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J Immunol 2007; 178:6236-6241; doi: 10.4049/jimmunol.178.10.6236
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The absence of B7-2-mediated costimulation protects NOD mice from the development of diabetes. Although the effects of B7-2 on T cell priming are well known, its impact on the function of APCs is not fully elucidated. We tested APC function and survival in mice lacking B7-2. A significant reduction in the phagocytic ability was observed in both splenic and pancreatic lymph node-associated dendritic cells (DCs) in B7-2 knockout (KO) mice. DCs from B7-2KO mice exhibited enhanced susceptibility to death, which was reflected by their reduced total cell numbers. Phenotypic analysis of APCs in B7-2KO mice revealed a significantly decreased proportion of CD8α⁺CD205⁺ DCs. Interestingly, an enhanced proportion of B7-H1⁺ and B7-DC⁺ DCs were observed in B7-2KO mice. Lastly, we found that B7-2 deficiency significantly diminished the PKC-ε response in APCs upon CD28-Ig stimulation. In conclusion our data suggests that B7-2 promotes the generation of a mature APC repertoire and promotes APC function and survival. The Journal of Immunology, 2007, 178: 6236–6241.

The capacity for initiating immunity or tolerance depends on the functional and maturation state of APCs such as dendritic cells (DCs) (1). For example, Ag targeting to immature lymph node resident DCs promotes tolerance, whereas upon DC activation, the same conditions cause induction of T cell responses (2). Thus, the immune status of DCs determines whether an Ag-specific immune response will lead to priming vs tolerance. The B7-1 and B7-2 family of costimulatory molecules play a critical role in the reciprocal conditioning of T cells and APCs by binding to their ligands CD28 and CTLA-4. Although there has been intensive work on the role of B7-2 on T cell immunity, its impact on the function of APCs is not fully elucidated. We tested APC function and survival in B7-2KO mice. Interestingly, an enhanced proportion of B7-H1⁺ and B7-DC⁺ DCs were observed in B7-2KO mice. Lastly, we found that B7-2 deficiency significantly diminished the PKC-ε response in APCs upon CD28-Ig stimulation. In conclusion our data suggests that B7-2 promotes the generation of a mature APC repertoire and promotes APC function and survival. The Journal of Immunology, 2007, 178: 6236–6241.

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The capacity for initiating immunity or tolerance depends on the functional and maturation state of APCs such as dendritic cells (DCs) (1). For example, Ag targeting to immature lymph node resident DCs promotes tolerance, whereas upon DC activation, the same conditions cause induction of T cell responses (2). Thus, the immune status of DCs determines whether an Ag-specific immune response will lead to priming vs tolerance. The B7-1 and B7-2 family of costimulatory molecules play a critical role in the reciprocal conditioning of T cells and APCs by binding to their ligands CD28 and CTLA-4. Although there has been intensive work on the role of B7-2 on T cell immunity, its impact on the function and maturation of DC have not been fully addressed. Recent work demonstrated that binding of CTLA-Ig to DCs induces signaling via B7, which in turn leads to IFN-γ-mediated indolamine 2,3-dioxygenase induction, causing inhibition of T cell proliferation (3). Furthermore, CD28-Ig engagement has been shown to induce immunostimulatory signals in DCs that are mediated by p38 MAPK and IL-6 production (4). The short cytoplasmic tail of B7-2 has been reported to contain three putative protein kinase C (PKC) phosphorylation sites (5, 6), suggesting that B7-2 may play a distinct role in signaling into DCs. Recently, Kin and Sanders (7) have proposed that activation of PI3K/Akt and phospholipase Cγ2/PKC-αβ signaling occurs upon B7-2 stimulation in B cells.

