Cell PRESS
Immunity
Article

Engagement of the Type I Interferon Receptor on Dendritic Cells Inhibits T Helper 17 Cell Development: Role of Intracellular Osteopontin

Mari L. Shinohara,[1,2] June-Ho Kim,[1] Virgilio A. Garcia,[1] and Harvey Cantor[1,2,*]
[1]Department of Cancer Immunology & AIDS, Dana-Farber Cancer Institute
[2]Department of Pathology, Harvard Medical School
44 Binney Street, Boston, MA 02115, USA
*Correspondence: harvey_cantor@dfci.harvard.edu
DOI 10.1016/j.immuni.2008.05.008

SUMMARY

Mechanisms that prevent inappropriate or excessive interleukin-17-producing T helper (Th17) cell responses after microbial infection may be necessary to avoid autoimmunity. Here, we define a pathway initiated by engagement of type I IFN receptor (IFNAR) expressed by dendritic cells (DC) that culminated in suppression of Th17 cell differentiation. IFNAR-dependent inhibition of an intracellular translational isoform of Osteopontin, termed Opn-i, derepressed interleukin-27 (IL-27) secretion and prevented efficient Th17 responses. Moreover, Opn-i expression in DC and microglia regulated the type and intensity of experimental autoimmune encephalomyelitis (EAE). Mice containing DC deficient in Opn-i produced excessive amounts of IL-27 and developed a delayed disease characterized by an enhanced Th1 response compared with the dominant Th17 response of Opn-sufficient mice. Definition of the IFNAR-Opn-i axis that controls Th17 development provides insight into regulation of Th cell sublineage development and the molecular basis of type I interferon therapy for MS and other autoimmune diseases.

INTRODUCTION

Efficient protection against microbial infection depends on an interaction between the innate and adaptive immune systems. Recognition of microbial pathogens by pattern-recognition receptors expressed by dendritic cells (DC) activates these cells to efficiently present pathogen-derived antigens to T cells, which initiate adaptive immune responses. Whereas the task of $CD8^+$ T cells is limited primarily to lysis of cells that display peptides derived from intracellular pathogens, the more complex activities of $CD4^+$ T cells are divided among several functionally distinct sublineages of $CD4^+$ T cells. T helper 1 (Th1) and T helper 2 (Th2) cells, discovered some 20 years ago, have evolved to enhance clearance of intracellular pathogens, including viruses and parasites, respectively. A recently defined third sublineage of Th cells termed Th17 cells may be equipped to protect against infection by extracellular bacterial and fungal pathogens including *Mycobacteria, Borrelia burgdorferi,* and *Candida albicans* (Burchill et al., 2003; Khader et al., 2007; Umemura et al., 2007). The nature of the Th17 cell response reflects, in part, the ability of its signature cytokine—interleukin-17 (IL-17)—to attract extensive inflammatory cellular infiltrates through interactions with chemokine receptors. Excessive Th17 cell responses after infection with, for example, *B. burgdorferi*, can result in widespread inflammatory tissue destruction and chronic autoimmune disease.

These considerations suggest that mechanisms that prevent inappropriate or excessive Th17 cell responses after microbial infection may be essential to avoid host immunopathology and associated autoimmunity. For example, DC may not produce sufficient quantities of cytokines that promote Th17 cell development unless particular infectious stimuli are present. A more reliable mechanism for controlling Th17 cell responses depends on active suppression of Th17 cell differentiation by antigen-presenting DC that may selectively allow antiviral Th1 cell responses.

The ability of antigen-presenting DC to differentially regulate generation of Th cell subsets is not well understood. Although recent reports have indicated that dectin-1-Syk-CARD9 signaling, triggered by detection of fungal β-glucans, in DC promotes Th17 cell responses (Leibundgut-Landmann et al., 2007), whether activated DC inhibit Th17 cell responses has not been established. We reasoned that because a molecular signature of viral infection is early expression of type I interferons (IFN-I), engagement of the IFN-I receptor (IFNAR) on antigen-presenting DC might constitutively pre-empt the ability of these DC to promote Th17 cell responses in the course of viral infections.

Here, we show that robust Th17 cell responses depended on the ability of a newly defined translational intracellular isoform of Osteopontin (Shinohara et al., 2008)—Opn-i—to inhibit DC-dependent secretion of the potent inhibitory cytokine IL-27. IFNAR-dependent inhibition of Opn-i expression resulted in derepression of IL-27 secretion and inhibition of the Th17 cell response in vitro and in vivo. Mice that contain Opn-i-deficient DC developed increased serum IL-27 amounts and a dominant pathogenic Th1 response in the spinal cord compared with the central nervous system (CNS) infiltrates that developed in Opn WT control mice. Definition of an IFNAR-Opn-i axis that regulates the Th17 cell response provides insight into the therapeutic

impact of IFN-I treatment of multiple sclerosis and other Th17 cell diseases (Paty et al., 1993; Yu et al., 1996) as well as a rationale for new approaches to identify IFNAR ligands with increased therapeutic efficacy in MS and related diseases.

RESULTS

IFNAR Engagement Inhibits Opn-i-Dependent Generation of Th17 Cells

Although some forms of microbial infection can efficiently stimulate robust Th17 cell responses, the Th17 cell response after conventional immunization is normally meager compared to the Th1 cell response (Park et al., 2005; Gocke et al., 2007). We investigated whether Th1 cell dominance is imprinted by DC at the outset of a polyclonal T cell response. In the absence of T cell-polarizing reagents, cultures containing resting DC or DC that had been preactivated with LPS contained >10-fold greater numbers of IFN-γ-producing $CD4^+$ T cells compared with IL-17 cell-producing cells (Figure 1A, top). Culture conditions that contained rIL-23, IL-4, and IFN-γ antibodies only modestly increased the Th17 cell response. However, the Th17:Th1 cell ratio increased substantially in cultures containing T cells and DC deficient in IFNAR (Figure 1A, bottom).

IFNAR Expression by DC Suppresses the Th17 Cell Response

Expression of the IL-6 and TGF-β cytokines is necessary for generation of Th17 cells from naive T cell precursors (Veldhoen et al., 2006; Bettelli et al., 2006), whereas IL-23 contributes to expansion of this RORγt-dependent Th cell subset (Park et al., 2005; Harrington et al., 2005; Ivanov et al., 2006) from activated or memory T cells (reviewed in Kastelein et al., 2007). To test the possibility that the above result reflected, in part, an increase in memory Th17 cells in *Ifnar1*$^{-/-}$ mice, we asked whether IFNAR-deficient antigen-presenting DC alone could drive T cells from IFNAR WT donors to differentiate into Th17 cells. To this end, we compared the ability of *Ifnar1*$^{-/-}$ DC or *Ifnar1*$^{+/+}$ DC to present MOG peptide to (*Ifnar1*$^{+/+}$) $CD4^+$ T cells expressing the MOG-specific T cell receptor (2D2) transgene (Figure 1B). Inclusion of *Ifnar1*$^{-/-}$ DC altered the Th1 cell and Th17 cell balance in favor of the latter (Figure 1B). Indeed, the Th17:Th1 cell ratios were increased approximately 3- to 5-fold in cultures containing *Ifnar1*$^{-/-}$ DC, independent of its state of activation. Enhancement of Th17 cell responses could not be attributed to IFNAR-dependent changes in inhibitory Th1 cell and Th2 cell cytokines (IFN-γ, IL-4) or to IL-23, because these cultures included IL-4 and IFN-γ antibodies and exogenous IL-23 (Figure 1B). IFNAR expression by DC was also sufficient to fully inhibit the MOG-specific Th17 cell response in the absence of Th17 cell-polarizing conditions (Figure S1 available online). We also examined Th17 cell expansion in cultures containing *Ifnar1*$^{+/+}$ or *Ifnar1*$^{-/-}$ T cells. Under both Th17 cell-neutral and Th17 cell-polarizing culture conditions, with or without activation of DC by rIFN-α, CpG, and LPS, cultures containing IFNAR-deficient T cells did not display substantially enhanced Th17 cell responses (Figures S2A and S2B).

We then tested the impact of *Ifnar1*$^{-/-}$ DC on activation of $CD4^+$ T cells expressing an OVA-specific TCR transgene, OT-2, in cultures containing IL-6, TGF-β, IL-23, and neutralizing IFN-γ and IL-4 antibodies, i.e., under conditions engineered to fully optimize Th17 cell development from both naive and memory T cell pools. Presentation of the OVA peptide by *Ifnar1*$^{-/-}$ DC provoked substantially enhanced Th17 cell responses compared to cultures containing *Ifnar1*$^{+/+}$ DC (Figure 1C), indicating that supplementation of cultures with exogenous IL-6 and IL-23 or inhibition of IFN-γ and IL-4 does not enhance responses of cultures containing *Ifnar1*$^{+/+}$ DC to the degree seen in cultures containing *Ifnar1*$^{-/-}$ DC, i.e., increased Th17 responses promoted by *Ifnar1*$^{-/-}$ DC do not reflect enhanced production of IL-6 or IL-23 or inhibition of IFN-γ and IL-4.

