

High-level expression of B7-H1 molecules by dendritic cells suppresses the function of activated T cells and desensitizes allergen-primed animals

Hee Kyung Kim,* Hongbing Guan,* Guorui Zu,* Hui Li,* Lizhi Wu,* Xu Feng,[†] Craig Elmetts,* Yangxin Fu,[‡] and Hui Xu*^{†,1}

Departments of *Dermatology and [†]Pathology, University of Alabama at Birmingham; and [‡]Department of Pathology, University of Chicago, Illinois

Abstract: A body of evidence indicates that expression of the programmed cell death 1 (PD-1) receptor by activated T cells plays an important role in the down-regulation of immune responses; however, the functions of its known ligands, B7-H1 (PD-L1) and B7-dendritic cell (DC; PD-L2), at the effector phase of immune responses are less clear. In the current study, we investigated the roles of B7-H1 in DC-mediated regulation of hapten-activated T cells and the delayed-type contact hypersensitivity response in primed animals. We found that the expression of B7-H1 and B7-DC was induced on activation of DC by hapten stimulation. Blockade of B7-H1, but not B7-DC, enhanced the activity of hapten-specific T cells. Interaction with a DC line that expresses high cell-surface levels of B7-H1 (B7-H1/DC) suppressed the proliferation of, and cytokine production by, activated T cells. In vivo administration of hapten-carrying B7-H1/DC desensitized the response of sensitized animals to hapten challenge, and this desensitization was hapten-specific. These data indicate that B7-H1 expressed by DC mediates inhibitory signals for activated T cells and suppresses the elicitation of immune responses. The ability of B7-H1/DC to inhibit the function of preactivated T cells in vivo suggests novel strategies for the treatment of immune response-mediated disorders. *J. Leukoc. Biol.* 79: 686–695; 2006.

Key Words: PD-1 · contact hypersensitivity · immunology · cytokines

INTRODUCTION

Activation of the immune response requires two signals, one mediated by antigens and one, by costimulatory molecules. Lack of costimulation reduces the activation of antigen-specific T cells and can result in tolerization of the immune system to antigen stimulation. Accumulating evidence suggests that factors other than the absence of costimulatory signals may contribute to the down-regulation of activated T cells [1]. Inhibitory molecules, which are expressed on activated T cells, play

important roles in the feedback control and down-regulation of immune responses [2–7]. A full understanding of the molecular interactions that inhibit activated T cells is of particular importance to the development of strategies for the treatment of patients with immune response-mediated disorders in which antigen-specific T cells are activated already.

The programmed cell death 1 (PD-1) receptor was defined originally as a death receptor, which is induced on activation of T cells [8–11]. In *PD-1* gene knockout mice, immune responses are exaggerated, and the mice develop autoimmune diseases spontaneously, implicating PD-1 in the mediation of inhibitory signals for immune T cells [12, 13]. Two PD-1 ligands have been identified: B7-H1 (PD-L1) and B7-dendritic cell (DC; PD-L2). B7-H1 is expressed widely by activated DC, T cells, B cells, and monocytes, as well as in peripheral tissues, whereas the expression of B7-DC appears to be restricted to activated DC [10, 14–16]. B7-H1 and B7-DC have been shown to be involved in the regulation of T cell activation and function [16–21]. Although there is evidence indicating that B7-H1 costimulation enhances T cell activation and enhances the development of immune responses [20, 22], other studies indicate that B7-H1 costimulation inhibits immune responses [16, 23–30]. In hapten-induced contact hypersensitivity responses (CHS), anti-B7-H1 but not anti-B7-DC antibody enhanced CHS responses when administered during sensitization, suggesting that B7-H1 molecules provide inhibitory signals for the immune response. However, administration of the antibody prior to challenge of sensitized animals did not show significant effects, although the antibody did suppress the proliferation of hapten-primed T cells in cultures [31]. In most of the studies using antibodies, fusion proteins, or gene-deficient mice, the primary focus is on the induction of immune responses. Less is known about the role of B7-H1 molecules in the regulation of immune responses at the elicitation phase, when activated T cells express an increased level of PD-1.