The phenotypic alterations commonly associated with DC maturation allow these cells to become potent activators of T cell immunity. Maturation of DCs involves several coordinated events leading to the up-regulation of the costimulatory molecules B7-1, B7-2, and CD40, including an increase in MHC expression and changes in DC migration and endocytic ability (8). In addition, CD205 is up-regulated upon DC maturation and participates in Ag uptake and enhances the efficiency of Ag presentation (9, 10). CD8α⁺ DCs play an essential role in the induction of proinflammatory T cell responses (11) and endocytosis (12). DCs that reside in lymphoid tissues use a range of unique receptors to acquire and present Ag, which include the lectin type receptor DEC-205 (CD205) and Fc (CD16/32) receptors (13). Alteration in the expression of negative costimulatory molecules such as B7-H1 and B7-DC (14) can impact on the capacity of APCs to generate negative signals. During inflammation, DCs initiate their maturation and survival programs (15) and disappear from lymph nodes after Ag presentation (16). Defects in DC apoptosis can lead to DC accumulation and the development of autoimmunity (17).

B7-2-mediated costimulation plays a pivotal role in T cell priming. Specifically, NOD mice deficient in B7-2 have been shown to be completely protected from pancreatic autoimmunity due to defective DC4 T cell priming (18, 19). These results led us to hypothesize that lack of the anti-islet T cell response may be associated with an intrinsic defect in the DC compartment. In this study we questioned the actions of B7-2 in modulating the immune phenotype and function of APCs. We show that B7-2 deficiency causes a reduction in survival, maturity, and signaling in APCs, indicating that B7-2 promotes autoimmunity by regulating APC subsets and function.

Materials and Methods

Mice

Female NODsh mice were obtained from The Scripps Research Institute Animal Facility (La Jolla, CA) and used at 5–8 wk of age (unless otherwise mentioned). The B7-2 knockout (KO) mice on the NOD genetic background were a gift from Dr. J. Bluestone (University of California, San Francisco, CA). All the experiments in the study are done using nondiabetic mice and were maintained at The Scripps Research Institute Animal Facility in pathogen-free conditions. All experiments were conducted in accordance with institutional guidelines for animal care and use.

Abs, reagents, and flow cytometric analyses

Single-cell suspensions from spleen and pancreatic lymph nodes (PLN) were prepared and followed by RBC lysis with ammonium chloride lysis buffer and washed with PBS. Cells (0.5–1.5 × 10⁶/sample) were incubated...
FIGURE 1. Reduced phagocytic ability of DCs in B7-2KO mice. Four- to 6-wk-old NOD and B7-2KO mice were injected with 200 μg of dextran-FITC per animal, sacrificed 1 h later and their spleens and PLN cells processed for flow cytometric analysis of dextran signal in APCs. Histograms (gated on the region R shown in the dot plot) depict the percentage of CD11c+ cells exhibiting dextran-FITC signal in PLN and spleen compartments. The overall level of phagocytosis in other APC populations (CD11b+CD11c- and CD11b+CD11c+) was found to be much lower (1–6%) and was not observed to be significantly different between the two groups (data not shown). The data are pooled from three independent experiments. b, Histograms and dot plots are gated on region R. The dotted lines in the histograms represent the isotype staining. The data are pooled from two to three independent experiments and are represented as mean percentage ± SEM.

with appropriate amounts of mAbs (1 μg/million cells) and stained for the indicated cell surface markers. Markers included CD8, CD11b, CD11c, CD16/32, CD40, MHC class I (kβ), MHC class II (I-Aα), B7-H1, and annexin and were obtained from BD Pharmingen. Anti-mouse B7-DC and CD205 were purchased from eBioscience and Serotec, respectively. Rabbit polyclonal anti-mouse PKC-ε was purchased from Santa Cruz Biotechnologies. The specificity of anti-PKC-ε Ab was checked by Western blot analysis using total splenocytes and a single ~90-kDa band was revealed, which is in agreement with the manufacturer’s observation regarding the expected size for PKC-ε. Anti-rabbit Cy5 Ab was obtained from Jackson Immunochemicals. FITC-labeled dextran was purchased from Sigma-Aldrich. The CD28-Ig and IgG3 have been previously described (4) and were a gift from Dr. C. Orabona (University of Perugia, Perugia, Italy).

