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J Immunol 2015; 194:4175-4184; Prepublished online 30 March 2015;
doi: 10.4049/jimmunol.1401766
http://www.jimmunol.org/content/194/9/4175

Supplementary Material
http://www.jimmunol.org/content/suppl/2015/03/28/jimmunol.1401766.DCSupplemental

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Impaired Dendritic Cell Function in a Spontaneous Autoimmune Polyneuropathy

Songhua Quan,* Hye-Jung Kim,*,† Danuta Dukala,* Jian Rong Sheng,* and Betty Soliven*

Spontaneous autoimmune polyneuropathy (SAP) in B7-2 knockout NOD mice mimics the progressive form of chronic inflammatory demyelinating polyradiculoneuropathy, and is mediated by myelin protein zero (P0)–reactive Th1 cells. In this study, we focused on the effect of B7-2 deletion on the function of dendritic cells (DCs) within the context of SAP. We found that development of SAP was associated with a preponderance or increase of CD11b+ DCs in peripheral lymph nodes and sciatic nerves. B7-2 deletion led to altered immunophenotypic properties that differ between CD11b+ DCs and CD8α+ DCs. Both DC subsets from B7-2 knockout NOD mice exhibited impaired capacity to capture fluorophore-labeled myelin P0, but diminished Ag-presenting function was observed only in CD11b+ DCs. Clinical assessment, electrophysiologic studies, and splenocyte proliferation studies revealed that absence of B7-2 on DCs was sufficient to cause impaired ability to induce tolerance to P0, which could be overcome by preconditioning with IL-10. Tolerance induction by Ag-pulsed wild-type NOD DCs was dependent on IL-10 and was associated with increased CD4+ regulatory T cells, whereas tolerance induction by IL-10–conditioned B7-2–deficient DCs was associated with increased percentages of both regulatory T cells and B10 cells in the spleen. We conclude that B7-2 deletion has an impact on the distribution of DC subsets in lymphoid organs and alters the expression of costimulatory molecules, but functional consequences are not uniform across DC subsets. Defective tolerance induction in the absence of B7-2 can be restored by preconditioning of DCs with IL-10. The Journal of Immunology, 2015, 194: 4175–4184.

Dendritic cells (DCs) are potent APCs and play a crucial role in the orchestration of immune responses, both in terms of T cell immunity and tolerance induction. There are two major categories of DCs (CD11c+ cells) in mice, as follows: 1) plasmacytoid DCs (pDCs [CD11c−CD11b−B220+]), also known as IFN-producing cells, and 2) conventional DCs. The latter includes migratory DCs and lymphoid organ resident DCs, which are classified into CD8α−CD11b+ and CD8α+ CD11b+ DC subsets. Although CD8α+ DCs and CD11b+ DCs have been shown to promote polarization of Th cells toward Th1 and Th2 cells, respectively, both DC subsets are capable of IL-12 production, albeit requiring different stimuli (1–5). The capacity of DCs for initiating immunity or tolerance depends on their maturational and functional state, and on specialized DC subsets. Mature DCs are characterized by upregulation of MHC class II, B7 family molecules, and CD40, resulting in enhanced immunogenicity (6). That DCs are required to maintain self-tolerance is supported by DC ablation studies showing the development of myeloid proliferative disease or fatal spontaneous autoimmunity depending on whether pDCs and Langerhans cells are spared or not (7, 8).

The role of DCs in the development of human autoimmune neuropathies such as Guillain Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) or their animal models has not been well elucidated. Press et al. (9) found increased number of CD11b+ DCs in the cerebrospinal fluid of Guillain Barré syndrome and CIDP patients, which correlated positively with the severity of clinical disability in CIDP. In experimental autoimmune neuritis in Lewis rats, disease severity can be attenuated by atorvastatin-modified DCs, which exhibit lower levels of costimulatory molecules B7-1, B7-2, and MHC class II (10).

