-17 and IFN-γ. Numbers in quadrants indicate the percentage of positive cells. (E) Percentage of IL-17- and IFN-γ-producing CD4+ T cells isolated from control and β8fl/fl × CD11c-cre mice. (F) MFI of IL-17 and IFN-γ staining in CD4+ T cells isolated from control and β8fl/fl × CD11c-cre mice. Data represent the mean ± SEM of 2 independent experiments and 3 to 5 mice per group. *P < 0.005; **P < 0.01.
Discussion

How TGF-β is regulated in the extracellular space and presented to naïve T cells during Th17 development has been unknown. Our results strongly suggest that the integrin αvβ8 on DCs is required for maximal Th17 development. This effect appears to be due to TGF-β activation by this integrin, since the defect in Th17 induction by DCs lacking αvβ8 can be completely rescued by the addition of exogenous TGF-β. Furthermore, these data suggest that activation of TGF-β by integrin αvβ8 on DCs may be a novel target in the development of therapeutics for autoimmune diseases.

We tested the in vivo relevance of TGF-β activation by integrin αvβ8 on DCs in the EAE disease model. The EAE model is widely used to study the formation of pathogenic T cells to self-molecules and has been utilized as a model of multiple sclerosis. Our result that overexpression of αvβ8 on DCs is regulated in the extracellular space and presented to naïve T cells during Th17 development has been unknown. Our results strongly suggest that the integrin αvβ8 on DCs in the CNS (2) are almost completely protected from TGF-β activation by this integrin, since the defect in Th17 induction by DCs lacking αvβ8 can be completely rescued by the addition of exogenous TGF-β. Furthermore, these data suggest that activation of TGF-β by integrin αvβ8 on DCs is required in the development of therapeutics for autoimmune diseases.

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mice lacking αvβ8 on DCs suggests that these mice may be protected from EAE in part due to impaired formation of MOG-reactive Th17 cells. This concept is also supported by our observation that at day 8 following EAE immunization, a time point before the onset of symptomatic EAE, Th17 cells failed to develop in the draining lymph nodes of mice lacking in αvβ8 on DCs. These data agree with a previous report that mice engineered to express a dominant-negative TGF-β receptor-II in T cells are protected from EAE through a defect in Th17 cell induction (15). Interestingly, transgenic mice that overexpress TGF-β within T cells develop more severe EAE than wild-type mice and also exhibit greater numbers of Th17 cells in the CNS (2). While Th17 cells are regarded as essential for EAE, several recent studies have called into question which type or types of CD4+ T cells are encephalitogenic in this model (16, 17). In the present study, the scarcity of Th17 cells found in the CNS and periphery of EAE-primed mice lacking αvβ8 on DCs, and their dramatic protection from EAE, indicates that TGF-β activated via αvβ8 on DCs is an important step in the generation of encephalitogenic CD4+ T cells.
The present study did not examine which cell type or types are responsible for producing the latent TGF-β required for Th17 development. A previous report from Li et al. showed that deletion of the Tgfb gene in T cells protects mice from EAE and Th17 development (26). In context with the findings presented here, these data suggest a model whereby T cells provide their own latent TGF-β, which then gets activated by αβ8 on DCs. However, DCs also produce significant amounts of TGF-β, and it has been reported that a subset of DCs in the CNS that preferentially induce Th17 cells also produce elevated levels of TGF-β (27). These data suggest that TGF-β signaling within the immune system may be regulated by both expression and activation of the latent form.

The spatial and temporal regulation of TGF-β in the extracellular space during Th17 development has been unknown. In the current study, we provide a partial answer to this question by showing that αβ8 integrin-mediated TGF-β activation by DCs can only induce antigen-specific T cells to become Th17 cells if the same cell that is expressing the integrin is also actively presenting antigen in the context of MHCII. Since TGF-β signaling in T cells has the potential to direct the formation of a pro-inflammatory (i.e., Th17) or immunosuppressive (i.e., Treg) response to antigen, it makes teleologic sense for this process to be tightly regulated in space and time. Our results suggest that one important factor is precise spatial regulation of delivery of the active TGF-β, potentially allowing the same DCs to provide active TGF-β and other polarizing cytokines (for example, IL-6 in the context of Th17 cell induction). Presentation of active TGF-β when T cells are in close proximity to DCs would allow for precise control of T cell fate at a time when T cells are receiving signals from costimulatory and co-inhibitory molecules as well as secreted cytokines.

Collectively, the data presented here indicate that integrin αβ8 on DCs facilitates the development of Th17 cells, and consequently contributes to IL-17–mediated CNS inflammation, through activation of TGF-β. This finding suggests that the αβ8 integrin, or the steps upstream and downstream of αβ8–mediated TGF-β activation, could be novel targets for treatment of multiple sclerosis and other autoimmune diseases.

