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Costimulator B7-DC Attenuates Strong Th2 Responses Induced by *Nippostrongylus brasiliensis*

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Abstract

The caliber and magnitude of T cell responses are regulated by costimulatory molecules following the engagement of TCRs and MHC molecules. B7-DC has the highest homology with B7-H1 in the B7 family, and both of them bind an immunoregulatory molecule, programmed death 1. Previous studies have demonstrated that B7-DC stimulates T cell proliferation and CTL generation, which sharply contrasts the inhibitory role of B7-H1. Th2 cytokines prompt B7-DC expression, which in turn enhances Th1 responses. In this study, we used an intestinal nematode, *Nippostrongylus brasiliensis,* to induce strong Th2 responses and to evaluate B7-DC function under Th2-polarizing conditions in vivo. By either blocking B7-DC expression during *N. brasiliensis* infection or by examining *N. brasiliensis*-infected B7-DC knockout mice, we observed enhanced eosinophilia, the overproduction of serum IgE, and increased Th2 cytokine production along with decreased Th1 cytokine production (particularly IFN-γ production), indicating that B7-DC inhibits Th2 responses. Our results further demonstrate that the inhibition of Th2 responses by B7-DC occurs independently of programmed death 1 but conceivably acts through an as yet unknown alternative receptor that enhances Th1 responses. Although the deficiency of B7-DC expression that enhanced the production of IL-13 paradoxically resulted in better protection against *N. brasiliensis* infection, our results show that B7-DC plays an important role in bolstering a robust Th1 response that is required for effective antiviral and anticancer immunity, even under a strong Th2-polarizing environment induced by *N. brasiliensis* infection.

Immediately following infection by most viruses, bacteria, or parasites, Ags from these pathogens are captured and processed by APCs to induce adaptive immune responses, which are primarily mediated by T cells. The specificity of the adaptive immune response is

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determined by the interaction between TCR and the MHC molecule bound by antigenic peptides. Subsequently, the quality and the quantity of T cell responses are determined by the costimulatory molecules represented by the B7 family molecules, leading to the following stimulatory or inhibitory immune responses. In recent years, seven molecules that belong to B7 family molecules have been identified (1, 2). B7-1 (CD80) and B7-2 (CD86) are prototypical B7 family molecules that costimulate T cells via interaction with CD28 and downregulate T cell responses via CTLA-4 (CD152). B7-H1 (CD274/PD-L1) and B7-DC (CD273/PD-L2) constitute a second pair of molecules that bind the same inhibitory receptor, programmed death (PD) 1 (CD279). Furthermore, the recent discovery of the inhibitory effect by CD80 binding to B7-H1 suggests that the function of B7 family molecules is much more complicated than originally thought in terms of the regulation of T cell immune responses (3).

The homology of B7-H1 and B7-DC is the highest among B7 family molecules, and their genes reside in close proximity to each other (103 and 120 kbp apart on chromosome 19 and 9 in mouse and human, respectively) (4). These two costimulatory molecules are regulated quite differently. For instance, B7-H1 is expressed ubiquitously and is strongly induced by IFN-γ, whereas the expression of B7-DC is highly restricted to dendritic cells (DCs) and activated macrophages and is strongly induced by IL-4 and IL-13 (5, 6). It is also interesting that previous in vivo studies using either knockout (KO) mice or Abs that block PD-1, B7-H1, and B7-DC have consistently shown similar inhibitory function for PD-1 and B7-H1, but not for B7-DC (7).

B7-DC was originally characterized as a strong stimulator of T cells, enhancing T cell proliferation and IFN-γ production with or without CD28 costimulation (4, 8). B7-DC facilitates CD40L expression on activated T cells and also has a synergistic effect on T cell proliferation and cytokine production with CD80 or CD86 expression in a PD-1–independent manner. Furthermore, mutant protein variants of B7-DC and B7-H1 with compromised binding to PD-1 retain their costimulatory ability despite this (9). Finally, a mouse plasmacytoma cell line transduced with B7-DC was much more vulnerable to an anticancer CTL response, which also occurs in a PD-1–independent manner (10). Other evidence of the stimulatory function of B7-DC has been established using the agonistic human anti-mouse B7-DC IgM Ab, which induces the maturation and migration of DCs, as well as enhanced tumor-specific immunity by T cells against many cancers (11, 12). B7-DC–deficient DCs demonstrated a lower capacity to stimulate T cell proliferation and Th1 cytokine production in vivo (13). In addition, B7-DC–deficient mice were more susceptible than wild-type (WT) mice to syngeneic cancer cell challenge due to the lower potency of tumor-associated Ag-specific CTLs. Taken together, these observations suggest the existence of at least one alternative receptor for B7-DC that enhances T cell proliferation and polarizes cells toward a Th1 immune response. In contrast, B7-DC can indeed bind to the inhibitory receptor PD-1 in vitro, suggesting the possibility of an inhibitory function of B7-DC in vivo as well (14).

If the expression of B7-DC is maximized in vivo, will its function be stimulatory or inhibitory? To answer this question, we investigated the invivo function of B7-DC under strong Th2-polarizing conditions using the intestinal nematode *Nippostrongylus brasiliensis.*

N. brasiliensis is a gastrointestinal parasite of rodents with a similar life cycle to the human hookworm (*Necator americanus* and *Ancylostoma duodenale*). We show in this study that Th2 responses, such as eosinophilia and hyper-IgE, were enhanced and IFN-γ production was reduced in B7-DC KO mice infected with *N. brasiliensis* and that this response was independent of PD-1. The exaggerated Th2 response, especially IL-13 production by T cells in B7-DC KO mice, enhanced the expulsion of *N. brasiliensis* from mouse intestines. This indicates that the inhibition of Th2 responses by B7-DC results in robust Th1 responses that in turn act as a negative feedback system of Th2 immune responses. This is the first demonstration of the mechanism by which B7-DC inhibits Th2 responses using an intestinal parasite infection model.