The B7-1/B7-2-CD28/CTLA4 pathway plays a crucial role in T cell priming, CD4/CD8 homeostasis, as well as homeostasis of regulatory T cells (Tregs) (11, 12). In NOD mice, B7-2 elimination leads to protection from diabetes that is attributed to defective CD4 T cell priming, but it triggers the development of a spontaneous autoimmune polyneuropathy (SAP) (13, 14). The latter is characterized by electrophysiologic findings of demyelinating features and axonal loss, as well as presence of inflammatory cells in sections of sciatic nerves and dorsal root ganglia (13, 14). These findings mimic those of human CIDP, although the latter can be relapsing-remitting or progressive. In contrast, SAP mice exhibit progressive weakness to the point of quadripareisis. We and other investigators found that SAP is mediated by myelin protein zero (P0)–reactive Th1 cells, and at least two epitopes are involved—P0 (180–199) and P0 (1–25) (13, 15–17). More recently, we have demonstrated that B cell autoreactivity to myelin P0 also contributes to the pathogenesis of SAP (18).

The goal of this study was to delineate the consequences of B7-2 deletion on DC function within the context of SAP, and to investigate whether absence of B7-2 on DCs is sufficient to cause defect in peripheral tolerance to myelin P0 in vivo. There is evidence that...
B7-2 promotes the survival and function of DCs. B7-2-deficient DCs exhibited enhanced susceptibility to death. In addition, there was reduced proportion of CD80b DCs and increased frequency of both B7-H1 and B7-DCs in pancreatic lymph nodes (PLN) of B7-2 knockout (KO) NOD mice (19). In this work, we report that the functional consequences of B7-2 deletion differ between CD11b DCs and CD8α DCs in some aspects. In addition, we provide evidence to support the concept that absence of B7-2 on DCs contributes to the loss of tolerance to myelin P0 in SAP, which could be overcome by preconditioning with IL-10.

Materials and Methods

Animals, clinical and electrophysiological assessment

Wild-type (WT) NOD, B7-2 KO NOD, IL-10 KO NOD, and Foxp3-eGFP-Cre NOD mice (The Jackson Laboratory, Bar Harbor, ME) were housed and bred in pathogen-free conditions in the Animal Barrier Facility. All animal use procedures were conducted in strict accordance with the National Institutes of Health and University of Chicago institutional guidelines. Female B7-2 KO NOD mice were used in this study, unless otherwise stated. Foxp3-eGFP-Cre mice were maintained on a C57BL/6J background with the following genotypes: C57BL/6J (EID Millipore, Billerica MA), as described previously (18). The purity of the His-tagged P0-ECD protein was confirmed by Western blot analysis using HRP-conjugated goat anti–6-His Ab (1:10,000; Bethyl Lab, Montgomery, TX). The final endotoxin level was reduced to <1 EU/μg using the ToxinEraser Endotoxin Removal Kit (Genscript, Piscataway, NJ). Splenic CD11c+ B cells were determined by V450-conjugated anti-mouse CD11c, FITC-conjugated anti-mouse CD11b (BioLegend), FITC-conjugated anti-mouse CD8α (BD Biosciences). PE-conjugated anti-mouse B7-1, MHC class II (anti-RT1b), CD40, and ligand of ICOS (ICOSL; BioLegend). Splenic CD1dhighCD5+ T cells were determined using V450-conjugated anti-mouse CD19, PE-conjugated anti-CD5, and Alexa-Fluor 647-conjugated CD1d (BioLegend). For the detection of Tregs, splenocytes were stained with FITC-conjugated anti-mouse CD4 and allophycocyanin-conjugated anti-mouse CD25 Abs, fixed, permeabilized, and subsequently stained with PE-conjugated anti-mouse Foxp3 (AbEis sce, San Diego, CA).