DC used in the experiment were not activated, so autocrine amounts of IFN-I, which are constitutively expressed by unstimulated DC (Taniguchi and Takaoka, 2001), may be sufficient to inhibit Th17 cell generation. Indeed, in the absence of exogenous rIFN-α, cultures containing *Ifnar1*$^{-/-}$ DC generated enhanced Th17 cell responses even under nonpolarized conditions (Figure 1D). We further examined the impact of increasing concentrations of IFN-α on the Th17 cell response of $CD4^+$ T cells expressing the OT-2 TCR transgene. Increasing concentrations of IFN-α resulted in progressively reduced Th17 cell responses in cultures containing *Ifnar1*$^{+/+}$ DC, whereas addition of this cytokine did not suppress the Th17 cell response in cultures containing *Ifnar1*$^{-/-}$ DC (Figure 1D). These data suggest that constitutive autocrine IFNAR signaling in DC is sufficient to suppress Th17 cell responses. In sum, IFNAR on DC negatively regulates Th17 cell responses upon antigen presentation to $CD4^+$ T cells.

IFNAR Expression by DC Alters the Balance between the Th17 Cell and Th1 Cell Response

We then asked whether the impact of *Ifnar1*$^{-/-}$ DC on the IL-17 response reflected a generalized increase in potency of APC activity. IFNAR deficiency did not alter expression of DC cell-surface molecules involved in T cell activation including MHC class I, class II, CD80, and CD40 (splenic DC, Figure S3A; BM-derived DC, Figure S3B) and did not affect development of plasmacytoid DC (pDC) or conventional DC (cDC) (data not shown). T cell proliferation to antigen-pulsed *Ifnar1*$^{-/-}$ and *Ifnar1* WT DC was not distinguishable under nonpolarized or two different Th17 cell-polarizing conditions (rIL-23 and IL-4 + IFN-γ Ab and rIL-6 + rTGF-β) (Figure S3C). Although strong Th17 cell responses were observed after stimulation of OT-2 T cells by *Ifnar1*$^{-/-}$ DC in cultures containing IL-6 and TGF-β (Figure 2A), T cell stimulation under Th1 cell-polarizing conditions (IL-12 and IL-4 Ab) by *Ifnar1*$^{-/-}$ DC produced substantially weaker Th1 cell responses compared to cultures containing *Ifnar1*$^{+/+}$ DC (Figure 2B). These findings indicate that enhanced induction of Th17 cell generation by *Ifnar1*$^{-/-}$ DC does not reflect a generalized increase in antigen-presenting activity. Instead, *Ifnar1*$^{-/-}$ DC acquire the ability to skew T cells toward the Th17 cell pathway and away from Th1 cell development.

IFNAR-Dependent Regulation of Opn-i Expression in DC and the Th17 Cell Response

Opn-deficient (*Spp1*$^{-/-}$) mice develop milder EAE compared to Opn-sufficient mice, and this has been attributed to expression of Opn-s by activated T cells (Chabas et al., 2001; Jansson et al., 2002; Shinohara et al., 2005, 2006; Hur et al., 2007). We have defined a translational isoform of Opn that is expressed in

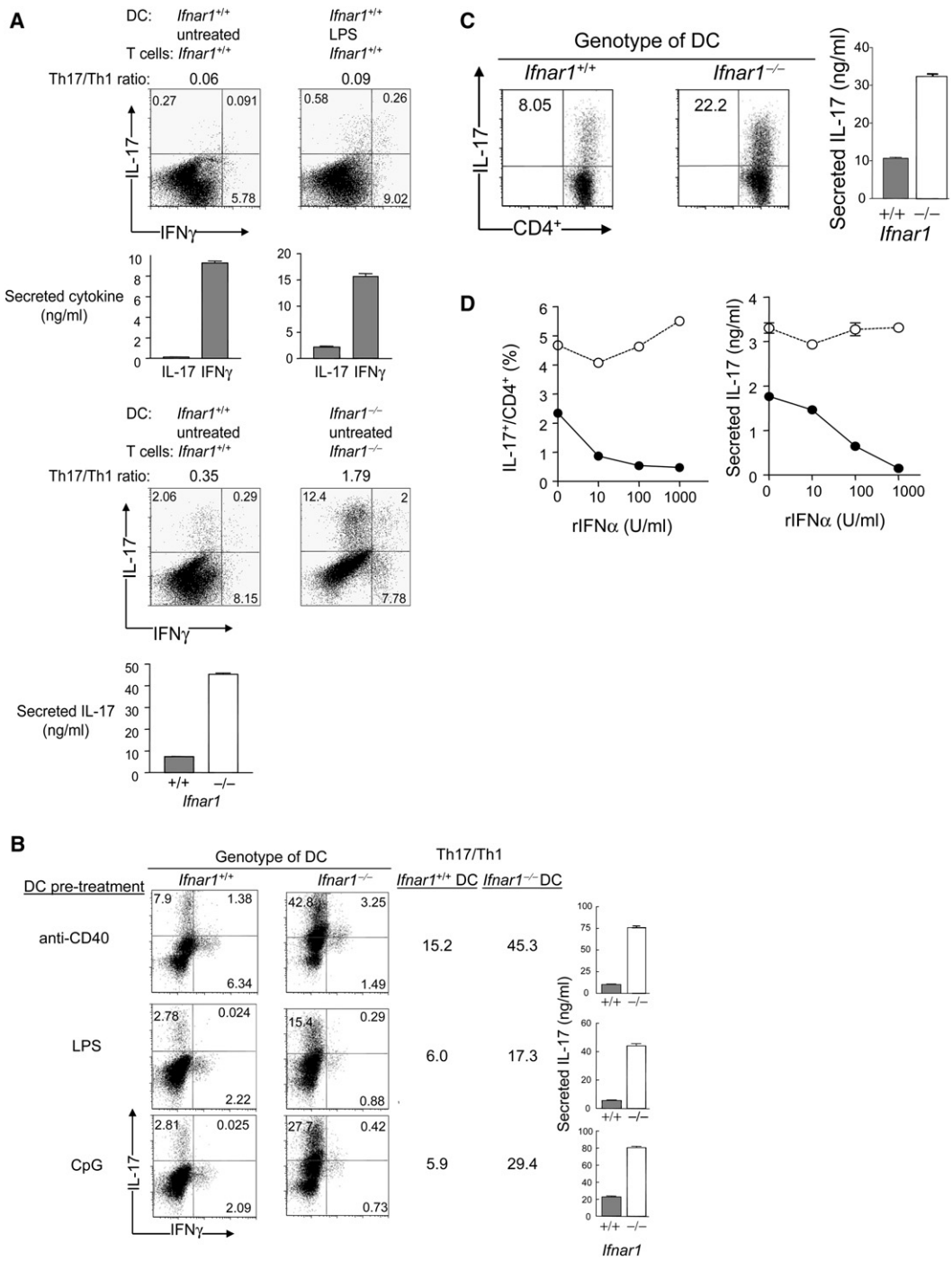

Figure 1. IFNAR Signaling in DC Suppresses Th17 Cell Generation

(A) Polyclonal T cell activation. Top: $CD4^+$ T cells (2×10^5 T cells/well) were incubated with soluble CD3ε Ab (1 μg/ml) and splenic DC for 5 days. $CD4^+$ T cells were then restimulated with CD3ε Ab (1 μg/ml) for 24 hr. Intracellular cytokines were stained with PE-conjugated IL-17 Ab or biotin-conjugated IFN-γ Ab followed by CyChrome-conjugated streptavidin. Events of flow cytometry were gated on $CD4^+$. DC were either unactivated or preactivated for 24 hr with LPS (1 μg/ml). Bottom: Cell cultures were polarized to Th17 cells with rIL-23 and IFN-γ + IL-4 Abs; data are representative of three experiments.

(B) Antigen-specific T cell activation of MOG-specific $CD4^+$ T cells expressing the 2D2 TCR transgene. 2D2 T cells were activated by BM-derived DC (*Ifnar1*$^{+/+}$ versus *Ifnar1*$^{-/-}$) with 1 μg/ml of MOG peptide. Events of flow cytometry were gated on $CD4^+$. Culture conditions were Th17 cell polarized with rIL-23 and with IFN-γ + IL-4 Ab. DC were pretreated with the indicated reagents for 4 hr. Data are representative of four experiments.

(C and D) $CD4^+$ T cells expressing the OT-2 TCR transgene were activated by BM-DC (*Ifnar1*$^{+/+}$ versus *Ifnar1*$^{-/-}$) and 1 μg/ml OT-2 peptide antigen. Cultures were skewed to Th17 cells with rIL-6, rTGF-β, rIL-23, and IFN-γ + IL-4 Abs (C), or not (D) with the indicated amounts of rIFN-α added to DC/T cell coculture. Data are representative of five experiments. Culture supernatants were harvested 24 hr after T cell restimulation with soluble CD3 Ab and the amounts of IL-17 were analyzed by ELISA in triplicate wells; shown as mean ± SEM.