For flow cytometric analyses, the dead cells were gated out on the basis of forward and side scatter. Appropriate isotype controls were used to determine the background staining. All values reported are mean ± SEM.

Ag capture

Ag uptake was conducted as previously described (20) with minor modifications. Briefly, 200 μl of FITC-dextran (1 mg/ml) was injected i.v. into the tail veins of mice. One hour later, mice were sacrificed, and spleens and PLNs were collected and analyzed for FITC signal by flow cytometry.

Immunofluorescence staining, confocal microscopy, and analysis

The adherent cells from total splenocytes from NOD and B7-2KO mice were prepared as previously described (21). Preparation was followed by staining the adherent cells with CD11c+ FITC and washing. One to 2 million of these cells were cultured in 48-well tissue culture plates in the presence of either CD28-Ig fusion protein or IgG3 (control) for 5 min at the concentration of 40 μg/ml in complete RPMI 1640. The reaction was stopped by 4% paraformaldehyde treatment for 12 min at room temperature; cells were centrifuged, followed by treatment with PBS containing 10 mM Tris (pH 7.4) for 5 min at room temperature. The cells were then permeabilized using permeabilizing solution (containing 0.2% saponin plus 4% paraformaldehyde) for 15 min at room temperature, followed by treatment with FACS buffer (containing 10 mM Tris (pH 7.4) plus 0.2% saponin) for 5 min and washed. The cells were stained with anti-PKC-ε (1/50, 1 h at room temperature), and anti-rabbit Cy5 (1/100, 45 min at room temperature) was used as the secondary Ab. Cells were washed three times with FACS buffer. Finally the cells were resuspended in minimum volume of Vectashield mounting medium (Vector Laboratories) for fluorescence containing DAPI (4',6'-diamidino-2-phenylindole) to visualize the nuclei. The cells were loaded on glass slides and analyzed using a confocal microscope (Radiance 2100 Rainbow; Bio-Rad). Instrument settings were kept constant for both NOD and B7-2KO groups throughout acquisition.

The confocal images were analyzed using ImageJ 1.33u program (National Institutes of Health), and the total area stained with PKC-ε was calculated. An appropriate threshold value (155 on a scale of 0–255) based on controls was applied to capture the real PKC-ε-associated signal, and the threshold settings were kept constant for both NOD and B7-2KO groups during analysis. The area stained with PKC-ε was calculated and expressed as the mean area ± SEM in squared microns.

Statistical analyses

The Student’s t test (unpaired, two-tailed) was used to determine the level of significance of the data using Statview software (Abacus Concepts). A value of p < 0.05 was considered as significant.
Results

Reduced phagocytic capability of APCs in B7-2KO mice

One important function of DCs is the phagocytosis of exogenous Ags. To address whether B7-2 impacts on Ag capture ability, we measured the phagocytic activities in DCs from NOD and B7-2KO mice in vivo by quantitatively comparing the uptake of fluorescently labeled polysaccharides (20, 22, 23). FITC-labeled dextran was injected i.v. into B7-2KO and control NOD mice. CD11c has been proposed to be the best marker for murine DC in lymphoid tissues (24). Consequently, the FITC signal in CD11c+ cells was assessed by flow cytometry after 1 h. We found a significantly reduced (p = 0.0003) percentage of CD11c+ dextran-positive cells in both the spleen and PLN compartments of mice lacking B7-2 (Fig. 1a). The overall level of phagocytosis in other APC populations (CD11b+CD11c+ and CD11b+CD11c−) was found to be much lower (1–6%) and was not observed to be significantly different between the two groups (data not shown), suggesting the phagocytic defect is found in CD11b+CD11c+ population (depicted by gated region in Fig. 1a). Indeed, initial uptake of foreign Ag has been previously shown to be preferentially associated with CD11b+CD11c+ DCs (25). We therefore concentrated on the CD11b+CD11c+ DC (designated as DCs) subset of APCs in our subsequent analyses.