For intracellular cytokine staining, splenocytes (1 × 10⁶/well) in 96-well plates were stimulated at 37°C in a humidified CO2 incubator for 4 h with Leukocyte Activation Cocktail (BD Pharmingen, San Jose, CA). This was followed by staining for cell surface CD4 and intracellular IFN-γ, IL-17, IL-10, or TNF-α using the Intracellular Cytokine Staining Starter Kit (BD Pharmingen, San Diego, CA). The percentage of IFN-γ+ , IL-17+, IL-10+, and TNF-α+ produces CD4+ T cells was analyzed by Fortessa flow cytometer and FlowJo software. With regard to B10 cells, splenocytes were incubated for 4 h in 96-well plates with LPS (10 μg/ml) in addition to Leukocyte Activation Cocktail. Cells were then stained with V450-conjugated anti-mouse CD4 followed by fixation and permeabilization using Cytofix Kit prior to staining with PE-conjugated anti-mouse IL-10 Ab (BD Biosciences).

Real-time RT-PCR

The total RNA was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA). Reverse transcription was performed from 1 μg total RNA, and the cDNA was used for SYBR Green real-time PCR. Amplification was performed with forward and reverse primers for transcripts of interest, which were designed using Primer3 software. The expression of each cytokine gene for each condition. The relative amounts of each product were calculated by the comparative Ct (2^−ΔΔCt) method, as described in User Manual 2 of ABI Prism 7900 Sequence Detection System (Applied Biosystems). Primer sequences used are listed in Supplemental Table 1.

Ag capture

Alexa546-labeled P0-ECD (200 μg) was injected i.v. into the tail veins of 6-wk-old WT NOD and B7-2 KO NOD mice. One hour later, animals were sacrificed, and splenocytes were isolated for flow cytometric analysis of Alexa546 signal in CD11b+ and CD11b+ DCs.

Adoptive transfer studies

DCs (CD11c+ cells) were purified from splenocytes using Dynal Mouse DC negative isolation kit (Invitrogen) and treated with P0 (180–199) (20 μg/ml) prior to AT. Each recipient mouse (5-mo-old female B7-2 KO NOD) was injected i.v. with 6 × 10⁶ WT, B7-2 KO, or IL-10 KO DCs from 3-mo-old donor animals. In a subset of experiments, purified B7-2 KO DCs were pretreated with mouse rIL-10 (200 ng/ml) for 3 d in R10 media. On day 3, P0 (180–199) (20 μg/ml) was added for 16 h (overnight) prior to AT.

Data analysis

Results from clinical severity, immunologic studies, grip strength measurements, and electrophysiology are expressed as mean ± SEM. Statistical significance for these data was determined by ANOVA, followed by Student t test and the Bonferroni method for multiple group experiments. Significance levels were set at p < 0.05.
Results
DC subsets, immunophenotypic properties, and cytokine expression

B7-2 KO NOD mice usually start to develop hindlimb weakness ∼5–6 mo of age. By 8 mo, 100% of female B7-2 KO NOD mice would exhibit clinical and electrophysiological findings of SAP (13). Flow cytometry was performed to investigate whether the development of SAP is accompanied by changes in the proportion of CD11b+ DCs and CD8α+ DCs in the spleen and peripheral LN (inguinal and axillary). Compared with the WT NOD mice, B7-2 KO NOD mice exhibited an increase in the percentage of total DCs and CD11b+ DCs in the peripheral LN (Peri-LN) at 8 mo but not at 2 mo (Fig. 1A). The proportion of CD11b+ DCs and CD8α+ DCs in the spleen did not differ between WT and B7-2 KO NOD mice. The percentage of pDCs in the spleen was also not affected by B7-2 elimination (n = 3, data not shown). The frequency of pDCs in Peri-LN was too low to permit accurate determination.