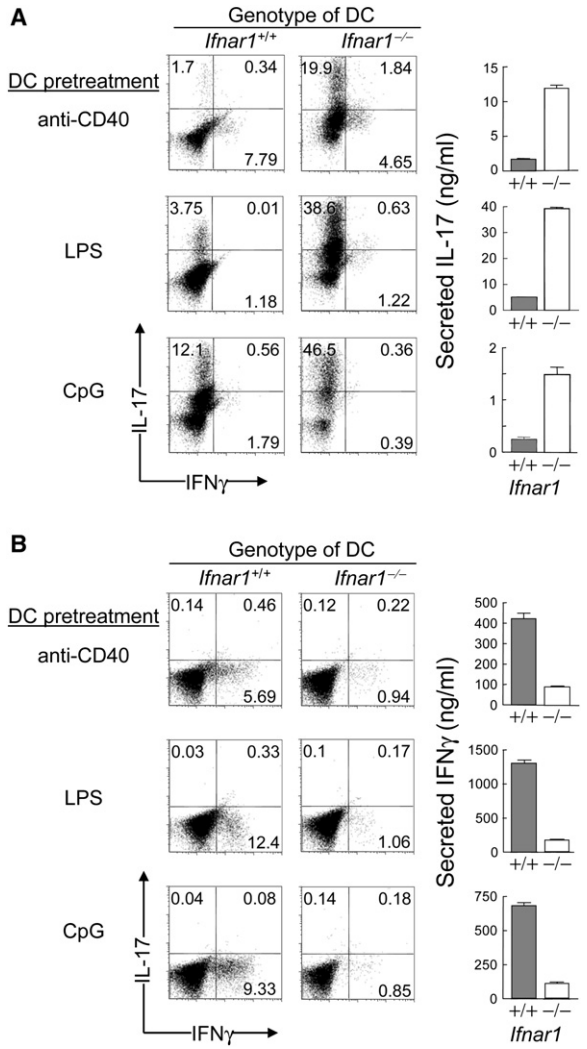

Figure 2. IFNAR Expression by DC Alters the Balance between the Th17 Cell and Th1 Cell Response

2D2 T cells were incubated with BM-derived DC and 1 μg/ml of OT-2 peptide in cultures skewed to Th17 cells (rIL-6 and rTGF-β [A]) or Th1 cells (rIL-12 and IL-4 Ab [B]). Supernatants harvested 24 hr after restimulation with soluble CD3 Ab were analyzed by ELISA in triplicate wells shown as mean ± SEM; representative of 5 and 3 experiments for (A) and (B), respectively.

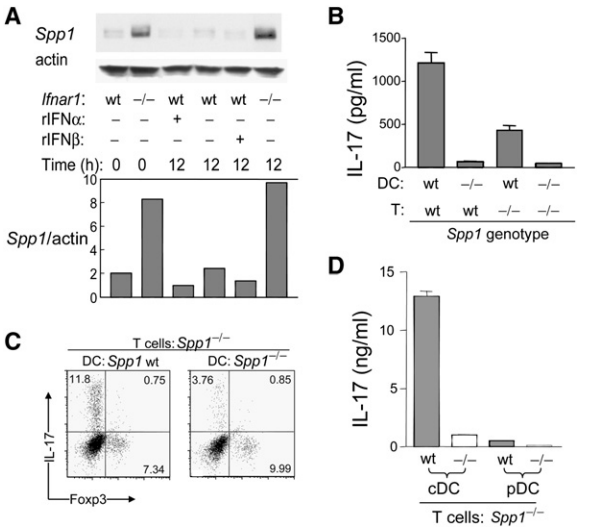

Figure 3. IFNAR Signaling Suppresses Opn-i Expression and Th17 Cell Generation

(A) BM-derived DC cultured with Flt-3L were harvested on day 7 and replated with or without IFN-α or rIFN-β (10 U/ml) as indicated. Opn-i was detected in DC lysates by immunoblot and quantified by densitometry. The bar graph summarizes the amounts of Opn normalized to actin; data are representative of three experiments.

(B–D) $CD4^+$ 2D2 Tg cells were stimulated by DC with 1 μg/ml MOG and rIL-23 and with IFN-γ + IL-4 Abs.

(B) Supernatants of coculture including spleen-derived DC and 2D2 T cells were harvested on day 6 after 24 hr of T cell restimulation with soluble CD3 Ab (1 μg/ml). The amount of IL-17 in supernatants was evaluated by ELISA in triplicate wells. Shown are mean ± SEM and representative of seven experiments.

(C) Opn-deficient ($Spp1^{-/-}$) $CD4^+$ 2D2 Tg T cells were incubated with BM-derived DC and 1 μg/ml MOG peptide. Events of flow cytometry were gated on $CD4^+$. Representative of seven experiments.

(D) Total BM-DC were sorted by FACS into pDC and cDC. IL-17 levels in the restimulated culture supernatants were determined by ELISA in triplicate wells shown as mean ± SEM. Data are representative of four similar experiments.

the cytosol of DC subsets, termed Opn-i (see above), so we asked whether Opn-i expression by DC contributes to the development of Th17 cells. IFNAR expression reduced the expression of Opn-i in DC to 20%–25%, as judged by immunoblotting (Figure 3A). Constitutive expression of IFNAR in the absence of added ligand (recombinant) IFN-α inhibits Opn-i expression, whereas addition of limiting concentrations of IFN (10 U/ml of IFN-α or IFN-β) results in a further increase (up to 2-fold) in suppression. Reduced Opn-i expression in $Ifnar1^{+/+}$ DC was associated with reduced promotion of the Th17 cell response; expression of Opn by cDC, rather than pDC, was critical to Th17 cell generation (Figures 3B–3D). We also noted that Opn-deficient T cells produced less IL-17 than Opn WT T cells, in cultures containing Opn WT DC, opening the possibility that secreted Opn (Opn-s) produced by activated T cells may augment the Th17 cell response, possibly via inhibition of T cell apoptosis, as delineated by Hur et al. (2007) (Figure 3B). Opn-deficient T cells were routinely used in these cocultures to study the isolated Opn-dependent contribution of DC to the response (Figures 3C and 3D). Generation of $Foxp3^+$ regulatory T cells (Treg) was not affected by the amount of Opn expression in DC in the absence of TGF-β (Figures 3C; Figure S4A), suggesting that Opn-i expression in DC regulates Th17 cell generation without affecting Treg production under these conditions. In addition, Opn expression in DC did not alter the ability of T cells to proliferate under IL-23-mediated Th17 cell-polarizing conditions (Figure S4B). These findings indicate that IFNAR-dependent regulation of the Th17 cell response reflects inhibition of Opn-i expression in DC.

If suppression of Th17 cell development by IFNAR signaling is mediated by inhibition of Opn-i expression, reconstitution of Opn-i expression in Opn-deficient DC should restore Th17 cell generation. We transduced Opn-deficient DC with a lentiviral Opn-i vector, lenti-ΔOpn, which restores expression of intracellular but not secreted Opn (Shinohara et al., 2006). Incubation of T cells with lenti-ΔOpn-transduced DC or lenti-GFP-transduced

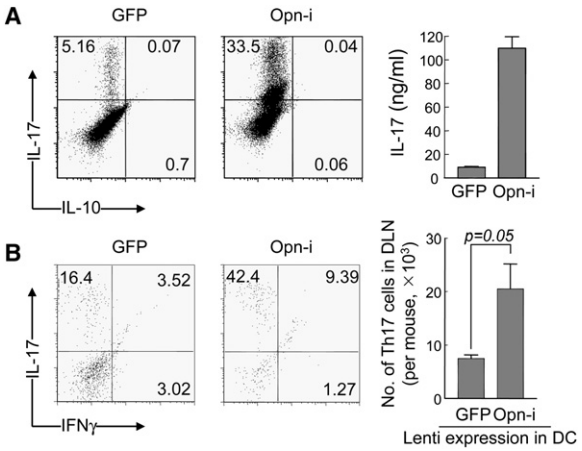

Figure 4. Reconstitution of Opn-i Expression in Opn-Deficient DC Rescues Th17 Cell Generation In Vitro and In Vivo

(A) BM-derived Opn-deficient DC transduced with lentivirus encoding either Opn-i or GFP (negative control) were incubated with Opn-deficient $CD4^+$ 2D2 cells with 1 μg/ml MOG peptide under Th17 cell-polarizing conditions (rIL-23, and IFN-γ + IL-4 Abs). Cells were stained with IL-17 Ab on day 6 and supernatants harvested 24 hr after restimulation with CD3 Ab and analyzed by ELISA; representative of two experiments.

(B) *Rag2*$^{-/-}$ hosts reconstituted i.v. with naive ($CD44^-CD62L^+$) $CD4^+$ 2D2 Tg T cells (1.5 × 10^5/mouse) immunized by s.c. injection of MOG-pulsed BM-DC (2 × 10^6/mouse). DLN cells harvested 7 days later were treated immediately with PMA and ionomycin for 4 hr followed by 2 hr of BFA treatment before cell staining for FACS analysis. Dot plot shows IL-17 and IFN-γ immunofluorescence of DLN cells gated on $CD4^+$. Numbers of DLN Th17 cells per mouse (n = 3) calculated from total numbers of DLN cells and proportions of $CD4^+$IL-17^+ double-positive cells. Error bars represent mean ± SD.

DC resulted in a 7-fold increase in the Th17 cell response and a 10-fold increase in IL-17 concentrations in culture supernatants. However, IL-10 was not detectable in either culture supernatant, and <1% of $CD4^+$ T cells expressed intracellular IL-10 (Figure 4A; Figure S5). These data suggested that IFNAR-dependent down-regulation of Th17 cell development reflects inhibition of Opn-i expression in DC.