Materials and Methods

Mice

WT, B7-DC KO, IFN-γ KO, and DO11.10 TCR transgenic mice (all on a BALB/c background) were maintained in accordance with the institutional guidelines of the Jikei Medical University (Tokyo, Japan), the Johns Hopkins University (Baltimore, MD), and the University of Texas Health Science Center at San Antonio (San Antonio, TX). WT, IFN-γ KO, and DO11.10 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained under specific pathogen-free conditions. All experiments were performed using 10–12-wk-old mice and were approved by the Animal Care and Use Committee of the Jikei Medical University, the Johns Hopkins University, and University of Texas Health Science Center at San Antonio.

Abs and reagents

FITC-conjugated anti-mouse PD-1 (RMP1-30), PE-conjugated anti-mouse B7-DC(TY25), B7-H1(MIH5), PD-1(J43), IL-17 (eBio13A), I-Edcross-reacting I-Ek (14-4-4S), CD86 (GL1), allophycocyanin-conjugated anti-mouse IFN-γ (XMG1.2), DO11.10 TCR (KJ1-26) and FoxP3 (FJK-16s), allophycocyanin-Cy7-conjugated anti-mouse CD4 (RM4-5) mAbs were purchased from eBioscience (San Diego, CA). FITC-conjugated anti-mouse CD3 (145-2c11), CD4 (RM4-5), CD45R/B220 (RA3-6B2), IFN-γ (R4-6A2), allophycocyanin-conjugated anti-mouse CD11c (HL3), IFN-γ (R4-6A2), and purified CD16/32 (2.4G2) mAbs were purchased from BD Biosciences (San Jose, CA). Purified anti-mouse B7-DC (TY25) and PD-1 (RMP1-14) mAbs were generated at Juntendo University, Tokyo, Japan. Recombinant mouse GM-CSF and B7-DC (PD-L2)-Fc were purchased from R&D Systems (Minneapolis, MN).

Induction of Th1, Th2, and Th17 cytokines and intracellular cytokine staining

Bone marrow-derived DCs (BMDCs) were prepared by culturing them with 1000 U/ml recombinant mouse GM-CSF (R&D Systems) for 7 d. $CD4^+$ DO11.10 T cells were purified using a magnetic isolation kit (Miltenyi Biotec, Auburn, CA). The purity of $CD4^+$ T cells was monitored by FACS and was always >90%. Fifty thousand $CD4^+$ DO11.10 T cells and the same number of BMDCs from WTor B7-DC KO micewere cultured with 0.2 μg/ml $OVA_{323–339}$ peptide for 5 d in the presence of recombinant human TGF-β (2 ng/ml) and mouse IL-6 (10 ng/ml) and/or mouse IL-23 (10 ng/ml), anti-mouse IL-4 mAb (10 μg/ml),

and anti-mouse IFN-γ mAb (10 μg/ml) for Th17 generation. For the generation of inducible regulatory T cell (Treg), $CD4^+CD25^-$ DO11.10 T cells were magnetically purified and cultured with BMDCs in the presence of $OVA_{323–339}$ peptide and recombinant human TGF-β (2 ng/ml). Four to six hours prior to cells being harvested, additional $OVA_{323–339}$ peptide (1.0 μg/ml) or PMA, ionomycin (Sigma-Aldrich, St. Louis, MO), and GolgiStop (BD Biosciences) were added to each well for restimulation. Intracellular IFN-γ and IL-17 were stained after surface staining of the CD4 receptor had been completed (15–18).

Adoptive transfer of Ag-loaded DCs or OVA-specific $CD4^+$ T cells

BMDCs from WT or B7-DC KO mice were incubated with 20 μg/ml chicken OVA (OVA protein) for 3 h, then washed three times with PBS and adoptively transferred by i.v. injection to naive WT or B7-DC KO mice (10^6 cells per mouse). Seven days later, whole spleen cells or magnetically sorted (MACS, Miltenyi Biotec) $CD4^+$ T cells from spleen were subsequently cultured in vitro for 48 h. The cytokine content in the culture supernatant was detected using a Cytokine Beads Array kit (BD Biosciences) or ELISA.

One million DO11.10 $CD4^+$ T cells were magnetically purified (Miltenyi Biotec) and adoptively transferred to naive mice on day −1. The mixture of $OVA_{323–339}$ peptide and IFA was emulsified and injected intradermally into mice. Anti–PD-1 RMP1-14 mAb was administrated by i.p. injection on days 0, 3, 5, 7, and 10. The percentage of transferred DO11.10 $CD4^+$ T cells in the leukocytes of peripheral blood was measured on days 3, 6, 9, and 12.

N. brasiliensis *infection and ex vivo experiments*

Mice were inoculated by s.c. injection with 750 third-stage larvae (L3) of *N. brasiliensis* on day 0 for primary infection and additionally on day 21 for secondary infection (19). The peripheral blood was collected from anesthetized mice 14 d after primary infection (which was 7 d after the second challenge). For Ag-specific responses, 1 μg DNP-conjugated *N. brasiliensis* adult Ag (DNP-*N. brasiliensis*) was mixed with 2 mg alum (Wako Pure Chemical Industries, Osaka, Japan). This mixture was administered by i.p. injection 2 wk after the second challenge of *N. brasiliensis.* The haplotype of the DNP-specific Ab in peripheral blood was determined by ELISA. Eosinophils were counted using phase-contrast light microscopy after staining with Hinkelmann's solution. The serum IgE concentration was determined by ELISA. The mesenteric lymph nodes (MLNs) were isolated from *N. brasiliensis*-infected mice 7 d after L3 inoculation. Eight million cells per well were cultured in 24 well plates for 48 h. The cytokine concentration in the culture supernatant was detected by ELISA. To block B7-DC or PD-1, either anti–B7-DC mAbs (TY25) or anti–PD-1 mAbs (RMP1-14) were administered by i.p. injection on days 0, 3, 5, 7, and 10. For the enumeration of egg production, feces from individual *N. brasiliensis*-infected mice were collected daily and fixed with 1% formaldehyde. The eggs were then counted using a phase-contrast microscope.