B7-2 deletion resulted in a higher proportion of B7-1+CD11b+ DCs in the spleen at 2 and 8 mo, and increased percentages of MHCII+, CD40+, and ICOSL+ CD11b+ DCs at 2 mo. For CD8α+ DCs, the only difference was an increased proportion of B7-1+ cells in the spleen of B7-2 KO mice at 8 mo (Fig. 1B). A higher proportion of B7-1+, MHCII+, and CD40+CD11b+ DCs was also noted in the Peri-LN of B7-2 KO NOD mice at 2 and 8 mo, whereas only increased percentages of B7-1+ and MHCII+ CD11b+ DCs were noted in PLN of B7-2 KO NOD at 2 mo, but not at 8 mo (Supplemental Fig. 1). Immunophenotypic properties were not assessed for CD8α− DCs from Peri-LN and PLN due to low frequency. Immunofluorescence studies on sciatic nerve sections from SAP mice revealed frequent colocalization of CD11c with CD11b, but not with CD8α. Rare pDCs (B220+CD11c+) were observed (Fig. 1C). Subsequent studies were focused on splenic CD11b+ DCs, CD8α+ DCs, or total DCs.

Effect of B7-2 elimination on DC function

The P0-ECD was purified and labeled with Alexa546, which was used to determine whether B7-2 elimination leads to altered capability of DCs to capture SAPAg in vivo. At 1 h after i.v. injection, both splenic CD11b+ DCs and CD11b− DCs from 6-wk-old B7-2 KO mice exhibited a significant reduction in the percentage of labeled P0-ECD+ cells when compared with those from WT NOD mice (Fig. 2A).

Next, we examined the ability of CD11b+ DCs to stimulate T cell proliferation at varying DC–T cell ratio (1:50, 1:10, 1:5). For these experiments, CD4+ T cells were isolated from symptomatic 8-mo-old B7-2 KO mice, whereas DC subsets were isolated from 2- to 3-mo-old B7-2 KO or WT NOD mice. As shown in Fig. 2B, B7-2 KO CD11b+ DCs exhibited a diminished capacity to stimulate T cell proliferation at baseline and in response to 20 μg/ml P0 (180–199) when compared with WT CD11b+ DCs.
contrast, there was a trend toward slightly enhanced T cell proliferative response to P0 (180–199) by B7-2 KO CD8α+ DCs compared with WT CD8α+ DCs, but the difference did not reach statistical significance.

Further studies on the functional consequences of B7-2 deletion were carried out using total splenic DCs. Real-time RT-PCR technique was used to compare the mRNA levels of IL-12 p40, TNF-α, TGF-β, IL-6, and IL-10 in purified, unstimulated WT and B7-2 KO DCs from 2- to 3-mo-old animals. Comparing B7-2 KO DCs versus WT DCs (n = 3 each), there was a significant decrease in the mRNA levels of IL-10, but not of other cytokines (Fig. 3A). Cytokine secretion was also determined by ELISA in DCs cultured in the presence of LPS (10 μg/ml) for 2 d. Lack of B7-2 on DCs led to decreased IL-10 production in response to LPS compared with WT NOD DCs, but did not affect the secretion of the other cytokines tested, including IL-12 p70 (5 ng/ml) alone or with TGF-β (5 ng/ml) plus IL-2 (200 U/ml). Paradoxically, addition of anti-CD28 mAb (5 μg/ml) led to decreased frequency of iTregs and did not overcome the effect of B7-2 deficiency on DCs (Fig. 3C).

To investigate the consequence of B7-2 deletion on DC function in vivo, we compared the capacity of WT versus B7-2 KO DCs to induce tolerance in adoptive transfer (AT) experiments.Recipient B7-2 KO NOD mice (5 mo old) were divided into three groups, as follows: group A received PBS injections (no AT); group B received P0 (180–199)-pulsed WT DCs, designated as WT DC (AT); group C received P0 (180–199)-pulsed B7-2 KO DCs, designated as B7-2 KO DC (AT). Clinical assessment (clinical score and grip strength measurements) revealed that the development of SAP was abrogated by AT of P0 (180–199)-pulsed WT DCs, but not by P0 (180–199)-pulsed B7-2 KO DCs (Fig. 4A). Induction of tolerance