In Vivo Th17 Cell Polarization Is Enhanced by Opn-i Expression in Antigen-Presenting DC

To test this pathway in vivo, we evaluated the ability of Opn-deficient and Opn-i-reconstituted DC to induce Th17 cell responses to the MOG peptide. Opn-deficient DC transduced with lenti-Opn-i were pulsed with MOG peptide before injection into *Rag2*$^{-/-}$ mice along with naive 2D2 T cells (sorted as $CD4^+CD62L^{hi}CD44^{lo}$). One week later, the Th17 cell response was defined immediately after harvesting cells from draining LN after a 4 hr pulse with PMA and ionomycin before FACS analysis. In the absence of pertussis toxin, no sign of EAE onset was observed in these mice. Immunization of mice with Opn-i-transduced DC provoked a ~2- to 3-fold increase in the Th17 cell response compared with GFP-transduced Opn$^{-/-}$ DC (Figure 4B).

Inhibition of Th17 Cell Differentiation by an IFNAR-Opn-i Pathway Is Mediated by IL-27

IFNAR-dependent regulation of the Th17 cell response did not reflect the impact of this receptor on cytokines that might require the Th17 cell response, including IL-6, TGF-β, IL-23, and IFN-γ (Figures 1A–1C, 2A, 2B, 3B–3D, and 4A). Because expression and secretion of IL-27 by DC can exert potent suppressive effects on Th17 cell generation (Batten et al., 2006; Stumhofer et al., 2006), we asked whether IFNAR-mediated inhibitory pathway depicted in Figure S6A resulted in increased IL-27 expression. Activation of *Ifnar1*$^{+/+}$ but not *Ifnar1*$^{-/-}$ DC by LPS resulted in progressively increasing concentrations of IL-27 (Figure 5A). Addition of limiting concentrations of IFN-α to Opn-deficient DC did not further enhance IL-27 responses. In contrast, addition of the same concentrations of IFN-α to Opn WT DC resulted in a dose-dependent induction in IL-27 secretion (Figure 5B, right), suggesting that Opn-i inhibition is a key element in enhanced IL-27 expression under these conditions. High concentration of DC without stimulation showed that IL-27 is induced by IFNAR expression and absence of Opn (Figure 5C, left and middle). Lentiviral expression of Opn-i confirmed that Opn-i promotes secretion of IL-27 in unstimulated DC (Figure 5C, right). Induction of IL-27*p28* mRNA expression was also confirmed by quantitative PCR in IFNAR-deficient and Opn-sufficient DC (Figure 5D).

To directly test the hypothesis that the IFNAR-Opn-i axis resulting in enhanced expression of IL-27 was responsible for suppression of Th17 cell responses, we measured the effects of neutralizing IL-27 antibody in these cultures. Addition of IL-27 neutralizing antibody to cultures containing Opn-deficient unstimulated DC, under two different Th17 cell-polarizing culture conditions, successfully restored the Th17 cell response (Figure 5E), i.e., reversed suppression of the Th17 cell response in cultures containing Opn-deficient DC. In contrast, treatment of cultures containing Opn WT DC with IL-27 neutralizing antibody had no effect on the Th17 cell response in these cultures (Figure S6B). Moreover, addition of IL-27 antibody to cultures containing IFN-α also restored the Th17 cell response (Figure 5F). These findings indicated that IFNAR-dependent suppression of Th17 cell responses is mediated by enhanced secretion of the inhibitory IL-27 cytokine.

Delayed EAE Onset in Opn-Deficient Hosts Associated with Increased IL-27 Expression and Altered Ratio of Th1 and Th17 Autoreactive Cells

These analyses suggested that promotion of Th17 cell responses by DC requires expression of Opn-i in these APC. Early experiments of Langrish et al. (2005) showed that IL-17 is essential to the development of severe EAE. We therefore investigated whether presentation of autoantigen by Opn-deficient APC might impair development of EAE. Opn-deficient *Rag2*$^{-/-}$ hosts reconstituted with naive ($CD4^+CD62L^{hi}CD44^{lo}$) MOG-specific 2D2 T cells displayed a substantially delayed onset of EAE compared to Opn WT *Rag2*$^{-/-}$ hosts (Figure 6A) characterized by delayed lymphocyte and macrophage infiltration into spinal cord (Figure 6B). We also analyzed mice on days 12 (Opn WT) and 17 (Opn-deficient) in a separate experiment from Figure 6A when Opn WT and Opn-deficient hosts displayed a clinical score of 3–3.5 for analysis of serum IL-27. Serum IL-27 concentrations of Opn WT hosts were 5–10 pg at day 5 and 17, whereas serum IL-27 amounts in Opn-deficient hosts were 40 pg/ml at day 5 and 80 pg/ml at day 17 (Figure 6C). Splenic DC from Opn-deficient *Rag2*$^{-/-}$ hosts also expressed higher amounts of IL-27p28

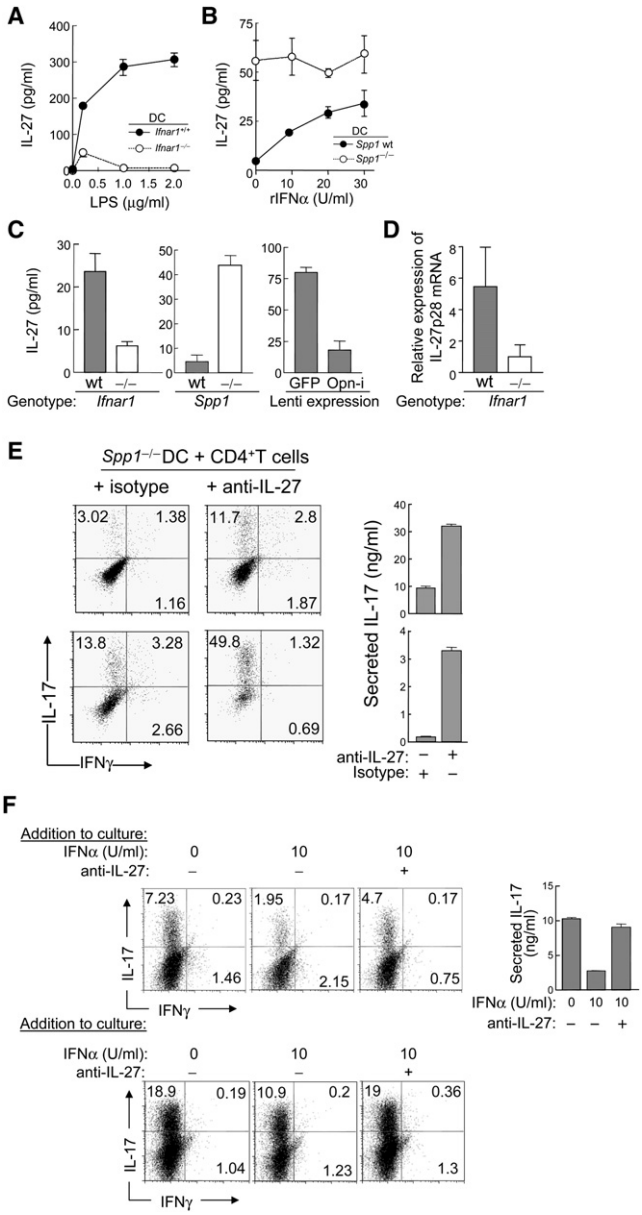

Figure 5. Suppression of IL-27 Expression in DC Contributes to Opn-i-Mediated Th17 Cell Differentiation

(A) BM-derived DC (*Ifnar1*$^{+/+}$ [closed circle] versus *Ifnar1*$^{-/-}$ [open circle]) were incubated (2 × 10^6 DC/ml) with the indicated concentration of LPS for 30 hr. Supernatants were evaluated for IL-27p28 amounts by ELISA in triplicate well; representative of three experiments.

(B) BM-derived DC (*Spp1* WT [closed circle] versus *Spp1*$^{-/-}$ [open circle]) were incubated with rIFN-α (5 × 10^6 DC/ml) for 42 hr. IL-27p28 amounts were determined. Representative of two experiments.

(C) Resting BM-derived DC were incubated for 48 hr. IL-27p28 amounts were determined by ELISA in triplicate wells. Left: Comparison of IL-27 secretion by *Ifnar1*$^{+/+}$ and *Ifnar1*$^{-/-}$ DC (10^7 DC/ml) in 44 hr culture supernatants. Middle: Comparison of IL-27 secretion by Opn WT and Opn-deficient (*Spp1*$^{-/-}$) DC (2 × 10^6 DC/ml) in 44 hr culture supernatants. Right: Comparison of IL-27 secretion by Opn-deficient DC transduced with lenti-GFP (control) or lenti-ΔOpn-i (2 × 10^6 DC/ml) in 24 hr culture supernatants; data representative of two experiments.

(D) Comparison of the amounts of IL-27*p28* mRNA in resting BM-derived DC from *Ifnar1*$^{+/+}$ or *Ifnar1*$^{-/-}$ mice Real-time PCR of total cDNA and results obtained from cycling threshold (−ΔΔ*Ct*) values normalized to β-actin.

(E and F) Two different Th17 cell skewing conditions were tested. Top: rIL-23 and IFN-γ + IL-4 Abs; bottom: rIL-6 and rTGF-β.

(E) 2D2 T cells were incubated with Opn-deficient BM-DC, 1 μg/ml MOG peptide, and 20 μg/ml IL-27 Ab or isotype IgG. 24 hr after T cell restimulation with soluble CD3 Ab on day 5, expression of IL-17 and IFN-γ was evaluated by flow cytometry (events gated on CD4$^+$). The amount of IL-17 in culture supernatants was measured by ELISA in triplicated wells (mean ± SEM). Representative of four experiments.