Results

Th17 and Treg generation by B7-DC KO DCs in vitro and in vivo

Activated $CD4^+$ T cells can differentiate into an ever-increasing number of distinct lineages (20). On the basis of cytokine production and function, Th1, Th2, Th17, and Treg lineages have been defined, with Th9 cells possibly being the newest lineage to be added to this group (21, 22). We demonstrated in previous studies that Th2 cytokines prompted B7-DC expression, which in turn enhances Th1 responses. This was based on the fact that a deficiency of B7-DC expression on DCs led to decreased Th1 and increased Th2 cell differentiation both invitro and invivo (13). In our current study, we evaluated the influence of B7-DC expression on the generation of the remaining two major $CD4^+$ T cell lineages, specifically Th17 and Tregs. Th17 cells are IL-17–producing $CD4^+$ T cells, which are widely regarded as a proinflammatory cytokine associated with many inflammatory diseases, such as experimental autoimmune encephalomyelitis (EAE), rheumatoid arthritis, asthma, systemic lupus erythematosus, and allograft rejection (23). In contrast, Tregs are a major immunosuppressive population that suppress T cell-mediated immune responses and are generally thought to prevent autoimmune diseases (24, 25).

Th17 cells were generated in vitro by culturing DO11.10 $CD4^+$ T cells with B7-DC sufficient or deficient BMDCs in the presence of the OVA peptide and pertinent cytokines. B7-DC deficiency on these BMDCs did not appear to affect the differentiation of Th17 cells on the basis of intracellular staining as IFN-γ^- and IL-17^+ cells (Fig. 1A). Based on phenotype, naturally occurring Tregs (Fig. 1*B*) and inducible Tregs (Fig. 1*C*) from the spleens of B7-DC KO mice were indistinguishable from the corresponding populations from WT mice. This suggests that Treg differentiation was also unaffected by B7-DC deficiency.

We initially wished to determine whether B7-DC deficiency had any effect on Th polarization after induction of mild immune responses in vivo. To this end, we immunized WT mice with WT BMDCs pulsed with whole OVA protein. Conversely, we immunized B7-DC KO mice with OVA protein-pulsed BMDCs from the corresponding KO mice. Whole spleen cells (Fig. 1*D*) or purified $CD4^+$ T cells (Fig. 1*E*) were cultured ex vivo, and cytokine production in the culture supernatant was measured. Interestingly, Th1 cytokines, such as IFN-γ and IL-2, were produced at a much lower level in the supernatants from B7-DC KO mice compared with WT mice. In contrast, Th2 cytokines, such as IL-4 and IL-5, were readily detected in the supernatants from B7-DC KO mice relative to WT mice. No appreciable differences were observed in the production of IL-17 (Fig. 1*D*, 1*E*) or TNF-α (data not shown) from WT and B7-DC KO spleens. The total number and proportion of leukocyte populations in spleen before ex vivo culture were also unchanged (data not shown). Moreover, apoptotic cells were only 2% to 3% of the total population of whole spleen cells and $CD4^+$ T cells isolated from either WT or B7-DC KO recipients (data not shown).

Th2 responses in B7-DC KO mice are exaggerated by parasite infection

To analyze the in vivo function of B7-DC under strong Th2-polarizing conditions, mice were infected with *N. brasiliensis*. Following s.c. inoculation of mice with the L3, the larvae

initially migrate to the lungs, where they molt to become fourth-stage larvae. They subsequently migrate to the gastrointestinal tract, where they develop into fully mature adult worms and lay eggs (26, 27). Intestinally localized mature adult worms are highly immunogenic and induce significant Th2 cytokine production, such as IL-4, -5 and -13, resulting in eosinophilia and hyper-IgE.

Two weeks after L3 inoculation, B7-DC KO mice had more than twice the level of eosinophilia and significantly elevated serum IgE compared with infected WT mice (Fig. 2*A*). One week after the second recall infection of *N. brasiliensis*, B7-DC KO mice showed an ever further increase in both eosinophilia and serum IgE levels (Fig. 2*B*). In another setting of the experiment, we observed similar kinetics of eosinophilia in B7-DC KO mice compared with WT mice after L3 inoculation (Fig. 2*C*). Hyper-IgE was also evident in B7-DC KO mice, but was less pronounced in this particular case (Fig. 2*D*). The Ag-specific Ab class switch was triggered by i.p. injection of DNP-conjugated *N. brasiliensis* Ag 2 wk after the second inoculation of L3 (Fig. 2*D*). The production of representative Th2 class-switching, Ag-specific IgE was greater in B7-DC KO mice compared with WT mice, with little difference in either IgG1 (Fig. 2*E*) or other IgG subclasses (IgG2a, IgG2b, and IgG3) observed (data not shown). After L3 inoculation, the total number of cells isolated from the MLNs of both WT and B7-DC KO mice increased significantly starting 5 d postinfection (Fig. 2*F*). Following L3 inoculation, all MLNs are generally considered to be draining lymph nodes. Interestingly, we observed a significant drop in IFN-γ secretion, with elevated levels of IL-4, IL-5, and IL-13 secretion from the MLNs isolated from B7-DC KO mice compared with WT mice (Fig. 2*G*). The source of these cytokines remains to be determined. Taken together, these data indicate that B7-DC dramatically inhibits Th2 responses that are strongly induced by *N. brasiliensis* infection.

DCs are generally considered as the principal cell population involved in Ag uptake and presentation, thereby initiating T cell-mediated immune responses (28). Therefore, we next examined the level of expression of key surface molecules on myeloid DCs (mDCs) and plasmacytoid DCs (pDCs), such as MHC class II, CD86, B7-H1, and B7-DC after *N. brasiliensis* infection (Fig. 3*A*, 3*B*). B7-DC expression on mDCs was significantly upregulated by *N. brasiliensis* infection to a far greater extent than that observed for infectious agents, such as *Leishmania*, *Schistosoma*, and *Listeria* (29–31). However, aside from the obvious deficiency of B7-DC on both mDCs and pDCs from B7-DC KO mice, no significant difference in the expression of these key molecules was observed. We also tested the expression of PD-1, a known B7-DC receptor, in *N. brasiliensis*-infected mice and found that there was no significant difference in PD-1 expression between WT and B7-DC KO mice (Fig. 3*C*). We speculate that the exaggerated Th2 responses in B7-DC KO mice result from the loss the B7-DC expression.

