This information is current as of May 1, 2022.

**Cutting Edge: Central Nervous System Plasmacytoid Dendritic Cells Regulate the Severity of Relapsing Experimental Autoimmune Encephalomyelitis**

Samantha L. Bailey-Bucktrout, Sarah C. Caulkins, Gwendolyn Goings, Jens A. A. Fischer, Andrzej Dzionek and Stephen D. Miller

*J Immunol* 2008; 180:6457-6461; doi: 10.4049/jimmunol.180.10.6457

http://www.jimmunol.org/content/180/10/6457

**References** This article cites 23 articles, 10 of which you can access for free at: http://www.jimmunol.org/content/180/10/6457.full#ref-list-1

**Why The JI?** Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Plasmacytoid dendritic cells (pDCs) have both stimulatory and regulatory effects on T cells. pDCs are a major CNS-infiltrating dendritic cell population during experimental autoimmune encephalomyelitis. However, unlike myeloid dendritic cells, they play a minor role in T cell activation and epitope spreading. We show that depletion of pDCs during either the acute or relapse phases of experimental autoimmune encephalomyelitis resulted in exacerbation of disease severity. pDC depletion significantly enhanced CNS myeloid dendritic cell-driven production of IL-17, IFN-γ, and IL-10 in an IDO-independent manner. The data demonstrate that pDCs play a critical regulatory role in negatively regulating pathogenic CNS CD4⁺ T cell responses, highlighting a new role for pDCs in inflammatory autoimmune disease. The Journal of Immunology, 2008, 180: 6457–6461.

Enhanced numbers of activated pDCs have been described in MS, and activated pDCs are associated with Sjögren’s syndrome, lupus, and psoriasis (7–11). It was therefore important to understand the role of pDCs in MS/EAE pathogenesis. Using a mAb (anti-mPCDA-1) to deplete pDCs from the CNS following R-EAE induction, we show that pDC depletion caused the rapid exacerbation of EAE severity in both the primary acute and relapse phases. Mechanistically, pDC depletion did not affect the frequency of myelin-specific CD4⁺ T cells in peripheral lymphoid organs but markedly enhanced CNS CD4⁺ T cell activation as well as IL-17 and IFN-γ production. Moreover, CNS pDCs suppressed CNS mDC-driven production of IL-17, IFN-γ, and IL-10 in an IDO-independent manner.

**Materials and Methods**

**Mice**

Female SJL/J mice were purchased from Harlan Sprague Dawley (SJL/JCrHsd) or Taconic Farms (SJL/JCrNiac). Mice were housed and cared for according to Northwestern University (Chicago, IL) Institutional Animal Care and Use Committee-approved protocols.

**Induction of EAE**

As previously reported (6).

**Peptides and antibodies**

PLP139–151 (HSLGKWLGHPDKF) was synthesized to >95% purity by Genemed Synthesis. Conjugated Abs were purchased from BD Pharmingen or eBioscience.

**Depletion of pDCs**

Mice received i.p. injections of 250 μg of anti-mouse plasmacytoid dendritic cell antigen-1 (anti-mPCDA-1) (clone JF05-1C2, rat IgG2b; Miltenyi Biotec) or purified rat IgG2b (eBioscience) every other day for four treatments.

**Isolation of cells from secondary lymphoid tissues and CNS**

As previously reported (6).
Staining was analyzed using a Leica DM5000B fluorescent microscope. Lumbar spinal cord sections from PBS-perfused mice as previously described. IFN-γ, IL-10, and IL-17 levels in the supernatants were assessed by cytokine bead array for levels of IL-10, IL-17, and IFN-γ. Cell populations were flow sorted as described in Ref. 6 to enrich for live CNS DCs (CD45<sup>hi</sup>CD3<sup>-</sup>CD11c<sup>+</sup>). 

**Flow cytometric analysis and gating**

For analysis of cytokines, CNS cells were cultured for 4 h in R10 medium (6) plus GolgiStop (BD Biosciences) according to the manufacturer’s recommendations. Cells were stained with five- or six-color Ab mixtures. Amine-reactive, fixable, live/dead viability dye was used according to the manufacturer’s instructions (Molecular Probes/Invitrogen) and dead cells were excluded. Data were acquired on an BD LSR II cytometer (BD Biosciences) and analyzed using FACSDiva (BD Biosciences) or Flow Jo (Tree Star) software.