A range of unique receptors such as the lectin-type receptor DEC-205 (CD205) and Fc (CD16/32) receptors are used by lymphoid tissue DCs to acquire and present Ag. CD205 has been shown to be involved in the uptake of carbohydrate conjugate Ags and enhances the efficiency of Ag presentation (9, 10). We therefore hypothesized that

FIGURE 2. B7-2 promotes DC survival. a, PLN cells from 6- to 8-wk-old mice were subjected to annexin staining as depicted by histograms. Cells are gated on CD11c+ DCs as shown in region (R) in Fig. 1a. The data are pooled from two independent experiments. Values shown represent mean percentage ± SEM. b, The total number of CD11c+ DCs (shown in R) in NOD and B7-2KO mice. For calculating total number of DCs, the percentage of CD11c+ cells was multiplied with the total cellular yield of PLNs. The data are pooled from 9–10 independent experiments and results are expressed as mean total number ± SEM.

FIGURE 3. Immunophenotypic analysis of DC subsets in B7-2KO mice. PLN cells from 5- to 8-wk-old animals were used. The histograms presented are gated on cells in region (R) shown in Fig. 1a. The dashed lines in the histogram represent the isotype staining. Data are pooled from two to four independent experiments. Values shown represent mean percentage ± SEM.
the diminished Ag uptake observed in B7-2KO mice may be accounted for by the reduced proportion of DCs exhibiting CD16/32 and CD205 expression. We found significantly reduced percentages of CD16/32<sup>+</sup> (p = 0.0003), CD8<sup>α</sup>CD205<sup>+</sup> (p = 0.040) cells as well as CD8<sup>α</sup>CD205<sup>−</sup> (p = 0.0005) cells within the DC compartment of PLNs in mice lacking B7-2 (Fig. 1b). In spleen, a similar reduction in the percentages of CD16/32<sup>−</sup> cells was observed in B7-2KO mice; however, no significant difference in the proportion of CD8<sup>α</sup>CD205<sup>−</sup>/CD8<sup>α</sup>CD205<sup>+</sup> cells was observed (data not shown). These observations suggest that B7-2 deficiency not only diminishes Ag uptake in vivo but also causes a reduction in the proportion of cells exhibiting Ag uptake receptors.

**B7-2 controls DC survival**

Accumulation of DCs resulting from defects in apoptosis can lead to the development of autoimmunity (17). We hypothesized that protection from pancreatic autoimmunity in B7-2KO mice may result from the enhanced apoptosis of the DC compartment. We therefore, quantitated the percentage of cells undergoing apoptosis in the DC compartment of B7-2KO mice by annexin staining both in spleen and PLNs. We found a significantly enhanced proportion of DCs exhibited annexin staining in the PLNs (p = 0.0006) and spleen (data not shown) of mice lacking B7-2 compared with NOD mice (Fig. 2a). In parallel, there was also a significant reduction in the total number of DCs in the PLN compartment in B7-2KO mice (4.4 ± 0.3 × 10<sup>5</sup> in B7-2KO mice vs 7.4 ± 0.5 × 10<sup>5</sup> in NOD, n = 30; p < 0.0001) (Fig. 2b). This finding suggests that B7-2 promotes DC survival in vivo.