The inhibition of Th2 responses by B7-DC is independent of PD-1

Following the administration of anti–B7-DC blocking mAb (TY25), *N. brasiliensis*-infected WT mice showed an elevated level of eosinophilia and hyper-IgE compared with WT mice receiving control IgG (Fig. 4*A*). We then evaluated the effect of anti–PD-1 blocking mAb (RMP1-14) on *N. brasiliensis*-infected WTand B7-DC KO mice. The blocking of PD-1

using this mAb has been shown to be sufficient to exaggerate the effects of asthma, insulitis, EAE, graft-versus-host disease, and chronic intestinal inflammation in mouse models (7, 32–35). Blocking PD-1 mAb also facilitates the rejection of organ transplantation (36, 37) and benefits anticancer immunity (38, 39).

In fact, the administration of anti–PD-1 mAb RMP1-14 completely blocked the binding of the B7-DC-Fc fusion protein to upregulated PD-1 but did not affect the binding of an anti–PD-1 mAb clone RMP1-30, which interacts with a different epitope and does not compete with RMP1-14 (Fig. 4*B*). This indicates that RMP1-14 can block PD-1 but does not deplete PD-1$^+$ cells. Following *N. brasiliensis* infection, we observed <20% elevation in either eosinophilia or hyper-IgE in the mice treated with RMP1-14 anti–PD-1 mAb compared with mice treated with control IgG, a difference that is not considered to be statistically significant (Fig. 4*C*). Furthermore, there was no significant difference in eosinophilia or hyper-IgE between B7-DC KO mice treated with anti–PD-1 mAb treatment or control IgG Ab (Fig. 4*D*). This suggested that the PD-1–B7-DC pathway might not influence the inhibition of Th2 responses, although the administration of anti–PD-1 mAb RMP1-14 itself indeed augmented the proliferation of Ag-specific T cells in vivo (Fig. 4*E*) as previously reported (40). No further exaggerated Th2 responses were observed when *N. brasiliensis*-infected IFN-γ KO mice were treated with anti–B7-DC mAb (Fig. 4*F*). These data indicate that the inhibition of Th2 responses by B7-DC during *N. brasiliensis* infection is independent of PD-1 signaling but is dependent upon Th1 responses.

Host protection against N. brasiliensis *is promoted in B7-DC KO mice*

Aside from the host's Th2 immune responses, intestinal nematode infection is often completely asymptomatic. However, heavy infestation may cause host malnutrition, and the larval migration stage may cause tissue damage and inflammation under such circumstances. Nematodes may also cause obstruction in the intestine, bile duct, or pancreatic duct. Therefore, it is important for the host to attempt to expel parasites, such as *N. brasiliensis*, during the course of its life cycle by inducing Th2 immune responses. Host protection against *N. brasiliensis* is reported to be entirely dependent on IL-13 and STAT6 signaling (26, 41–44).

It has already been well established that the female *N. brasiliensis* lays eggs around day 6 postinfection, and the eggs must be passed in the feces until all adult worms have been expelled by approximately day 10 (27, 45). However, the expulsion of *N. brasiliensis* was significantly accelerated in B7-DC KO mice compared with WT mice (Fig. 5), and this correlated with enhanced IL-13 production in the MLNs (Fig. 2*G*). The total number of expelled eggs is a reflection of the number of adult *N. brasiliensis* in the intestine, with an absence of expelled eggs indicating that all adult parasites have been expelled from the host intestine. The accelerated and complete expulsion of *N. brasiliensis* is also a reflection of the inhospitable environment in the intestines of B7-DC KO mice. Although B7-DC plays an important role in tuning the Th1 responses that are beneficial to antivirus and anticancer immune responses, the deficiency of B7-DC is, paradoxically, helpful for host immune responses against *N. brasiliensis* infection.

Discussion

In this study, we demonstrate in vivo induction of Th1, Th2, and Th17 cytokines using the adoptive transfer of OVA protein-pulsed BMDCs. Consistent with our previous invitro and invivo data, lower IFN-γ production was observed in the B7-DC KO mice compared with WT mice. Blocking or deleting B7-DC in mice results in markedly reduced Th1 polarization with enhanced Th2 polarization without influencing Th17 or Treg differentiation. This is consistent with the data showing that B7-DC KO mice do not have any greater propensity to develop EAE, whereas B7-H1 and PD-1 KO mice developed much more severe EAE compared with WT mice (46). It is interesting that the regulation and function of B7-DC in response to *N. brasiliensis* only affects switching between Th1 and Th2 responses. On the other hand, the PD-1–B7-H1 axis does not seem to affect Th2 responses induced by *N. brasiliensis*.

In this study, we have established that B7-DC plays a critical inhibitory role in controlling the exaggerated Th2 responses induced by *N. brasiliensis* infection. This is primarily based on the fact that *N. brasiliensis*-infected B7-DC KO mice have increased eosinophilia, hyper-IgE, and production of IL-4, IL-5, and IL-13 compared with *N. brasiliensis*-infected WT mice. Except for the deletion of B7-DC expression in B7-DC KO mice, we were unable to detect changes in the expression of other important surface molecules on either mDCs or pDCs from either B7-DC KO or WT mice. At this point, we infer that the primary mechanism by which Th2 responses are attenuated might notbe mediated through PD-1 but by stimulating Th1 responses by means of an alternative receptor. However, our experiments remain equivocal on this point. Therefore, in the absence of further evidence, we must admit that it is premature to conclude the independency of PD-1 on Th2 responses.