**FIGURE 2.** Effect of B7-2 deletion on Ag capture and T cell proliferation induced by DCs. (A) Reduced Ag capture by DCs from B7-2 KO mice compared with those from WT NOD mice. Six-week-old animals were injected i.v. with 200 μg Alexa546-labeled P0-ECD and sacrificed 1 h later. Splenocytes were processed for flow cytometric analysis of Alexa546 signal in CD11b+ DCs and CD11b− DCs. Values shown in the histogram represent mean percentage ± SEM (n = 3). *p < 0.003 for both CD11b+ DCs and CD11b− DCs comparing B7-2 KO versus WT NOD. (B) Proliferative responses of CD4+ T cells from SAP mice (8 mo) alone or cocultured with splenic DCs (3 mo) with or without myelin P0 (180–199) (20 μg/ml). Left panel, CD11b+ DC–CD4 T cell cocultures. Comparing WT versus B7-2 KO CD11b+ DC at 1:5 ratio, *p < 0.05 with or without P0 (180–199) (n = 3 each). Right panel, CD8α+ DC–CD4 T cell cocultures. Comparing WT versus B7-2 KO CD8α+ DC, p > 0.05 with or without P0 (180–199) (n = 5).
was confirmed by electrophysiological studies showing dramatic improvement in distal latencies, conduction velocities, and distal amplitudes of sciatic motor response (Fig. 4A). Of note, WT DCs that were not pulsed with P0 (180–199) did not induce tolerance to the development of SAP (n = 5) (Supplemental Fig. 2).

Animals from AT experiments were sacrificed at 12 wk post-transfer (∼8 mo of age) for immunologic studies. Splenocyte proliferation induced by 20 μg/ml P0 (180–199) or P0-ECD was decreased in tolerized mice (WT DC [AT] group) compared with B7-2 KO DC (AT) group or PBS (no AT) group. There was no difference in the percentage of CD4+IFN-γ+ T cells or CD4+IL-17+ T cells in spleens and Peri-LN of animals from these three groups. However, there was a reduction in splenic CD4+TNF-α+ T cells, and an increase in CD4+IL-10+ T cells in spleens and Peri-LN of WT DC (AT) group (Fig. 4B). To delineate the effect of AT of Ag-pulsed DCs on regulatory mechanisms, we examined the frequency of CD4+ Tregs in spleens and Peri-LN of animals from three study groups. The frequency of CD4+ Tregs (expressed as percentage of CD25+Foxp3+ cells in CD4+ T cells) was significantly increased in the spleen of tolerized mice (WT DC [AT] group) compared with the other two groups. In Peri-LN, a significant difference in the frequency of Tregs was observed only when comparing WT DC (AT) group to PBS (no AT) group, but not when comparing to B7-2 KO DC (AT) group (Fig. 4C). The effect of DCs on splenic B cell subsets with regulatory activity was also investigated. B cells that express IL-10 after 4–5 h of exposure to phorbol ester and ionomycin are designated as B10 cells, and are found predominantly within CD1dhighCD5+CD19+ subset (22). In contrast to the effect on Tregs, AT of Ag-pulsed WT DCs or B7-2 KO DCs had no effect on the frequency of CD1dhighCD5+ subset in B cells (CD19+) or in the percentage of B10 cells in total splenocytes or Peri-LN cells (n = 7–10, data not shown).

Given that B7-2 KO DCs had lower IL-10 expression compared with WT DCs, we investigated whether its defect in tolerance induction can be corrected by treatment with IL-10 (200 ng/ml) for 3 d in vitro prior to AT. P0 (180–199) was added on the third day of preconditioning. As shown in Fig. 5A, AT of IL-10–conditioned B7-2 KO DCs led to induction of tolerance to myelin P0 when compared with PBS (no AT) group. Improvement in clinical scores, grip strength, and electrophysiologic parameters was demonstrated in tolerized animals, which was accompanied by decreased splenocyte proliferative response to P0 (180–199) or P0-ECD, and by increased frequency of splenic CD4+ Tregs and B10 cells (Fig. 5B). We examined the effect of IL-10 preconditioning in vitro on the proportion of MHCII+, CD4+, B7-1-, PDL1+, and PDL2+ splenic DCs. Pretreatment of splenic DCs from 3-mo-old B7-2 KO NOD
mice with IL-10 for 3 d led to reversal of immunophenotypic properties to mimic those of WT NOD DCs. Furthermore, IL-10–preconditioned B7-2 KO DCs exhibited improved capacity to generate iTregs from CD4+eGFP cells upon anti-CD3 stimulation compared with unconditioned B7-2 KO DCs (Fig. 5C). IL-10 pre-treatment for 3 d did not have an effect on Ag uptake by WT or B7-2 KO NOD DCs in vitro (Supplemental Fig. 3). Thus, IL-10 preconditioning converted B7-2 KO DCs back to WT NOD phenotype in some, but not all aspects. To further confirm the crucial role of IL-10 in the induction of tolerogenic DCs, splenic DCs were purified from 3-mo-old IL-10–deficient NOD mice for AT into 5-mo-old B7-2 KO NOD mice. Deletion of IL-10 led to impaired capacity of WT DCs to induce tolerance to myelin P0, as shown in Fig. 6.