**CD4<sup>+</sup> T cell activation assay**

Cell populations were flow sorted as described in Ref. 6 to >98% purity. Sorted populations were >95% pure. APCs (2 x 10<sup>5</sup>) were cocultured with 10<sup>6</sup>CD4<sup>+</sup>T cells from the CNS or spleen for 96 h with R10 medium, 5 µg/ml antiCD3, and 200 µM 1-methyl-L-tryptophan (1-MT; Sigma-Aldrich) prepared according to Ref. 12. Carrier solution alone was added as a control. Cells were assessed for viability by flow cytometry as described above, and cytokines in the supernatants were assessed by cytokine bead array for levels of IL-10, IL-17, and IFN-γ according to manufacturers instructions (Upstate Biotechnology).

**ELISPOT assays**

ELISPOT assays were performed as previously described (13) with 10<sup>6</sup> cells plus 10 µM PLP<sub>139-151</sub>.

**Immunohistochemistry**

Immunohistochemistry was performed on 6-µm-thick frozen cerebellar and lumbar spinal cord sections from PBS-perfused mice as previously described (13). Staining was analyzed using a Leica DM5000B fluorescent microscope and Advanced SPOT software.

**Statistical analysis**

Differences between groups were determined using an unpaired Student’s <i>t</i> test and the Mann Whitney <i>U</i> test.

**Results and Discussion**

**Anti-mPDCA-1 mAb efficiently and specifically depletes CNS-infiltrating pDCs during EAE**

pDCs are a minor subset of dendritic cells (DCs) in the secondary lymphoid tissues of most mouse strains (14), comprising 23% of DCs and 1.15% of cells in the lymph nodes (LNs) of SJL/J mice (data not shown). Strikingly, CNS infiltrates during EAE contain 37.7% pDCs, 5.4% of the total CNS mononuclear cell population (6). To investigate the role of pDCs during EAE, anti-mPDCA-1 mAb (15) was used to deplete pDCs during EAE onset. One day after pDC depletion, pDCs were depleted from LNs (not shown) (15) and CNS (92.4 ± 0.9% of CNS pDCs) (Fig. 1, A and B). During the relapse phase of EAE, 9 days following pDC depletion the number of CNS pDCs returned to control levels in pDC-depleted animals (Fig. 1A), and, importantly, anti-mPDCA-1 treatment did not affect the numbers of CNS mDCs or macrophages (Fig. 1B). Following pDC depletion, a few mPDCA-1<sup>+</sup> cells remained in the meningeal area of the CNS; however, no mPDCA-1<sup>+</sup> pDCs were detected in the parenchyma of the spinal cord or cerebellum (Fig. 1C). Inefficient clearing of pDCs from the blood vessel-rich areas of the CNS correlated with low level mPDCA-1 expression on blood CD11c<sup>+</sup>B220<sup>+</sup> pDCs (data not shown).

**pDCs regulate the severity of EAE**

The depletion of pDCs at the onset of R-EAE resulted in a significant exacerbation of peak clinical disease (Fig. 2A). Clinical scores in pDC-depleted mice returned to control levels 2–3 days after the last anti-mPDCA-1 mAb treatment, consistent with a report showing that pDC numbers recover 3–5 days following anti-mPDCA-1 depletion (15). That pDC depletion caused an immediate increase in clinical severity with a rapid return to control levels upon pDC reconstitution suggests that pDCs have a direct, acute regulatory effect on CNS autoimmune disease. pDCs were then depleted during the primary relapse of EAE, leading to enhanced EAE and the abrogation of secondary remission (Fig. 2B).