To induce and maintain Th1 cells, it is well established that there are several key factors, such as CD80, CD86, ICOS/CD278, IL-12, STAT4, and T-bet (47), that modulate Th1 polarization. It is possible that downstream signaling by an alternative receptor engaging B7-DC may selectively lead to the upregulation of T-bet expression and the inhibition of GATA-3 expression in responder T cells. Several studies have demonstrated that cross-linking B7-DC by human anti-mouse IgM Ab results in the activation, maturation, and migration of IL-12–producing DCs (12, 48, 49). Therefore, it is also possible that the local production of IL-12 by DCs in response to reverse signaling by B7-DC is also important in generating Th1 responses in *N. brasiliensis* infection. Paradoxically, the loss of B7-DC expression significantly enhanced protection against *N. brasiliensis*. Although it has been well established that host protection against *N. brasiliensis* is entirely dependent on IL-13 and STAT6 signaling (26, 41–44), this should be confirmed in a B7-DC KO mouse model. Confirmation would either involve attenuating IL-13 by the administration of blocking Abs or by deriving a double B7-DC KO and IL-13 KO mouse.

The purpose of our current study was to evaluate the role of B7-DC under highly Th2-polarizing conditions. In this study, we have shown that B7-DC expression still supports Th1 responses under such circumstances, a finding that has also been corroborated by several other studies as well. For instance, DCs from B7-DC KO mice are less potent in priming T cells to produce Th1 cytokines both in vitro (4) and in vivo (13). In addition, IgG2a class

switching and anticancer immune responses are impaired in B7-DC KO mice (13). Finally, experiments involving Th1-dominant in vivo conditions, such as in contact hypersensitivity experiments, have shown that B7-DC KO mice or mice treated with anti–B7-DC blocking Abs were indistinguishable from WT mice (T. Shin, unpublished observations).

Matsumoto et al. (6, 32) have demonstrated that B7-DC regulates Th2 immune responses through a feedback loop in an allergic asthmatic mouse model. However, the effects of allergic asthma are largely confined to the bronchi and lungs, which might not apply to other organs. *N. brasiliensis* infection affects the host as a whole, with strong Th2 induction that leads to eosinophilia, hyper-IgE, and Th2 cytokine production in serum. In this study, we have demonstrated that B7-DC modulates strong primary and secondary Th2 responses induced by *N. brasiliensis* infection. This is consistent with a study by Liang et al. involving *Leishmania mexicana* infection in B7-DC KO mice. They observed that B7-DC KO mice developed larger lesions than WT mice in which Th1 responses were generally protective, suggesting that B7-DC KO mice either failed to induce the appropriate Th1 responses or they failed to inhibit exaggerated Th2 responses (29).

In conclusion, B7-DC is strongly induced by Th2 cytokines, which in turn specifically enhances Th1 responses. Although either B7-DC deficiency or the blocking of B7-DC may help clear an *N. brasiliensis* infection, B7-DC plays an important role, under strong Th2-polarizing conditions, in tuning Th1 polarization, a response that is in fact beneficial to antiviral and anticancer immunity.

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Abbreviations used in this paper

BMDC	bone marrow-derived dendritic cell
DC	dendritic cell
EAE	experimental autoimmune encephalomyelitis
EPG	egg output per gram of feces
KO	knockout
L3	third-stage larvae
mDC	myeloid dendritic cell
MLN	mesenteric lymph node
PD	programmed death
pDC	plasmacytoid dendritic cell

Treg	regulatory T cell
WT	wild-type

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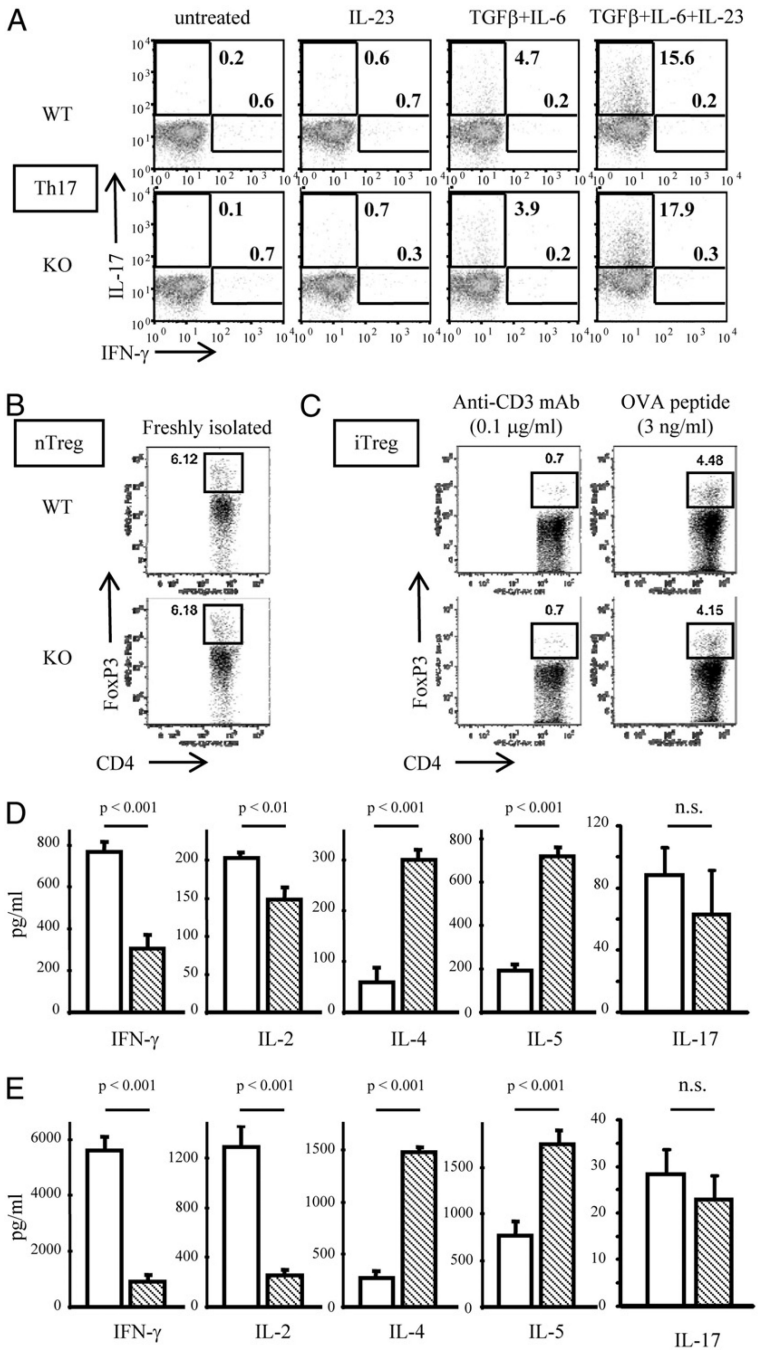

FIGURE 1.