**Discussion**

B7-2 plays an important role in the survival and function of DCs. Absence of B7-2 leads to impaired protein kinase C-ε response when stimulated with CD28-Ig (19). In this study, we have demonstrated that deletion of B7-2 leads to age-dependent alterations in immunophenotypic properties that differ between CD11b+ DCs and CD8α+ DCs. We found increased percentages of B7-1, MHC class II, CD40, and ICOSL+ splenic CD11b+ DCs, but no corresponding changes in CD8α+ DCs at 2 mo of age. At 8 mo, increased percentages of B7-1*CD11b+ DCs and B7-1*CD8α+ DCs were noted in B7-2 KO NOD mice. We observed some tissue heterogeneity in the expression/regulation of costimulatory molecules when comparing Peri-LN and PLN, although it
may not be sufficient to explain the absence of diabetes in SAP mice. For example, the percentage of B7-1+ CD11b+ DCs was increased in Peri-LN of B7-2 KO NOD mice at 2 and 8 mo when compared with age-matched WT NOD mice, whereas it was increased in PLN at 2 mo, but unchanged at 8 mo. Enhanced expression of MHC class II, B7-1, and CD40 would lead to augmented positive signals to T cells, contributing to enhanced autoimmunity. In contrast, increased ICOSL-ICOS signaling in mice is associated with induction of Th2 cells or Tregs, although it can also induce Th1 cells and support Th17 cells under certain circumstances (23–26). Studies using bone marrow DCs from NOD mice suggest that there is an inherent bias toward high co-stimulation and Th1 induction compared with DCs from other mouse strains, although impaired DC maturation in NOD mice has also been reported (27–30).

We found that B7-2 KO NOD mice exhibited an increase of CD11b+ DCs in Peri-LN during the symptomatic phase. This finding raises the possibility that the main DC subset involved in the pathogenesis of SAP is CD11b+ DCs, and is consistent with observations in human CIDP in which increased number of CD11b+ DCs in the cerebrospinal fluid correlates positively with clinical disability (9). Whether a specific DC subset is linked to autoimmunity versus tolerance has been the subject of intense investigations, and results are not always concordant from one experimental model of autoimmune disease to another (6, 31). Insulitis is attenuated by ablation of CD11b+ DCs,

![Figure 5](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/)