(F) DC and OT-2 T cell cocultures under the same skewing conditions used in (E) were treated with rIFN-α with or without IL-27 Ab (20 μg/ml) and stimulated with OVA peptide for 5 days, followed by 24 hr T cell restimulation with soluble CD3 Ab. Expression of IL-17 and IFN-γ was evaluated by flow cytometry (events gated on CD4$^+$). The amount of IL-17 in culture supernatants was analyzed by ELISA in triplicate wells is shown (mean ± SEM); data are representative of two experiments.

than DC from Opn WT *Rag2*$^{-/-}$ hosts (Figure S7), and brains of Opn-deficient hosts expressed 10-fold higher IL-27*p28* mRNA amounts than Opn WT hosts (data not shown).

Increased amount of serum IL-27 in Opn-deficient hosts beginning at day 5 (Figure 6C) was associated with substantially reduced Th17 cell responses in draining LN—2.54% (Opn-deficient) versus 17.6% (Opn WT) Th17 cells (Figure 6D). Analysis of the spinal cord and brain later in the course of EAE in Opn-deficient hosts revealed a 2-fold increase in the proportions of Th1 cells (~20% to 48%) and a doubling of CD4$^+$ T cells (approximately 9 × 10^5 to 2.1 × 10^5), representing a 4-fold enrichment of the CNS Th1 cell response in Opn-deficient hosts (Figure 6E). This is consistent with findings that IL-27 can enhance Th1 cell responses in other settings (Pflanz et al., 2002; Cao et al., 2008) and suggests that suppression of the Th17 cell response in Opn-deficient *Rag2*$^{-/-}$ hosts is apparent before onset of disease.

Opn Expression in Microglia Is Essential for Th17 Cell-Mediated Progression of EAE

Because restimulation of T cells by APC in CNS is necessary for full development of EAE (Tompkins et al., 2002; Kawakami et al., 2004), we asked whether microglia also required expression of Opn-i to induce Th17 cell responses to MOG peptide (Figure 7A). Lentiviral-mediated reconstitution of Opn-deficient microglia with Opn-i resulted in substantially increased production of IL-17 and decreased production of IL-27p28 (Figure 7B).

To test the contribution of Opn expression by CNS-resident microglia to EAE, we induced EAE in irradiation BM chimeras, in which host (radiation-resistant) microglia were Opn WT or Opn deficient whereas peripheral hematopoietic APC were derived from donor (*Spp1*$^{-/-}$) BM cells (Greter et al., 2005; Heppner et al., 2005). Opn deficiency in the CNS compartment dramatically reduced the intensity of EAE in radiation chimeras in which the hematopoietic compartment of Opn-deficient or Opn WT hosts was reconstituted with 1.7 × 10^7 Opn-deficient BM cells (Figure 7C), suggesting that expression of Opn in microglia is necessary for efficient development of EAE. These findings may also be relevant to MS pathogenesis, because overexpression of Opn in MS plaques is a notable feature of active disease (Steinman and Zamvil, 2003).

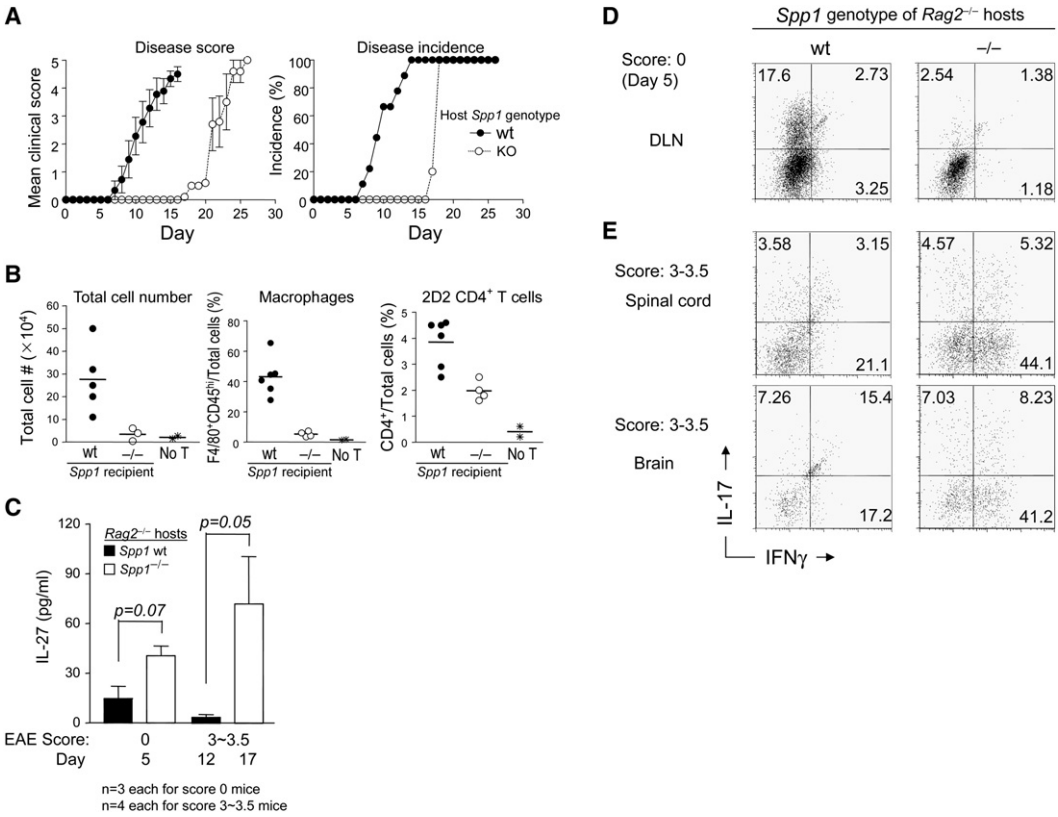

Figure 6. Opn Deficiency in Non-T Cells Delays EAE Onset

(A) EAE development in *Rag2*$^{-/-}$ hosts. Naive CD4$^+$ 2D2 Tg Opn-deficient T cells (1.4 × 10^5 cells/mouse) were adoptively transferred into Opn WT or Opn-deficient *Rag2*$^{-/-}$ hosts. Disease development and incidence are shown for each group. Opn WT, closed circle, n = 9; *Spp1*$^{-/-}$, open circle, n = 8; error bars denote mean ± SEM. Data are representative of three experiments.

(B–E) Analysis of mice at different time points in separate experiments.

(B) CD4$^+$ 2D2 T cells from B6 2D2 Tg mice were adoptively transferred to either Opn WT or Opn-deficient B6 *Rag2*$^{-/-}$ mice, as described in (A). Twenty days later, the total number of spinal-cord-infiltrated cells, spinal-cord-infiltrated macrophages (%) (F4/80$^+$CD45^{hi}), and total CD4$^+$ T cells were enumerated. Increased lymphocyte infiltration was observed in Opn WT hosts (closed circle) compared with Opn-deficient (open circle) hosts. Negative control (asterisk) mice are *Rag2*$^{-/-}$ mice without T cell transfer. Average disease scores of the Opn WT and Opn-deficient (*Spp1*$^{-/-}$) hosts used for these experiments (at day 20) were 3.8 and 1.2 for Opn WT and Opn-deficient hosts, respectively. Horizontal lines denote mean values.

(C) Serum IL-27 analysis. *Rag2*$^{-/-}$ hosts were analyzed when EAE scores were either 0 (day 5) or 3–3.5 (day 12 and day 17 for Opn WT and Opn-deficient hosts, respectively). Error bars represent mean ± SD from values of 3 mice (score 0) or 4 mice (score 3–3.5) each of Opn WT and Opn-deficient *Rag2*$^{-/-}$ hosts.

(D) Th17 and Th1 cell development. Cells from DLN were harvested from mice on day 5 (score = 0), treated immediately with PMA and ionomycin followed by BFA treatment, and subject to FACS analysis; dot plots were gated on CD4$^+$ cells.

(E) Th cell subsets in CNS. Cells from spinal cord or brain were harvested from score 3–3.5 mice on day 12 and 17 from Opn WT and Opn-deficient *Rag2*$^{-/-}$ hosts, respectively, treated immediately as described in (D). Panels represent results of 3 mice (score 0) or 4 mice (score 3–3.5) each from Opn WT and Opn-deficient *Rag2*$^{-/-}$ hosts.

DISCUSSION

It has taken two to three decades after the original description of Th1 and Th2 cells for Th17 cells to be identified as a new lineage of effector CD4$^+$ T cells. This delayed recognition reflects, in part, the relative paucity of Th17 cells after conventional immunization with antigen. In the absence of a cytokine milieu required to induce and expand Th17 cell development, stimulation of T cells from antigen-immunized mice often generates less than 1% Th17 cells, compared to much higher proportions of Th1 cells (Park et al., 2005; Gocke et al., 2007). Because generation of murine Th17 cells from naive cells requires IL-6, TGF-β (Veldhoen et al., 2006; Bettelli et al., 2006), and IL-23 for Th17 cell expansion (Park et al., 2005; Harrington et al., 2005), a relative insufficiency of these cytokines may dampen the Th17 cell response. However, a more efficient mechanism responsible for pre-emption of Th17 cell responses probably depends on the IFNAR-dependent inhibitory pathway described in this report.