B7-DC does not affect Th17 or Treg generation but regulates Th1/Th2 responses. *A*, Purified DO11.10 $CD4^+$ T cells and BMDCs from WT or B7-DC KO mice were incubated in Th17-polarizing conditions in vitro for 4 d in the presence of OVA peptide. Only $CD4^+$-gated cells are shown in each panel. *B*, Freshly isolated spleen cells from WT or B7-DC KO mice were stained for CD4 and FoxP3. *C*, $CD4^+CD25^-$ cells were purified from spleen and lymph nodes of DO11.10 mice and cultured with TGF-β and BMDCs from either WT or B7-DC KO mice for 4 d in vitro in the presence of anti-CD3 mAb or OVA peptide. *D* and *E*, OVA

protein-pulsed BMDCs from WT mice were administered by i.v. injection into WT mice (open bars, $n = 5$). Alternatively, OVA protein-pulsed BMDCs from B7-DC KO mice were administered by i.v. injection into B7-DC KO mice (hatched bars, $n = 5$). Seven days later, whole spleen cells (*D*) or purified $CD4^+$ T cells from spleen (*E*) were isolated from these mice and cultured in vitro. Cytokine production in culture supernatants was measured 48 h later. These data are representative of three to four independent experiments.

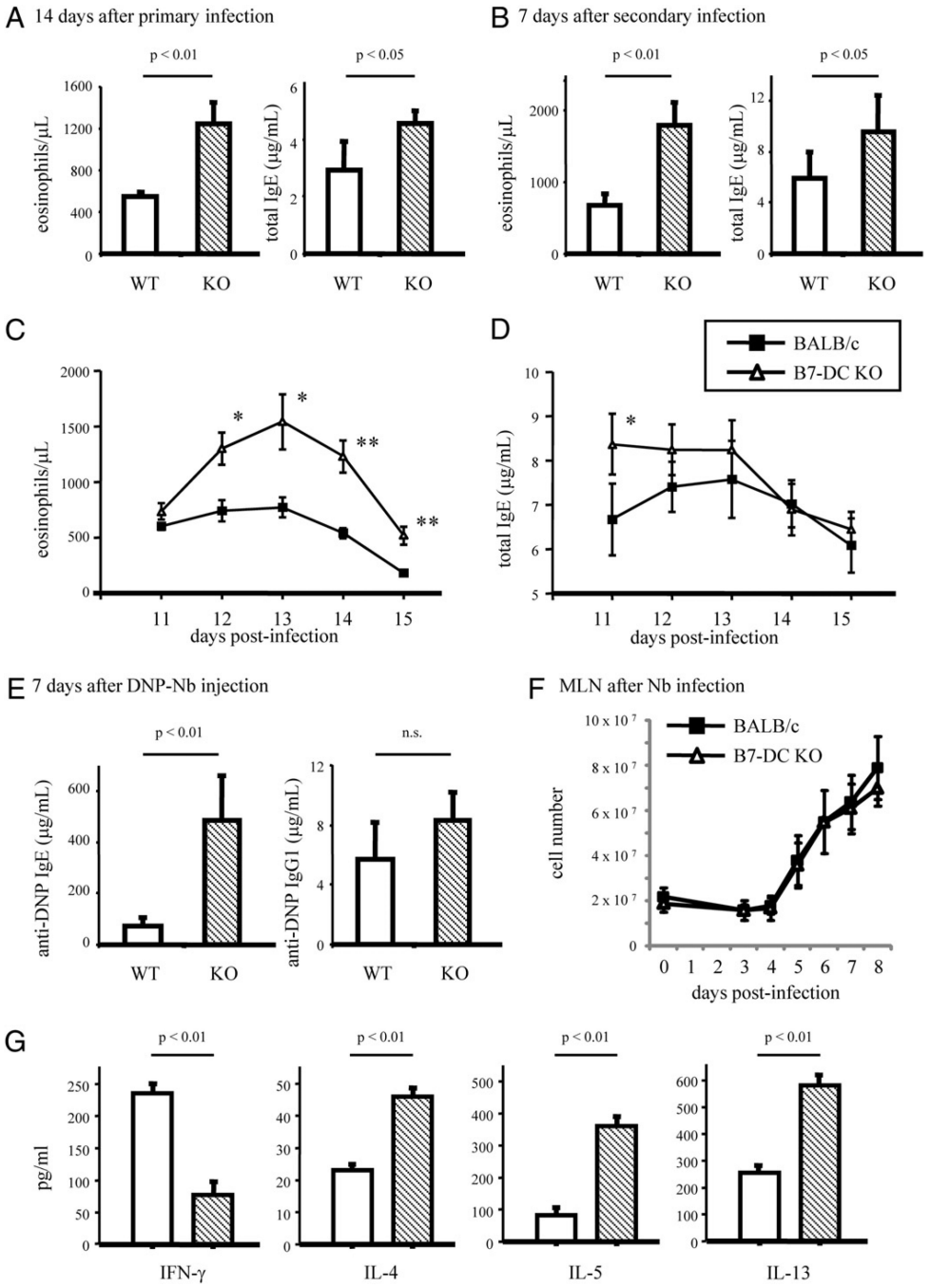

FIGURE 2.