**FIGURE 5.** Preconditioning of B7-2 KO DCs with IL-10 restores their capacity to induce tolerance to myelin P0. AT of DCs was performed in 5-mo-old B7-2 KO NOD mice, as described in Fig. 4. Statistical analysis was made comparing B7-2 KO DC–IL-10 group versus PBS (no AT) group in (A) and (B). (A) Disease severity. Grip strength measurements and sciatic nerve electrophysiology were performed at 12 wk post-AT. For mean clinical scores, *p < 0.0001 (n = 8–9); for grip strength, *p < 0.0001 (n = 8–9); and *p < 0.0003 for all electrophysiologic parameters (DL, CV, dAMP) (n = 6–7). (B) Immunologic studies at 12 wk post-AT. Tolerance induction by B7-2 KO DC (IL-10) was associated with decreased splenocyte proliferation in response to P0 (180–199) or P0-ECD (20 μg/ml), and increased percentages of Tregs (CD25+Foxp3+CD4+) and B10 cells in the spleen, but not in the Peri-LN. *p < 0.0002 (n = 4–5) for splenocyte proliferation, *p < 0.003 (n = 5–6) for splenic Tregs, and *p < 0.003 (n = 6–7) for splenic B10 cells. (C) Characterization of IL-10–preconditioned B7-2 KO DCs. Left panel, IL-10 pretreatment of B7-2 KO DCs led to altered immunophenotypic properties that mimicked those of WT DCs. Cells were gated based on CD11c staining, and then analyzed for MHC class II, CD40, B7-1, PDL1, and PDL2 expression. Comparing B7-2 KO versus B7-2 KO DC-10, *p < 0.0002 for MHCII, CD40, and B7-1 (n = 4 each). Right panel, Improved capacity of B7-2 KO DCs to generate iTregs from sorted CD4+Foxp3+ (eGFP+) cells by IL-10 preconditioning (*p < 0.002 and *p < 0.0002 [n = 3 each]). Experimental conditions for iTregs were as described in Fig. 3C.
FIGURE 6. Failure of IL-10–deficient NOD DCs to induce tolerance to myelin P0. Splenic DCs were purified from 3-mo-old IL-10 KO NOD mice for AT into 5-mo-old B7-2 KO NOD mice, as described in Fig. 4. (A) Mean clinical scores. (B) Grip strength measurements. (C) Data from sciatic nerve electrophysiology. (D) Splenocyte proliferation. Data represent mean ± SEM (n = 6 for each group).

but accelerated by loss of pDCs in NOD mice (32). The protective effect of pDCs is also observed in experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) peptide [aa35–55] (33). In contrast, CD11b+ DCs in the CNS preferentially induce Th17 cells in EAE induced by proteolipid protein in SJL/J mice, whereas they are associated with induction of Th2 cells and CD4+ Tregs in MOG EAE in C57BL/6 or SJL/J mice (3, 34, 35). Therefore, it appears that CD11b+ DCs can be pathogenic or tolerogenic depending on the context, cytokine milieu, and the disease model.

DCs capture, process, and present self- and exogenous Ags to induce immunity or tolerance. The aberrant autoreactivity to P0 in SAP is not due to enhanced Ag uptake or presentation. We found that B7-2 deletion resulted in reduced uptake of Alexa546-labeled P0-ECD by both splenic CD11b+ DCs and CD8α+ DCs, which confirmed some, but not all the findings from the work by other investigators (19). In the latter study, reduced endocytosis of FITC-labeled dextran (polysaccharide) was limited to B7-2–deficient CD8α+ DCs, which correlated with decreased proportion of CD8α+ DCs expressing Ag uptake receptors such as CD205 and CD16/32 (19). As expected, impaired costimulation from the absence of B7-2 on CD11b+ DCs led to a decreased proliferative response to P0. That upregulation of B7-1 is insufficient to compensate for the lack of B7-2 in CD11b+ DCs would support the concept that B7-1 and B7-2 differentially regulate immune responses despite some overlapping functions. In contrast to CD11b+ DCs, absence of B7-2 on CD8α+ DCs did not result in diminished T cell proliferative response to P0 (180–199) and P0-ECD, but was not associated with perturbations in Th1/Th17 polarization. Nonetheless, altered T cell cytokine profile was observed in that the percentage of IL-10+ CD4+ T cells was increased in the spleen and Peri-LN, whereas the percentage of TNF-CD4+ T cells was reduced in the spleen of tolerized animals. IL-10 is secreted not only by Th2 cells, but also by macrophages, B cells, and activated CD4+ T cells (40–43). However, only DCs exhibit strong capacity to directly expand Tregs (44). Exogenous TGF-β is required for induction of Tregs by CD11b+ DCs, but not when CD8α+CD205+ DCs are used (45). We found that B7-2–deficient DCs were less effective than WT DCs not only in maintaining the proliferation of splenic Tregs, but also in the generation of iTregs de novo in the presence of TGF-β. IL-2 did not exert any additive effect on the generation of iTregs under our experimental conditions.