**Immunophenotypic distinctions in B7-2-deficient DCs**

The impact of B7-2 on DC survival and function led us to surmise that protection from pancreatic autoimmunity may be associated with altered phenotypic characteristics of APCs. These changes could include changes in their state of activation (CD8<sup>α</sup> expression) (26), availability of alternative costimulatory molecules such as B7-1 and CD40, alterations in MHC (class I and class II) expression, or enhanced capacity to provide negative costimulation via B7-H1 and B7-DC molecules (14). CD8<sup>α</sup> DCs play an essential role in the induction of proinflammatory T cell responses (11) and endocytosis (12). We asked whether the proportion of CD8<sup>α</sup><sup>+</sup> DCs was altered in B7-2KO mice. Interestingly, we found a significantly reduced proportion (p < 0.0001) of CD8<sup>α</sup> DCs in the PLN of B7-2KO mice compared with NOD controls. Conversely, the proportion of CD8<sup>α</sup><sup>−</sup> DCs was significantly enhanced in B7-2KO mice. No significant difference in the proportion of CD8<sup>α</sup><sup>+</sup> DCs was observed in the spleen compartment of B7-2KO mice (data not shown). Interestingly, a significantly higher proportion (p = 0.0001) of MHC class II<sup>+</sup> DCs were found in the PLN of B7-2KO mice, although MHC class I expression was not significantly altered. (Fig. 3). In spleens, no significant difference in the proportion of MHC class II<sup>−</sup> DCs was observed (data not shown); however, a marginal but statistically significant increase in the percentage of MHC class I<sup>−</sup> DCs was seen (87.2 ± 1.2% in NOD vs 92.6 ± 0.8% in B7-2KO mice, n = 8; p = 0.002) in B7-2KO mice.

Changes in the expression of negative costimulatory molecules such as B7-H1 and B7-DC can affect the capacity of APCs to generate negative signals by binding to their putative-negative regulator PD-1 on T cells (14). We tested whether B7-2 affected the expression of B7-H1 and B7-DC. We found a significantly increased (p = 0.001) frequency of both B7-H1<sup>+</sup> and B7-DC<sup>−</sup> DCs in the spleens of B7-2KO mice (Fig. 3). In spleen, no significant difference in the proportion of B7-DC<sup>−</sup> DCs was observed (data not shown), however, a marginal but statistically significant decrease in the percentage of B7-H1<sup>+</sup> DCs was seen (34.2 ± 1.7% in NOD vs 28.9 ± 2.6% in B7-2KO, n = 8; p = 0.035) in B7-2KO mice. We next asked whether the availability of other costimulatory molecules, namely B7-1 and CD40, is altered in DCs devoid of B7-2. A modest but statistically significant (p = 0.008) increase in the percentage of B7-1<sup>−</sup> DCs was observed in B7-2KO mice, both in PLN (Fig. 3) and spleen compartments (data not shown). However, there was no significant difference in the percentage of CD40<sup>−</sup> DCs between B7-2KO and NOD controls, in the PLN and spleen compartments (data not shown). Thus, these observations demonstrate that B7-2 impacts the expression of negative costimulatory molecules and distribution of DC subsets in vivo.

**Reduced surface localization of PKC-ε in the absence of B7-2**

Our results indicate that B7-2 deficiency affects the phenotypic and phagocytic characteristics of DCs in vivo. The existence of three putative PKC phosphorylation sites in the cytoplasmic tail of B7-2 suggests that it may be able to transduce a signal directly in the APCs. PKC-ε has also been shown to play a critical role in phagocytosis (27, 28). Because PKC activation involves its stabilized localization with the cell membrane (29), we asked whether the membrane localization of PKC-ε was affected in B7-2-deficient APCs. For this purpose, adherent splenocytes from NOD and B7-2KO mice were first surface stained with CD11c-FITC and then treated in vitro with either CD28-Ig fusion protein or IgG3 (control) for 5 min. The cells were then fixed, permeabilized, and stained with anti-PKC-ε Ab and examined by confocal microscopy. At least 100 cells were individually analyzed in each group for the calculation of area stained with PKC-ε. The images depict CD11c (FITC, green), nuclei (DAPI, blue), and PKC-ε (Cy5, red) stained cells.