Th2 responses to *N. brasiliensis* infection are greatly augmented in B7-DC KO mice. *A–E*, WT ($n = 5$) or B7-DC KO ($n = 5$) mice were inoculated by s.c. injection with L3 larvae on day 0 (*A, C, D*) or day 0 and 21 (*B*). The number of eosinophils and the concentration of IgE in peripheral blood were measured on day 14 (*A*), day 28 (*B*), or as indicated (*C* and *D*). *E*, DNP-conjugated *N. brasiliensis* adult Ag was administrated via i.p. injection on day 35 following secondary *N. brasiliensis* infection. The haplotype of the DNP-specific Ab was measured 7 d after DNP-*N. brasiliensis* injection. *F*, The total cell number in MLNs of WT

($n = 5$) and B7-DC KO ($n = 5$) mice before and after L3 inoculation was determined. *G*, MLNs were extracted 7 d after primary *N. brasiliensis* infection of WT ($n = 5$) or B7-DC KO ($n = 5$) mice. The single-cell suspension of MLNs was cultured in vitro for 48 h prior to measuring cytokine production in the culture supernatant. For reference, an uninfected mouse has <50 eosinophils per microliter of blood, and its serum IgE level is typically undetectable (data not shown). *A*–*G*, These data are representative of three to four independent experiments. *$p < 0.05$; **$p < 0.01$.

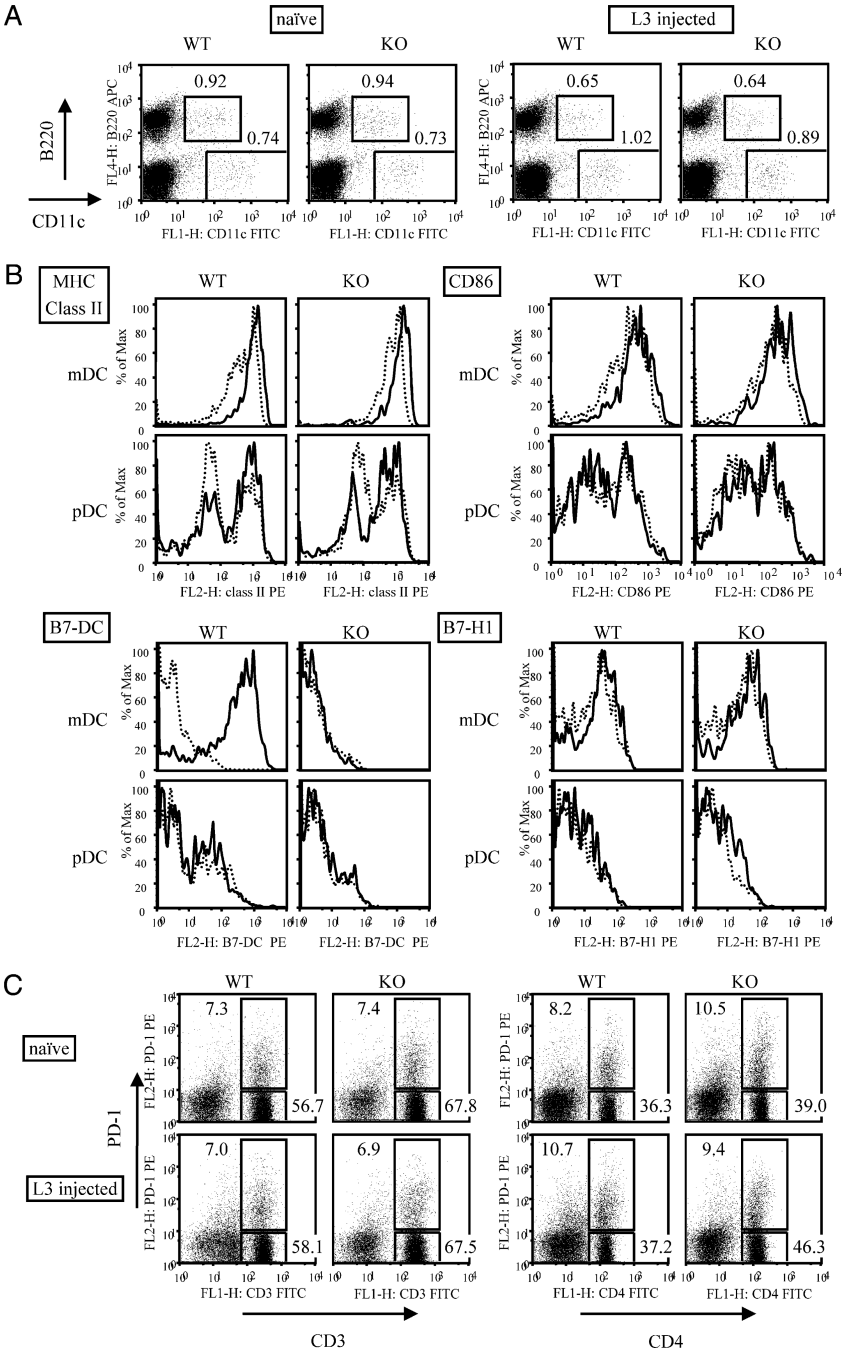

FIGURE 3.

Indistinguishable surface marker profile on DCs from either WT or B7-DC KO mice. A single-cell suspension of MLNs was prepared from WT and B7-DC KO mice 7 d after L3 s.c. injection. A, Phenotypic profiles of mDCs ($CD11c^+B220^-$) and pDCs ($CD11c^+B220^+$). *B*, MHC class II, CD86, B7-H1, and B7-DC expression on mDCs and pDCs. *C*, PD-1 expression on T cells. Solid lines indicate 7 d after L3 inoculation, and dashed lines indicate untreated controls. These data are representative of three independent experiments.