### Table 1
Costimulatory and coinhibitory protein expression on DCs from β8<sup>fl/fl</sup> × CD11c-cre mice

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>CD11c-cre</th>
<th>Pvalue</th>
</tr>
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<tbody>
<tr>
<td>CD40</td>
<td>10,539 ± 287</td>
<td>11,158 ± 288</td>
<td>0.09</td>
</tr>
<tr>
<td>CD80</td>
<td>407 ± 7</td>
<td>722 ± 221</td>
<td>0.10</td>
</tr>
<tr>
<td>PD-L1</td>
<td>49,069 ± 3,690</td>
<td>48,896 ± 2,318</td>
<td>0.48</td>
</tr>
<tr>
<td>CD86</td>
<td>6,048 ± 231</td>
<td>5,894 ± 195</td>
<td>0.31</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>6,144 ± 29</td>
<td>5,901 ± 143</td>
<td>0.07</td>
</tr>
<tr>
<td>OX40L</td>
<td>2,463 ± 420</td>
<td>2,570 ± 516</td>
<td>0.44</td>
</tr>
<tr>
<td>PD-L2</td>
<td>1,022 ± 71</td>
<td>1,136 ± 41</td>
<td>0.11</td>
</tr>
<tr>
<td>ICOSL</td>
<td>1,054 ± 89</td>
<td>965 ± 54</td>
<td>0.22</td>
</tr>
<tr>
<td>MHCII</td>
<td>3,824 ± 446</td>
<td>3,895 ± 236</td>
<td>0.45</td>
</tr>
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</table>

DCs were isolated from the draining lymph nodes of control or β8<sup>fl/fl</sup> × CD11c-cre mice 7 days after immunization for EAE and stained with antibodies for the costimulatory or coinhibitory proteins listed above. Stained cells were analyzed by flow cytometry, and the MFI of each label was determined. Data represent the MFI ± SEM of labeled DCs from at least 4 mice in each group.

An absolute requirement for TGF-β in Th17 development has been controversial. A combination of TGF-β and IL-6 was first reported to be sufficient to induce Th17 differentiation in mice (2–4). Subsequently, 2 groups reported that TGF-β was not required for the conversion of naive T cells to Th17 cells in humans (18, 19). However, the human naive T cells used in those studies were isolated using protocols that may have introduced TGF-β contamination and were cultured with serum containing TGF-β. Subsequently, 3 groups used rigorous methods to exclude TGF-β contamination and showed that TGF-β is required for Th17 differentiation by human T cells (20–22). However, a recent study that used mice lacking the transcription factors required to drive Th1 and Th2 differentiation suggested that TGF-β may function indirectly in Th17 conversion by suppressing Th1 and Th2 factors, which in turn suppress RORγt (23). Nonetheless, TGF-β appears to be crucial for Th17 induction in vivo and at the site of T cell priming, as administration of a TGF-β–blocking antibody delivered with antigen and CFA abrogates Th17 induction and EAE (15). The data presented in this study are also in line with previous findings on a requirement for TGF-β in the generation of intestinal lamina propria Th17 cells. Lamina propria Th17 cells are absent in Tgfb<sup>−/−</sup>CREC<sup>−/−</sup> knockin mice, which have impaired TGF-β activation through inhibition of covalent TGF-β attachment to latent TGF-β binding protein 1 (24). Furthermore, Atarashi et al. demonstrated that an αβ8–expressing CD11c<sup>+</sup> DC lamina propria subset preferentially induces Th17 generation from naive T cells in vitro (25).

We were concerned that the increased production of IFN-γ by T cells from mice lacking αβ8 on DCs might contribute to the markedly impaired Th17 cell induction in these animals. Our finding that mice deficient in both IFN-γ and αβ8 on DCs still have a dramatic defect in Th17 cell induction excludes that explanation. We also saw that some MOG-specific TCR transgenic T cells adoptively transferred into mice lacking αβ8 on DCs were able to express IL-17, albeit at lower levels than those transferred into control mice. This finding suggests that the presence αβ8 on DCs may not be an absolute requirement for the generation of Th17 cells, or alternatively, that a small number of memory cells were present in the naive transferred cells.
For intracellular cytokine analysis, cells were incubated for 4 hours and then stained for IL-17 and IFN-γ (clone M5/114.15.2), anti-B220 (clone RA3-6B2), anti-mPDCA1 (clone RM4-5), anti-CD8 (clone 53-6.7), anti-CD11c (clone N418), anti-MHCII (clone eBio17B7), anti–IFN-γ (clone XMG1.2), anti–PD-L1 (clone MIH5), anti–PD-L2 (clone 122), anti–ICOSL (clone HK5.3), anti–CD40 (clone 1C10), anti–CD80 (clone 16-10A1), anti–CD86 (clone GL1), anti–ICAM-1 (clone YN1/1.7.4), and anti–OX40L (clone RM134L). MOG tetramer staining. CD4+ T cells expressing a MOG-specific TCR were stained with PE-labeled I-A(b) GWYRSPFSRVVH tetramer (NIH Tetramer Core Facility at Emory University) as previously described with modifications (29). Briefly, cells (1 × 10^6/ml) from the draining lymph nodes and spleen of mice at day 7 after EAE immunization were incubated with a 1:100 dilution of tetramer in HEPES-buffered DMEM plus 2% FBS for 1 hour at room temperature. Cells were then washed and incubated with anti-PE microbeads (Miltenyi Biotec) in HEPES-buffered DMEM plus 2% FBS for 30 minutes at 4°C. After washing, tetramer-labeled cells were enriched by passing over a magnetic column (LS column; Miltenyi Biotec) according to the manufacturer’s instructions. Eluted cells were stained for cell surface markers and analyzed on an LSRII as described above.