The negative impact of IFNAR engagement on Th17 cell responses was mediated through inhibition of Opn-i expression, which in turn enhanced IL-27-dependent inhibition of Th17 cell development. We have not ruled out the possibility that IFNAR signaling in T cells may also inhibit expansion or survival of Th17 cells. Although extremely low amounts of IFN-I were sufficient to effectively initiate this IFNAR-dependent pathway in DC and attenuate Th17 cell development, increasing amounts of IFN-I, e.g., after certain viral infections, may be necessary to fully inhibit Th17 cell responses. According to this view, IFN-I

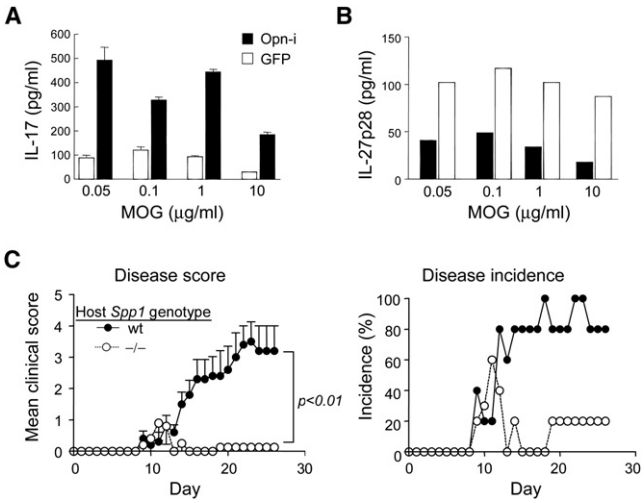

Figure 7. Microglia Require Opn-i Expression to Induce Th17 Cells

Opn-deficient microglia were infected with lenti-Opn-i or lenti-GFP and incubated $2D2^+CD4^-$ T cells and MOG peptide.

(A and B) Reconstitution of Opn-deficient microglia with Opn-i rescues IL-17 production by $CD4^+$ 2D2 T cells.

(A) IL-17 concentrations in culture supernatants 24 hr after T cell restimulation on day 4 were determined by ELISA in triplicate wells.

(B) Concentrations of IL-27p28 in day 4 cultures before T cell restimulation. The amounts of IL-27p28 were determined by ELISA in triplicate wells; empty and filled bars denote supernatants from Opn-i-transfected and GFP-transfected DC, respectively, and data are representative of two experiments.

(C) EAE development is inhibited in irradiated Opn-deficient hosts. Total BM cells (1.7×10^7 cells/mouse) from $2D2^+$ Tg Opn-deficient mice were i.v. transferred to irradiated (800 rads) hosts. Ten weeks later, EAE was induced. Disease development (mean clinical score) and incidence (%) is shown for each group (n = 5). Error bars in disease score denote mean ± SEM and t test values on the last day of observation are shown. Mice displayed >95% engraftment of donor bone marrow according to immunofluorescence with Ly5.2 Ab (donor BM cells were obtained from $B6.Ly5.2^+$ Opn-deficient mice expressing the 2D2 TCR transgene) 10 weeks after reconstitution and before disease induction, as judged by immunofluorescence of peripheral blood cells.

constitutively produced at low amounts in the absence of viral infection may chronically dampen Th17 cell responses, whereas increased IFNAR signaling secondary to rapid increases in IFN-I amounts after viral infections may be necessary for increased IL-27-dependent inhibition of Th17 cell responses. In contrast, certain bacterial or fungal infections that disrupt this inhibitory pathway may provoke robust Th17 cell responses and increase the risk of autoimmune disease. It may be relevant that Th17 cell generation is also suppressed by expression of the IFN-γ receptor on APC (Batten et al., 2006), and the potential similarities between IFN-α- and IFN-γ-dependent suppression of the Th17 cell response via interactions with DC deserve further investigation.

IL-27, a potent inhibitor of Th17 cell development, can also induce IL-10-producing T cells (Awasthi et al., 2007; Fitzgerald et al., 2007). Our DC-T cell cocultures do not produce sufficiently high IL-27 concentrations to enhance IL-10-producing cells, suggesting that inhibition of Th17 cell responses that we observe does not reflect enhanced production of IL-10. Huber et al. (2008) have shown that IL-27, in addition to being a potent inhibitor of Th17 cell development (Stumhofer et al., 2006), also inhibits TGF-β-mediated expansion of Treg cells. Low concentrations of IL-27 sufficient to suppress Th17 cell responses in cultures containing Opn-deficient DC did not substantially alter TGF-β-dependent generation of $Foxp3^+$ Treg cells in this study, a finding that emphasizes the striking susceptibility of the Th17 cell response to low concentrations of IL-27 and also indicates that Treg cell induction is not required to inhibit the Th17 cell response in our experiments.

Previous studies have indicated that Opn-i expression in pDC promotes expression of IFN-α and contributes to early protective responses against HSV-1 infection and tumor growth (Shinohara et al., 2006). Here, we show that Opn-i expression by conventional DC (cDC) (and microglia) contributes to Th17 cell commitment in vitro and in vivo in the setting of EAE. Opn expression is induced after TLR9 engagement of pDC but not cDC (Shinohara et al., 2006), so Th17 cell generation may be negatively regulated by pDC-dependent production of IFN-α through engagement of IFNAR expressed by cDC. Additional experiments are necessary to evaluate the contribution of an inhibitory interaction between DC subsets: IFN-I-producing pDC (which do not themselves promote detectable Th17 cell responses) and IFNAR-producing cDC in regulating the Th17 cell response.

Opn (*Spp1*) gene expression in T cells is essential for efficient Th1 cell development (Ashkar et al., 2000; O'Regan et al., 2000; Chabas et al., 2001; Shinohara et al., 2005; Sato et al., 2005; Renkl et al., 2005), whereas expression in cDC promotes Th17 cell differentiation. The contribution of Opn to the development of these Th cell lineages reflects cell-type-specific expression of the two Opn isoforms. Secretion of Opn (Opn-s) by T cells leads to an interaction with its receptors on macrophages that upregulates IL-12 production and enhances Th1 cell development. In addition, Opn-deficient T cells produced less IL-17 than did Opn WT T cells in cultures containing Opn WT DC, suggesting that secreted Opn (Opn-s) may also augment the Th17 cell response under some circumstances, possibly through a direct interaction that inhibits apoptosis (Hur et al., 2007). In addition to inhibition of IL-27, Opn-i may also promote Th17 cell responses through enhancement of DC-T cell interactions. Opn-i-dependent polarization of the actin cytoskeleton may enhance formation of immunological synapses necessary for efficient T cell activation and helper lineage commitment (Al Alwan et al., 2003; Shapiro et al., 2003; Benvenuti et al., 2004a, 2004b; Maldonado et al., 2004; West et al., 2004).

We tested two different models of EAE: (1) naive 2D2 $CD4^+$ T cell transferred into $Rag2^{-/-}$ hosts and (2) irradiation BM chimera reconstituted with 2D2 BM cells (in both models, transferred cells were Opn deficient to rule out excess Opn-s). EAE development was robust in the former system, characterized by efficient T cell activation and infiltration into the CNS (data not shown), allowing us to examine full-blown EAE in Opn-deficient mice. This model also allowed analysis of the role of Opn in APC and revealed that Opn-deficient APC promoted a dominant Th1 cell type of EAE. Enhanced Th1 cell responses in the CNS of Opn-deficient $Rag2^{-/-}$ hosts were associated with increased IL-27 secretion by DC and/or microglia, which may have resulted in enhanced IFN-γ responses (Pflanz et al., 2002; Cao et al., 2008). Analyses of Th17 and Th1 cell components of CNS cellular infiltrates at different stages of EAE induced by different autoantigens may define clinically distinct

subsets of this murine model of MS and provide insight into clinical subtypes of MS.

Definition of the IFNAR signaling pathway in DC leading to suppression of Th17 cell responses described in this report provides insight into the essential elements that govern the Th17 cell response. In addition, these findings are relevant to the use of IFN-β as the leading treatment for patients suffering from MS (Paty et al., 1993; Hafler et al., 2005). The ability of IFN-β, an innate immune cytokine with antiviral properties, to inhibit autoimmune inflammation and reduce the incidence of disease relapse has been poorly understood. Suppression of the development of proinflammatory Th17 cells provides a mechanistic explanation for the therapeutic effects of type I interferon therapy in MS. These findings also suggest that analysis of the effects of IFNAR engagement by antibodies or mutant IFNs that differentially engage the Opn-IL-27 pathway described here may yield new and effective therapies for MS.