**FIGURE 1.** Anti-mPDCA-1 efficiently and specifically depletes CNS pDCs during EAE. A, SJL mice were treated with anti-mPDCA-1 or the isotype-matched control rat IgG2b (RlgG2b) mAb on days 8–14 after EAE induction. One day (day 15) and 9 days after pDC depletion (day 24), the effect of treatment on gated live CNS DCs (CD45<sup>hi</sup>CD3<sup>-</sup>CD11c<sup>+</sup>) was determined. B, Day 15 analysis of CNS CD11b<sup>+</sup>mDCs, CD11b<sup>-</sup>B220<sup>+</sup>pDCs, and CD11b<sup>-</sup>CD11c<sup>-</sup> macrophages (MΦ). Data are mean ± SEM of four animals per group, representative of 5 experiments. The <i>p</i> values were determined by Mann-Whitney <i>U</i> test. C, Day 15 immunohistochemical analysis of a cerebellum (I and II) and a lumbar spinal cord (III and IV) stained for mPDCA-1 (red), CD4 (green), and 4',6'-diamidino-2-phenylindole (blue). Dotted line shows the meningeal edge of spinal cord tissue. mPDCA-1<sup>+</sup> pDCs are circled. Photographs at original magnifications of ×200 (I and II) and ×100 (III and IV) are representative of three mice.
The depletion of pDCs could have affected the priming of pathogenic T cells in the periphery (16), explaining the clinical outcome of pDC depletion (Fig. 2, A and B). However, the frequency of IL-17-, IFN-γ-, and IL-2-producing CD4^+ T cells specific for the immunizing peptide in the LNs 1 day following the final mPCDA-1 mAb injection was unchanged compared with controls (Fig. 2 C). In fact, PLP_{139-151}-specific Th17 cells were significantly reduced in the spleens of pDC-depleted mice, which may be reflective of reduced EAE severity (3) and not enhanced severity as observed in the clinical experiments.

pDCs modulate the activation and frequency of Th1 and Th17 cells in the CNS

Clinical EAE generally correlates with the number and activation status of CNS-infiltrating effector CD4^+ T cells and inversely with regulatory T cell (Treg) numbers (17). We found that pDC depletion did not significantly affect the numbers of CNS CD4^+ T cells and Foxp3^+ CD4^+ Tregs (p = 0.2; Fig. 3A). We previously showed that CNS pDCs isolated during EAE poorly activate both naive and activated myelin-specific T cells in comparison to mDCs (6). Because there was little change in the T cell numbers, it is unlikely that the primary function of pDC during EAE is to prime and expand CD4^+ T cells in the CNS.

Strikingly, however, in contrast to peripheral responses the CNS CD4^+ T cells were highly activated and produced more IL-17 and IFN-γ in the absence of pDCs. Following pDC depletion, CNS CD4^+ T cells were significantly more activated than controls as assessed by down-regulation of CD45RB and up-regulation of CD25 (Fig. 3B). Endogenous production of...
inflammatory cytokines by CNS CD4\(^+\) T cells was determined by incubating CNS isolates, which contain pathogenic T cells and DCs presenting endogenous myelin peptides (6), with GolgiStop for 4 h and then analyzing the accumulated cells expressing IFN-\(\gamma\) and IL-17. The frequency of CNS Th17 cells was increased by an average of 1.6 \(\pm\) 0.38-fold and that of Th1 cells by 1.6 \(\pm\) 0.27-fold, and more cytokine per cell (enhanced mean fluorescence intensity) were produced in pDC-depleted mice (Fig. 3C). Thus, CNS pDCs promote the accumulation of CD4\(^+\) T cells and Tregs in the target organ but strongly modulate the activation status of CD4\(^+\) T cells and, importantly, the frequency of CNS Th1 (IFN-\(\gamma\)) and Th17 (IL-17) cells.

CNS pDCs actively suppress IL-17, IFN-\(\gamma\), and IL-10 production by CNS CD4\(^+\) T cells in an IDO-independent manner

We next sought to determine the mechanism by which pDCs regulate CNS CD4\(^+\) T cell activation and cytokine production. pDCs have been implicated in inducing T cell anergy through IFN-\(\alpha\) and IL-10 production (18) or TGF-\(\beta\) (19). Using quantitative PCR, we have previously shown that CNS pDCs expressed low levels of TGF-\(\beta\) transcripts (6) and similar levels of IFN-\(\alpha\)/mRNA (not shown) compared with other CNS APCs. In addition, IL-10 levels were lower in CNS pDC-APCs T cell cocultures (Fig. 4) and CNS pDCs stimulated with CD40L (not shown) compared with CNS mDCs. Based on the low production by CNS pDCs, it is unlikely that TGF-\(\beta\), IL-10, or IFN-\(\alpha\)/is a dominant pathway for pDC suppression of CD4\(^+\) T cells in the CNS.