Findings from our in vitro studies were confirmed by results from our AT experiments, which revealed that the lack of B7-2 on DCs is sufficient to cause defect in tolerance induction in our model. Results from clinical, electrophysiological, and immunologic studies from B7-2 KO DC (AT) group were similar to those obtained from PBS (no AT) group. Tolerance induction by Ag-pulsed WT DCs correlated with decreased splenocyte proliferative response to P0 (180–199) and P0-ECD, but was not associated with perturbations in Th1/Th17 polarization. Nonetheless, altered T cell cytokine profile was observed in that the percentage of IL-10+ CD4+ T cells was increased in the spleen and Peri-LN, whereas the percentage of TNF-CD4+ T cells was reduced in the spleen of tolerized animals. IL-10 is secreted not only by Th2 cells, but also by Th1 and Th17 cells as part of a negative feedback regulation of CD4+ effector responses (46). There is some evidence that the ratio of IL-10 to the relevant effector cytokine (IFN-γ or IL-17) dictates the outcome of the immune response. Whereas C57BL/6 mice recover rapidly from MOG-induced EAE, IL-10–deficient mice do not recover and develop a progressive form of EAE (47). Proteolipid protein–specific CD4+ T cells that express IL-10 under the control of IL-2 promoter can prevent and suppress EAE (48). In experimental autoimmune...
neuritis. IL-10 was also suppressive even when administered after the onset of clinical disease (49).

Absence of B7-1 or B7-2 on DCs has been reported to abrogate the suppressive action of IL-10–treated DCs and DC-derived exosomes in the delayed-type hypersensitivity model (50). In contrast, we found that the defect in tolerance induction by splenic DCs lacking B7-2 in the SAP model could be reversed by preconditioning with IL-10, and was associated with increased frequency of both Tregs and B10 cells in the spleen. Conversely, IL-10–deficient DCs exhibited impaired capacity to induce tolerance to myelin P0. Induction of regulatory B cells such as B10 cells by tolerogenic DCs has recently been reported not only in NOD mice, but also in phase 1 clinical trial of tolerogenic autologous DC administration in type 1 diabetes (51, 52). In our current study, increased frequency of Tregs without concomitant increased frequency of B10 cells is sufficient to induce tolerance to myelin P0, as observed in animals tolerized with Ag-pulsed WT DCs.

DC-derived IL-10 can act in a paracrine or autocrine manner. It is plausible that lower expression of IL-10 in B7-2 KO DCs contributes partially to the loss of tolerance to P0. We found that IL-10 preconditioning had no effect on Ag uptake. Our data on altered immunophenotypic properties of B7-2 KO DCs by IL-10 are in agreement with those reported by other investigators in WT DCs (50, 53–57). In addition, IL-10 also upregulates other inhibitory molecules in human DCs, such as Ig-like transcripts 3 and 4 (55, 56). In addition, IL-10 also upregulates other inhibitory molecules in human DCs, such as Ig-like transcripts 3 and 4 (55, 56). The role of B7-2 in the development of SAP in NOD mice, mainly due to myelin P0, is characterized by massive infiltration of CD8+ T cells and macrophages, severe demyelination, and axonal damage in the sciatic nerves with less extensive involvement in the spinal cords (62). The outcome from perturbations of B7-2 expression or signaling ultimately depends on the balance of autoreactivity versus regulation.

Acknowledgments

B7-2 KO NOD mice were generated in the laboratory of Dr. J. A. Bluestone (University of California, San Francisco). IL-10 deficient NOD mice were generously provided by Dr. D. Mathis (Juvenile Diabetes Research Foundation Center on Immunological Tolerance in Type 1 Diabetes, Harvard Medical School).

Disclosures

The authors have no financial conflicts of interest.

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