EXPERIMENTAL PROCEDURES

Animals and Reagents

Opn-deficient (*Spp1*$^{-/-}$) mice (Rittling et al., 1998) were backcrossed to C57BL/6 (B6) for 15 generations; B6 2D2 MOG-specific T cell receptor transgenic mice (Bettelli et al., 2003) were a gift from V.K. Kuchroo (Brigham and Women's Hospital, Boston, MA). B6 OT-2 TCR transgenic mice and B6 Ly5.2 mice (Jackson Labs, Bar Harbor, ME) and 129/SvEv IFNAR1-deficient mice (B&K Universal, Hull, England) were used. Antibodies to IL-17 (TC11-18H10) and IFN-γ (XMG1.2) for flow cytometry were from BD PharMingen; Foxp3 (FJK-16 s) and IL-10 (JES5-16E3) Abs were from eBioscience; IL-27p28 Ab (BAF1834), recombinant proteins for mouse IL-23, IL-6, Flt-3L, and human TGF-β1 were from R&D systems; and recombinant mouse IFN-α was from HyCult. Lentiviral constructs of *Opn* without the signal peptide (ΔOpn) were as described (Shinohara et al., 2006). MOG_{35-55} peptide (MEVG WYRSPFSRVVHLYRNGK) and $OVA_{329-339}$ peptide (ISQAVHAAHAEINEAGR) were from New England Peptides. All work involving vertebrate animals was reviewed and approved by the DFCI IACUC and conducted in accordance with AAALAC guidelines.

EAE Induction

Induction was performed as described (Shinohara et al., 2005) with MOG peptide in CFA s.c. on day 0 and pertussis toxin i.v. on days 0 and 2. Mice were assessed daily for clinical signs of disease in a blinded fashion and graded as described previously. Mean clinical scores and mean maximal scores were recorded daily and calculated by adding scores of individual mice and dividing by number of mice in each group, including mice with no sign of disease.

Preparation of CD4$^+$ T Cells, DC, Microglia, and Spinal Cord Lymphocytes

Performed as described previously (Shinohara et al., 2006). Naive T cells were FACS sorted as CD4$^+$CD62L^{hi}CD44^{lo} and splenic DC were derived after collagenase treatment and positive selection with CD11c MACS beads (Miltenyi). BM-derived DC were prepared according to an in vitro culture method (Gilliet and Liu, 2002) with modifications (Shinohara et al., 2006), whereas further purification of pDC and cDC is described in Shinohara et al. (2006). Primary microglia were prepared as previously described (Dalpke et al., 2002), resulting in >98% Mac-1$^+$F4/80$^+$ cells. To prepare lymphocytes from spinal cord, single cells were prepared by Percoll spin after collagenase treatment of spinal cord.

APC-T Cell Coculture

1 × 10^5 DC/well and 2 × 10^5 CD4$^+$ T cells/well were incubated in 200 μl/well of complete RPMI media with antigen or soluble CD3ε Ab (BD PharMingen). For microglia-T cell cocultures, 2.5 × 10^5/well each of microglia and 2D2 T cells were incubated in 500 μl/well with RPMI media. Th17 cell polarization entailed rIL-23 (100 ng/ml) and IFN-γ + IL-4 Ab (10 μg/ml each) or rIL-6 (20 ng/ml) and rTGF-β (3 ng/ml) at culture initiation. Culture supernatants in Figure 1C included rIL-23 (10 ng/ml), rIL-6 (20 ng/ml), rTGF-β (1 ng/ml), and IFN-γ + IL-4 antibodies (10 μg/ml each). In some experiments, IL-27 was neutralized with IL-27 Ab (20 μg/ml). For Th1 cell polarization, rIL-12 (5 ng/ml) and IL-4 Ab (10 μg/ml) were added during set up of cocultures.

Irradiation BM Chimeric Mice

B6 Opn WT and deficient mice were irradiated (800 rads) 24 hr before i.v. transfer of total BM cells from Opn-deficient 2D2 mice (1.7 × 10^7 cells/mouse). Ten weeks later, EAE was induced as indicated above.

In Vivo Development of Th17 Cells

B6 *Rag2*$^{-/-}$ mice were transferred i.v. with Opn-deficient naive 2D2 CD4$^+$ T cells (1.5 × 10^5/mouse; CD4$^+$CD62L^{hi}CD44^{lo}). BM-derived DC (infected by lentivirus, below) were pulsed with 20 μg/ml of MOG for 3 hr at 37°C and then extensively washed. Mice were immunized with 2 × 10^6/mouse of MOG-pulsed DC by s.c. injection. Draining LN cells were excised 7 days later and treated with PMA and ionomycin (4 hr) and brefeldin A (in the last 2 hr) before FACS analysis.

Lentiviral Opn-i Transfection

Opn-i and GFP constructs were lentivirally transfected into DC or microglia as described (Shinohara et al., 2006).

ELISA and Intracellular Cytokine Flow Cytometry

After antigen-specific stimulation with DC for 5–6 days, T cells were restimulated for 24 hr with soluble CD3ε Ab (145-2C11, BD PharMingen) before culture supernatants were analyzed for IL-17 and IL-27p28 concentrations by ELISA (R&D Systems). Intracellular cytokine and Foxp3 staining was performed on cells treated with ionomycin (500 ng/ml) and PMA (50 ng/ml) for 4 hr and brefeldin A (10 μg/ml) for the last 2 hr. Cells were stained for cell-surface markers, fixed and permeabilized with Fix/Perm solution (eBioscience), followed by FcBlock (2.4G2, BD PharMingen) and intracellular staining. Gating of intracellular cytokine staining was determined by immunofluorescence with an isotype-matched control Ig (Ab control) and, in some experiments, immunofluorescence of an (IL-17$^-$) CD8$^+$ T cell line with IL-17 Ab (cell control) was used as well.

Immunoblotting of DC Lysates

BM-derived DC were prepared as described above. On day 7 of culture, DC were harvested and cell lysates were either immediately prepared (0 hr samples) or prepared 12 hr after replating cells in RPMI complete medium with or without recombinant IFN-α or IFN-β (12 hr samples). 10 μg/lane of DC lysate was applied to an SDS-PAGE gel and proteins were blotted onto a PDVF membrane before immunodetection was performed with Opn Ab (O-17, IBL, America).

RNA-cDNA Preparation and Real-Time PCR

After washing DC with PBS, total RNA was extracted from cells with an RNeasy kit (QIAGEN). cDNA synthesis was initiated by priming total RNA with oligo (dT) before RNA was reverse-transcribed with MMLV RT (Ambion). The resultant cDNA was used for real-time PCR analyses with an ABI 7700 (Applied Biosystems). QuantiTect SYBR Green PCR (QIAGEN) was used to detect IL-27*p28*, and β-actin was used as an internal control. (IL-27*p28* forward: 5′-CTC TGC TTC CTC GCT ACC AC-3′, reverse; 5′-GGG GCA GCT TCT TTT CTT CT-3′; β-actin primers forward: 5′-TGT TAC CAA CTG GGA CGA CA-3′, reverse; 5′-CTG GGT CAT CTT TTC ACG GT-3′). Error bars indicate the maximum and minimum values calculated from SD and -*ΔΔCt* values from triplicate PCR reactions, according to Applied Biosystems protocols.

SUPPLEMENTAL DATA

Supplemental Data include seven figures and can be found with this article online at http://www.immunity.com/cgi/content/full/29/1/68/DC1/.

ACKNOWLEDGMENTS

The authors wish to thank S.J. Turley and K.W. Wucherpfennig for critical reading of the manuscript, D. Laznik for technical assistance, and A. Angel for assistance with the manuscript and figures. This work was supported in part by research grants from the National Institutes of Health (AI 12184, AI 48125) to H.C. and an NRSA Fellowship (T32 CA70083) to M.L.S.

Received: November 16, 2007
Revised: March 7, 2008
Accepted: May 1, 2008
Published online: July 10, 2008

REFERENCES

Al Alwan, M.M., Liwski, R.S., Haeryfar, S.M., Baldridge, W.H., Hoskin, D.W., Rowden, G., and West, K.A. (2003). Cutting edge: dendritic cell actin cytoskeletal polarization during immunological synapse formation is highly antigen-dependent. J. Immunol. *171*, 4479–4483.

Ashkar, S., Weber, G.F., Panoutsakopoulou, V., Sanchirico, M.E., Zawaideh, S., Rittling, S.R., Denhardt, D.T., Glimcher, M.G., and Cantor, H. (2000). Eta-1 (osteopontin): an early component of Type 1(cell-mediated) immunity. Science *287*, 860–864.

Awasthi, A., Carrier, Y., Peron, J.P., Bettelli, E., Kamanaka, M., Flavell, R.A., Kuchroo, V.K., Oukka, M., and Weiner, H.L. (2007). A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. Nat. Immunol. *8*, 1380–1389.

Batten, M., Li, J., Yi, S., Kljavin, N.M., Danilenko, D.M., Lucas, S., Lee, J., de Sauvage, F.J., and Ghilardi, N. (2006). Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. Nat. Immunol. *7*, 929–936.

Benvenuti, F., Hugues, S., Walmsley, M., Ruf, S., Fetler, L., Popoff, M., Tybulewicz, V.L., and Amigorena, S. (2004a). Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming. Science *305*, 1150–1153.

Benvenuti, F., Lagaudriere-Gesbert, C., Grandjean, I., Jancic, C., Hivroz, C., Trautmann, A., Lantz, O., and Amigorena, S. (2004b). Dendritic cell maturation controls adhesion, synapse formation, and the duration of the interactions with naive T lymphocytes. J. Immunol. *172*, 292–301.

Bettelli, E., Pagany, M., Weiner, H.L., Linington, C., Sobel, R.A., and Kuchroo, V.K. (2003). Myelin oligodendrocyte glycoprotein-specific T cell receptor transgenic mice develop spontaneous autoimmune optic neuritis. J. Exp. Med. *197*, 1073–1081.

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., and Kuchroo, V.K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature *441*, 235–238.