Methods

Mice. C57BL/6 ββ<sup>−/−</sup> × CD11c-cre (14) and C57BL/6 2D2 TCR transgenic (28) mice have been previously described. C57BL/6 SCID mice (strain B6.CB17-Pkdc<sup>−/−</sup>/Sj) were purchased from The Jackson Laboratory and crossed to C57BL/6 ββ<sup>−/−</sup> × CD11c-cre. FVB/NJ mice were purchased from The Jackson Laboratory. Mice used for all experiments were 8–12 weeks old and were housed under specific pathogen-free conditions in the Animal Barrier Facility of the University of California, San Francisco. All experiments were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco.

Flow cytometry. For intracellular cytokine analysis, cells were incubated for 4 hours at 37°C in complete RPMI containing 50 ng/ml PMA (Sigma-Aldrich), 1 μM ionomycin (Sigma-Aldrich), and 2 μM monensin (eBioscience). Cells were then washed with ice-cold PBS and blocked for 20 minutes at 4°C with PBS containing 2 μg/ml α-CD16/32 (UCSF hybridoma core) and 10% heat-inactivated normal rat serum (Sigma-Aldrich). After washing with PBS, cells were incubated for 30 minutes in PBS with cell-surface antibodies and Aqua Live/Dead Fixable Dead cell stain (Invitrogen). Cells were then fixed and permeabilized with eBioscience Fix/Perm solution according to the manufacturer’s instructions. Intracellular cytokines were labeled with antibodies for 45 minutes at 4°C in permeabilization buffer (eBioscience). Cells were then analyzed on an LSRII (BD Biosciences), and postanalysis of flow cytometry data was performed with FlowJo software (Tree Star Inc.). The following antibodies were used in experiments and were purchased from BD Biosciences—Pharmingen or eBioscience: anti–CD4 (clone RM4-5), anti–CD8 (clone 53-6.7), anti–CD11c (clone N418), anti–MHCIi (clone M5/114.15.2), anti–B220 (clone RA3-6B2), anti–mPDCA1 (clone eBio927), anti–CD44 (clone IM7), anti–Thy1.1 (clone HIS51), anti–IL-17 (clone eBio17B7), anti–IFN-γ (clone XMG1.2), anti–PD-L1 (clone MIH5), anti–PD-L2 (clone 122), anti–ICOSL (clone HK5.3), anti–CD40 (clone 1C10), anti–CD80 (clone 16-10A1), anti–CD86 (clone GL1), anti–ICAM-1 (clone YN1/1.7.4), and anti–OX40L (clone RM134L).

Lymphocyte isolation from colonic lamina propria. Lymphocytes were isolated from the colonic lamina propria as previously described with slight modifications (30). Briefly, colons were removed, washed with ice-cold PBS, and cut into 2- to 3-cm-long pieces. Pieces were predigested in 5 ml of HBSS containing 5 mM EDTA and 1 mM DTT and rotated (180 rotations/min) for 20 minutes at 37°C to remove debris and intraepithelial lymphocytes. Pieces were then washed with PBS and predigested a second time. Pieces were next cut to 0.5 cm and digested in serum-free RPMI containing 0.1 U/ml lliberase blendzyme 3 (Roche) and 10 U/ml DNase I (Roche) at 37°C for 20 minutes with rotation. After vortexing, the supernatants were...
Mice were given s.c. injections into the flanks and nape. Naive Thy1.1 DCs were isolated from control, β8β8 × CD11c-cre, and MHCII-mismatched (H2q MHC haplotype from FVB strain) mice and cultured in different combinations with naive CD4+ 2D2 TCR transgenic T cells under Th17-driving conditions without exogenous TGF-β. Supernatants were collected and analyzed by ELISA for the presence of IL-17. Data are means ± SEM of the ratio of IL-17 produced between control and β8β8 DCs. *P < 0.05.

Figure 6
Th17 development through integrin αββ8 on DCs is dependent on MHCII-TCR engagement. DCs were isolated from control, β8β8 × CD11c-cre, and MHCII-mismatched (H2q MHC haplotype from FVB strain) mice and cultured in different combinations with naive CD4+ 2D2 TCR transgenic T cells under Th17-driving conditions without exogenous TGF-β. Supernatants were collected and analyzed by ELISA for the presence of IL-17. Data are means ± SEM of the ratio of IL-17 produced between control and β8β8 DCs. *P < 0.05.


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