**FIGURE 4.** Reduced APC-associated PKC-ε response in the absence of B7-2-mediated costimulation. Adherent splenocytes from 4- to 6-wk-old NOD and B7-2KO mice were first surface stained with CD11c-FITC and then treated in vitro with either CD28-Ig fusion protein or IgG3 (control) for 5 min. The cells were then fixed, permeabilized, and stained with anti-PKC-ε Ab and examined by confocal microscopy. At least 100 cells were individually analyzed in each group for the calculation of area stained with PKC-ε. The images depict CD11c (FITC, green), nuclei (DAPI, blue), and PKC-ε (Cy5, red) stained cells.

a. Typical staining profile of CD28-Ig-stimulated NOD (i) and B7-2KO (ii) adherent cells. The image in i is a typical representation of cells stimulated with IgG3 (magnification ×1260). b. The area of PKC-ε stained (in squared microns) between NOD (□) and B7-2KO (□) groups. Error bars represent SEM. The data are pooled from two independent experiments.
confocal microscopy. The total surface area (in squared micrometers) of PKC-ε staining was calculated on a per cell basis. These analyses revealed that upon IgG3 treatment, a significantly reduced mean area of PKC-ε staining was observed in cells from B7-2KO mice treated as with compared with NOD control mice (2.0 ± 0.16 mm² in B7-2KO cells vs 3.4 ± 0.2 mm² in NOD cells, n = 100–110 cells per group; p < 0.0001). Upon CD28-Ig stimulation, a significant increase in the PKC-ε signal was observed in both groups. However, B7-2KO cells showed significantly reduced up-regulation of PKC-ε response compared with NOD control cells (5.2 ± 0.3 μm² in B7-2KO cells vs 7.6 ± 0.4 μm² in NOD cells, n = 106–123 cells per group; p < 0.0001) (Fig. 4). These data demonstrate that PKC-ε represents a downstream event in B7-2-mediated signaling inside APCs.

Discussion
In this study, we describe the role of B7-2 in regulating the APC function, survival, and phenotype in vivo. We show that B7-2 expression promotes Ag uptake and survival. Phenotypically, B7-2 deficiency led to the prevalence of a nonimmunogenic APC repertoire. We provide evidence that PKC-ε represents a component of B7-2-mediated signaling inside APCs.

DCs in lymphoid tissue use a range of unique receptors to acquire and present Ag, which include mannose (CD205) and Fc (CD16/32) receptors (13). CD205 is not only up-regulated upon CD28- and CD205-expressing cells. Interestingly, B7-2KO cells exhibited a preponderance of CD8α+/H9255 cells per group; 0.3

0.3

terestingly, B7-2KO mice exhibited a preponderance of CD8

0.0001

B7-2KO mice treated as with compared with NOD mice (2.0

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0.0001

Upon CD28-Ig stimulation, a significant increase in the PKC-ε signal during B7-2 deficiency may be the reduced generation of membrane diacylglycerol, which is proposed to be the primary lipid responsible for the surface association of PKC (42). Phospholipase Cγ2, which is responsible for producing membrane diacylglycerol, has been shown to be activated via B7-2 stimulation in B cells (7). There is no evidence for a direct interaction between PKC-ε and B7-2, but our data suggest that signals emanating from B7-2 could play an important role in controlling PKC-ε homeostasis inside monocytes. Several reports describe the correlation between PKC-ε levels in APCs with their phagocytic ability (27, 43). Thus, the reduced membrane-associated PKC-ε could contribute to the poor phagocytic function we observed in vivo. Detailed intramolecular binding studies are needed to define the interplay between PKC-ε and B7-2 in purified DCs.

In conclusion, our data demonstrate that B7-2 regulates DC maturation and function, thereby promoting the development of immunity and autoimmunity.

Acknowledgments
We thank members of Sarvetnick laboratory and Malin F. Tullberg for valuable comments. Sandrine Dubernet and William Kiosses are thanked for their expertise with confocal staining and interpretation of data. Lori Sterling, Amy Maday, Cody Fine, and Patrick Secrest are thanked for technical help. This manuscript no. 18528 is from the Department of Immunology, The Scripps Research Institute.

Disclosures
The authors have no financial conflict of interest.

References