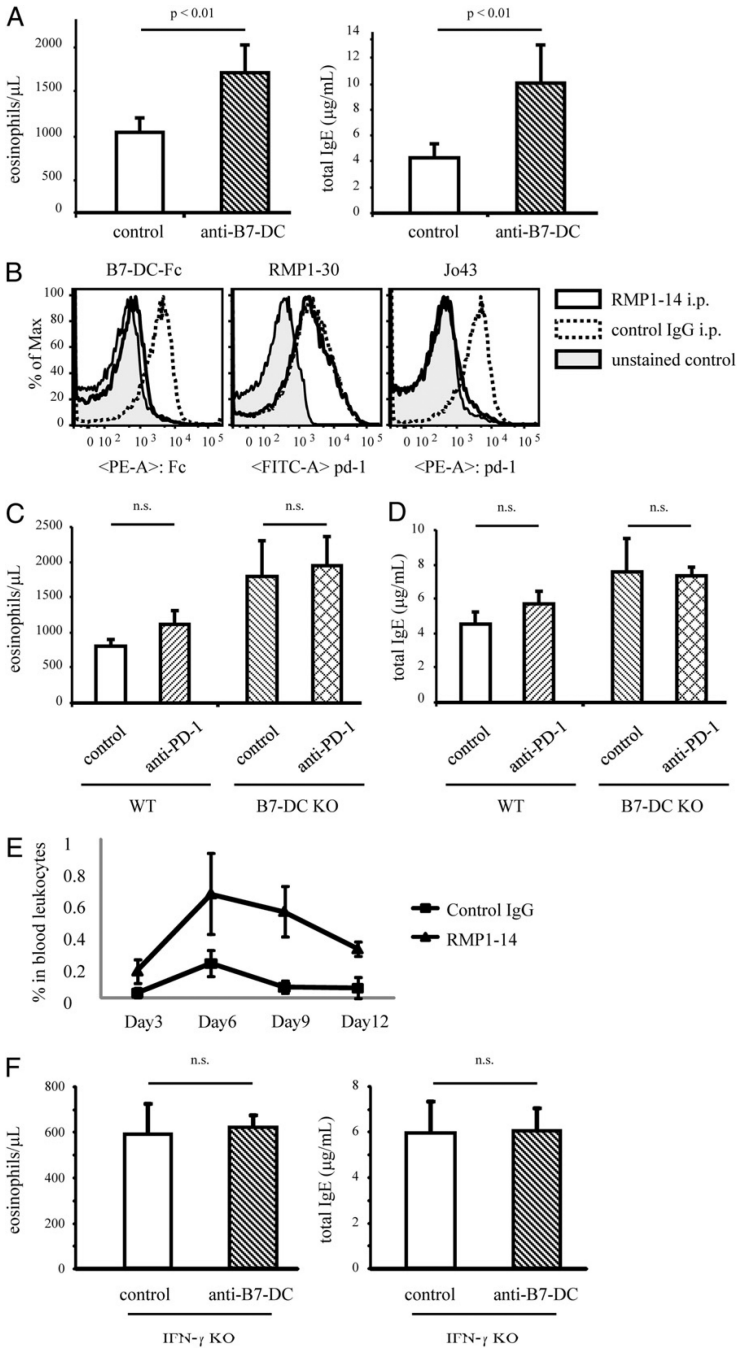

FIGURE 4.

Exaggerated Th2 responses caused by *N. brasiliensis* infection in B7-DC KO mice are independent of PD-1. *A, C, D,* and *F*, WT ($n = 5$), B7-DC KO ($n = 5$), or IFN-γ KO ($n = 5$) mice were inoculated by s.c. injection with L3 on day 0. Every 3 d starting at day −1, 150 μg anti–B7-DC mAb (TY25) (*A* and *F*) or anti–PD-1 mAb (RMP1-14) (*C* and *D*) was administered to each mouse via i.p. injection. The number of eosinophils and concentration of IgE in peripheral blood were measured on day 14. *B*, Mice were injected with 10 μg anti-CD3 mAb on day 0 via i.v. injection, and 150 μg anti–PD-1 mAb (RPM1-14) ($n = 5$) or

control IgG ($n = 5$) was administered i.p. on day 1. On day 3, single-cell suspensions of spleens were stained with B7-DC–Fc-secondary-PE or anti–PD-1 mAb (RMP1-30 or Jo43) and analyzed by flow cytometry. $CD3^+B220^-$ cells are shown. *E*, Purified OVA-specific $CD4^+$ DO11.10 T cells were adoptively transferred to naive recipient mice on day −1. These mice were treated with anti–PD-1 mAb (RPM1-14) ($n = 5$) or control IgG ($n = 5$) on the same schedule as in *C* and *D*. Mice were immunized with cognate OVA peptide-containing IFA on day 0. Transferred $CD4^+Do11.10^+$ T cells in the leukocyte gate of the peripheral blood were measured on the indicated day. These data are representative of three to four independent experiments.

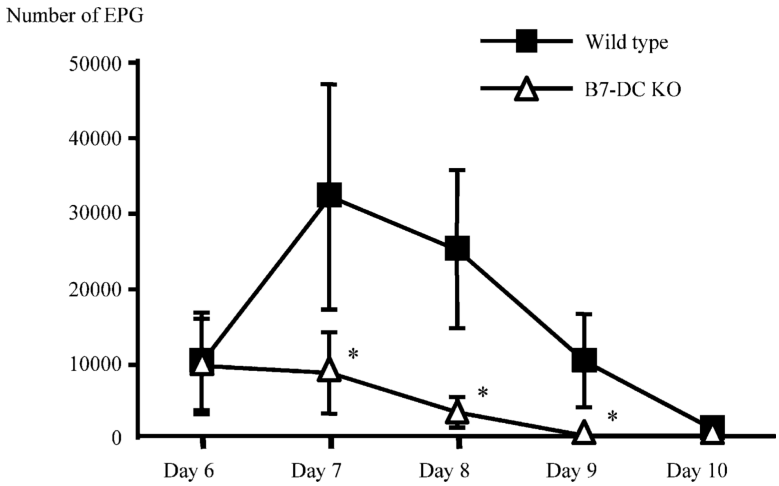

FIGURE 5.

B7-DC deficiency accelerated the clearance of adult *N. brasiliensis* from intestine. WT (n = 5) and B7-DC KO (n = 5) mice were inoculated by s.c. injection with L3 on day 0. The number of eggs in feces was enumerated using phase-contrast microscopy at the indicated time intervals. These data are representative of three independent experiments. EPG, egg output per gram of feces. *$p < 0.05$.