Burchill, M.A., Nardelli, D.T., England, D.M., DeCoster, D.J., Christopherson, J.A., Callister, S.M., and Schell, R.F. (2003). Inhibition of interleukin-17 prevents the development of arthritis in vaccinated mice challenged with *Borrelia burgdorferi*. Infect. Immun. *71*, 3437–3442.

Cao, Y., Doodes, P.D., Glant, T.T., and Finnegan, A. (2008). IL-27 induces a Th1 immune response and susceptibility to experimental arthritis. J. Immunol. *180*, 922–930.

Chabas, D., Baranzini, S.E., Mitchell, D., Bernard, C.C., Rittling, S.R., Denhardt, D.T., Sobel, R.A., Lock, C., Karpuj, M., Pedotti, R., et al. (2001). The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science *294*, 1731–1735.

Dalpke, A.H., Schafer, M.K., Frey, M., Zimmermann, S., Tebbe, J., Weihe, E., and Heeg, K. (2002). Immunostimulatory CpG-DNA activates murine microglia. J. Immunol. *168*, 4854–4863.

Fitzgerald, D.C., Zhang, G.X., El Behi, M., Fonseca-Kelly, Z., Li, H., Yu, S., Saris, C.J., Gran, B., Ciric, B., and Rostami, A. (2007). Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat. Immunol. *8*, 1372–1379.

Gilliet, M., and Liu, Y.J. (2002). Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. J. Exp. Med. *195*, 695–704.

Gocke, A.R., Cravens, P.D., Ben, L.H., Hussain, R.Z., Northrop, S.C., Racke, M.K., and Lovett-Racke, A.E. (2007). T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. J. Immunol. *178*, 1341–1348.

Greter, M., Heppner, F.L., Lemos, M.P., Odermatt, B.M., Goebels, N., Laufer, T., Noelle, R.J., and Becher, B. (2005). Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat. Med. *11*, 328–334.

Hafler, D.A., Slavik, J.M., Anderson, D.E., O'Connor, K.C., De Jager, P., and Baecher-Allan, C. (2005). Multiple sclerosis. Immunol. Rev. *204*, 208–231.

Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005). Interleukin 17-producing $CD4^+$ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat. Immunol. *6*, 1123–1132.

Heppner, F.L., Greter, M., Marino, D., Falsig, J., Raivich, G., Hovelmeyer, N., Waisman, A., Rulicke, T., Prinz, M., Priller, J., et al. (2005). Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat. Med. *11*, 146–152.

Huber, M., Steinwald, V., Guralnik, A., Brustle, A., Kleemann, P., Rosenplanter, C., Decker, T., and Lohoff, M. (2008). IL-27 inhibits the development of regulatory T cells via STAT3. Int. Immunol. *20*, 223–234.

Hur, E.M., Youssef, S., Haws, M.E., Zhang, S.Y., Sobel, R.A., and Steinman, L. (2007). Osteopontin-induced relapse and progression of autoimmune brain disease through enhanced survival of activated T cells. Nat. Immunol. *8*, 74–83.

Ivanov, I.I., McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17^+ T helper cells. Cell *126*, 1121–1133.

Jansson, M., Panoutsakopoulou, V., Baker, J., Klein, L., and Cantor, H. (2002). Attenuated experimental autoimmune encephalomyelitis in Eta-1/osteopontin-deficient mice. J. Immunol. *168*, 2096–2099.

Kastelein, R.A., Hunter, C.A., and Cua, D.J. (2007). Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu. Rev. Immunol. *25*, 221–242.

Kawakami, N., Lassmann, S., Li, Z., Odoardi, F., Ritter, T., Ziemssen, T., Klinkert, W.E., Ellwart, J.W., Bradl, M., Krivacic, K., et al. (2004). The activation status of neuroantigen-specific T cells in the target organ determines the clinical outcome of autoimmune encephalomyelitis. J. Exp. Med. *199*, 185–197.

Khader, S.A., Bell, G.K., Pearl, J.E., Fountain, J.J., Rangel-Moreno, J., Cilley, G.E., Shen, F., Eaton, S.M., Gaffen, S.L., Swain, S.L., et al. (2007). IL-23 and IL-17 in the establishment of protective pulmonary $CD4^+$ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. Nat. Immunol. *8*, 369–377.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., and Cua, D.J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J. Exp. Med. *201*, 233–240.

Leibundgut-Landmann, S., Gross, O., Robinson, M.J., Osorio, F., Slack, E.C., Tsoni, S.V., Schweighoffer, E., Tybulewicz, V., Brown, G.D., Ruland, J., and Reis e Sousa, C. (2007). Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. Nat. Immunol. *8*, 630–638.

Maldonado, R.A., Irvine, D.J., Schreiber, R., and Glimcher, L.H. (2004). A role for the immunological synapse in lineage commitment of CD4 lymphocytes. Nature *431*, 527–532.

O'Regan, A.W., Nau, G.J., Chupp, G.L., and Berman, J.S. (2000). Osteopontin (Eta-1) in cell-mediated immunity: teaching an old dog new tricks. Immunol. Today *21*, 475–478.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat. Immunol. *6*, 1133–1141.

Paty, D.W., Li, D.K., and UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group (1993). Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology *43*, 662–667.

Pflanz, S., Timans, J.C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E., et al. (2002). IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. Immunity *16*, 779–790.

Renkl, A.C., Wussler, J., Ahrens, T., Thoma, K., Kon, S., Uede, T., Martin, S.F., Simon, J.C., and Weiss, J.M. (2005). Osteopontin functionally activates dendritic cells and induces their differentiation toward a Th1-polarizing phenotype. Blood *106*, 946–955.

Rittling, S.R., Matsumoto, H.N., McKee, M.D., Nanci, A., An, X.R., Novick, K.E., Kowalski, A.J., Noda, M., and Denhardt, D.T. (1998). Mice lacking osteopontin show normal development and bone structure but display altered osteoclast formation in vitro. J. Bone Miner. Res. *13*, 1101–1111.

Sato, T., Nakai, T., Tamura, N., Okamoto, S., Matsuoka, K., Sakuraba, A., Fukushima, T., Uede, T., and Hibi, T. (2005). Osteopontin/Eta-1 upregulated in Crohn's disease regulates the Th1 immune response. Gut *54*, 1254–1262.

Shapiro, S., Galboiz, Y., Lahat, N., Kinarty, A., and Miller, A. (2003). The "immunological-synapse" at its APC side in relapsing and secondary-progressive multiple sclerosis: modulation by interferon-beta. J. Neuroimmunol. *144*, 116–124.

Shinohara, M.L., Jansson, M., Hwang, E.S., Werneck, M.B.F., Glimcher, L.H., and Cantor, H. (2005). T-bet-dependent expression of osteopontin contributes to T cell polarization. Proc. Natl. Acad. Sci. USA *102*, 17101–17106.

Shinohara, M.L., Lu, L., Bu, J., Werneck, M.B.F., Kobayashi, K., Glimcher, L.H., and Cantor, H. (2006). Osteopontin expression is essential for IFN-α production by plasmacytoid dendritic cells. Nat. Immunol. *7*, 498–506.

Shinohara, M.L., Kim, H.-J., Kim, J.-H., Garcia, V.A., and Cantor, H. (2008). Alternative translation of Osteopontin generates intracellular and secreted isoforms that mediate distinct biological activities in dendritic cells. Proc. Natl. Acad. Sci. USA *105*, 7235–7239.

Steinman, L., and Zamvil, S. (2003). Transcriptional analysis of targets in multiple sclerosis. Nat. Rev. Immunol. *3*, 483–492.

Stumhofer, J.S., Laurence, A., Wilson, E.H., Huang, E., Tato, C.M., Johnson, L.M., Villarino, A.V., Huang, Q., Yoshimura, A., Sehy, D., et al. (2006). Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. Nat. Immunol. *7*, 937–945.

Taniguchi, T., and Takaoka, A. (2001). A weak signal for strong responses: interferon-alpha/beta revisited. Nat. Rev. Mol. Cell Biol. *2*, 378–386.

Tompkins, S.M., Padilla, J., Dal Canto, M.C., Ting, J.P., Van Kaer, L., and Miller, S.D. (2002). De novo central nervous system processing of myelin antigen is required for the initiation of experimental autoimmune encephalomyelitis. J. Immunol. *168*, 4173–4183.

Umemura, M., Yahagi, A., Hamada, S., Begum, M.D., Watanabe, H., Kawakami, K., Suda, T., Sudo, K., Nakae, S., Iwakura, Y., and Matsuzaki, G. (2007). IL-17-mediated regulation of innate and acquired immune response against pulmonary *Mycobacterium bovis* bacille Calmette-Guerin infection. J. Immunol. *178*, 3786–3796.

Veldhoen, M., Hocking, R.J., Atkins, C.J., Locksley, R.M., and Stockinger, B. (2006). TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity *24*, 179–189.

West, M.A., Wallin, R.P., Matthews, S.P., Svensson, H.G., Zaru, R., Ljunggren, H.G., Prescott, A.R., and Watts, C. (2004). Enhanced dendritic cell antigen capture via toll-like receptor-induced actin remodeling. Science *305*, 1153–1157.

Yu, M., Nishiyama, A., Trapp, B.D., and Tuohy, V.K. (1996). Interferon-beta inhibits progression of relapsing-remitting experimental autoimmune encephalomyelitis. J. Neuroimmunol. *64*, 91–100.