pDCs are known to produce the T cell inhibitor IDO in response to IFNs (12, 20). To determine whether CNS pDCs produce IDO that suppresses CD4\(^+\) T cells during EAE, CNS pDCs and mDCs isolated during acute R-EAE were cocultured with CD4\(^+\) T cells from the CNS and spleen of the same animals with or without 1-MT. pDCs were poor stimulators of CNS CD4\(^+\) T cell survival and expansion ex vivo, and the addition of 1-MT had little effect on CNS T cell viability in the presence of pDCs or mDCs (not shown). In agreement with our previously published work (6), CNS mDCs supported the highest levels of IL-17, IFN-\(\gamma\), and IL-10 production by both splenic and CNS-derived T cells (Fig. 4). The IDO inhibitor 1-MT enhanced mDC-induced CD4\(^+\) T cell IL-17 and IFN-\(\gamma\) secretion and decreased IL-10 production. However, 1-MT had no affect on cytokine production in CD4\(^+\) T cells cultured with CNS pDCs. Most profoundly, when CNS pDCs were cocultured with CNS mDC and CNS T cells, production of IL-17, IFN-\(\gamma\), and IL-10 was significantly suppressed (Fig. 4D). These results indicate that CNS pDCs regulate CD4\(^+\) T cell cytokines in an active manner that dominates that of mDCs driving Th17 cells in the CNS (6) and that regulation is via an IDO-independent pathway. IFN-\(\beta\) modulates IFN-\(\gamma\) and IL-17 production by human PBMCs (21); thus, pDC production of IFN-\(\beta\) (22) may play a primary role in pDC modulation of Th1/Th17 activation during R-EAE concordant with the observation that IFN-\(\beta\) treatment is therapeutic in both EAE and MS (23, 24). We are currently investigating the role of IFN-\(\beta\) production by pDCs during EAE.

In summary, we demonstrate an acute, dominant regulatory role for pDCs in CNS autoimmune disease. pDC depletion results in exacerbated EAE but, once pDCs return to normal levels, relapse severity returns to control levels. Normal relapses suggest that the priming of naive T cells in the CNS is unaffected. pDCs suppress mDC-dependent induction/expansion of CNS Th17 and Th1 cells (Fig. 4D). pDC suppression of T cell cytokine production is IDO independent but is not due to the killing of T cells, because in CNS mDC/pDC cocultures CNS CD4\(^+\) T cells have enhanced viability (not shown). These data support a dominant regulatory role for pDCs during EAE in that pDCs recruited to the CNS limit pathology by regulating T cell activation and cytokine production. Treatments that support and expand regulatory pDCs may therefore be attractive therapies for T cell-mediated autoimmune diseases.

**FIGURE 4.** pDCs actively suppress mDC-supported IFN-\(\gamma\), IL-17, and IL-10 production by CNS CD4\(^+\) T cells. On day 14, 10^5 CD4\(^+\) T cells from the CNS (A) or spleen (B) were cultured with 2 \(\times\) 10^4 irradiated splenic APCs (Sp), sorted CNS pDCs, mDCs, or a 1:1 mixture of pDCs and mDCs in the absence (open bars) or presence (filled bars) of 1-MT. The amount of secreted IFN-\(\gamma\), IL-17, and IL-10 in the culture supernatants was determined 4 days later. The results are representative of three experiments where 15–20 perfused mice were pooled. ND, Not done; RlgG2b, rat IgG2b.
Acknowledgments
We thank Mat Degutes (Northwestern University, Chicago, IL) for technical assistance, James Marvin (NWU) for cell sorting, Dr. Xunrong Luo (Northwestern University), and Drs. Qizhi Tang and Jeff Bluestone (University of California, San Francisco, CA) for helpful comments and discussion.

Disclosures
Drs. Fischer and Dzionek are currently employees of Miltenyi Biotec GmbH, which produced the mPDCA-1 antibody used in the study to deplete plasmacytoid dendritic cells.

References