DC are primary antigen-presenting cells, which play important roles in the induction and regulation of immune responses

¹ Correspondence: Department of Dermatology, University of Alabama at Birmingham, VH 566B, 1670 University Blvd., Birmingham, AL 35294. E-mail: xuhui@uab.edu

Received August 4, 2005; revised November 28, 2005; accepted December 17, 2005; doi: 10.1189/jlb.0805436.

[32–35]. Depending on their level of maturation and functional status, DC can act to support or to inhibit immune responses. The mechanisms by which DC inhibit immune responses are being given increasing attention [32, 36–38]. A recent study has demonstrated that the DC, which infiltrate tumors, express B7-H1 and that blockade of B7-H1 molecules by specific antibodies enhances the efficiency of DC-based antitumor immunotherapy [39]. Similar results have been reported in other studies in which blockade of B7-H1 molecules enhances proliferation of, and cytokine production by, T cells stimulated with DC [40]. These studies implicate the expression of B7-H1 by DC in the provision of inhibitory signals for T cell activation and the suppression of immune responses. The possibility that B7-H1 signals mediated by DC may inactivate antigen-specific T cells and suppress ongoing immune responses has not been explored, however, although antigen-specific inactivation of T cells would be an ideal strategy for treatment of autoimmune diseases and prevention of graft rejection in transplantation.

In the present study, we have examined the role of B7-H1 in DC-mediated regulation of T cell activation and hapten-induced delayed-type CHS responses. The objectives were to determine whether B7-H1 costimulation mediated by DC inactivates hapten-specific T cells and suppresses the elicitation of this T cell-mediated immune response in presensitized animals in an antigen-specific manner. As the use of DC-based therapies has shown promise as an immunotherapeutic strategy, our findings advance the understanding of the mechanisms underlying DC-mediated immune regulation and provide insights that will promote the development of new strategies.

MATERIALS AND METHODS

Animals and reagents

C57BL/6 mice were purchased from Charles River Laboratories (Boston, MA) and A/J mice, from Harlan (Indianapolis, Indiana). Female mice of 6–8 weeks of age were used in the experiments. All animal procedures were performed according to National Institutes of Health guidelines under protocols approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Hybridoma lines 2.4G2 (CD16/32), GK1.5 (anti-CD4), and Lyt-2 (anti-CD8) were purchased from American Type Culture Collection (Manassas, VA). The antibodies were purified from culture supernatants by affinity chromatography using a protein G-coupled Sepharose column (Gamma-Bind Plus, Pharmacia, Uppsala, Sweden). Functional grade and phycoerythrin (PE)-labeled monoclonal rat anti-mouse B7-H1 (MIH5) and B7-DC (TY25) and biotin-labeled anti-PD-1 antibodies were purchased from eBiosciences (San Diego, CA). Fluorescein isothiocyanate (FITC)- or PE-labeled anti-mouse CD11c, CD4, CD8, CD44, B7.1, B7.2, Ia/IE, CD40, Cychrome-5 (Cy5)-labeled streptavidin, and isotype-matched control antibodies were purchased from Pharmingen (San Diego, CA). Dinitrofluorobenzene (DNFB), dinitrobenzenesulfonic (DNBS) acid, sodium salt, FITC (isomer I), normal rat immunoglobulin G (IgG), and recombinant mouse interleukin (rmIL)-2 were purchased from Sigma Chemical Co. (St. Louis, MO).

The mouse Langerhans cell line XS106 was kindly provided by Dr. Akira Takashima (University of Texas Southwestern Medical School, Dallas). This cell line originates from A/J mice and expresses a mature Langerhans cell phenotype. The cell line was maintained in culture as described previously [41].

Generation of a B7-H1-transfected DC line

To establish a B7-H1-transfected DC line, recombinant retrovirus encoding mouse B7-H1 was generated. The U3nlsLacZ plasmid and the package cell

line, 293GPG, were kindly provided by Dr. Dan Ory (Washington University, St. Louis, MO), and recombinant retrovirus was prepared as described [42]. Briefly, mouse B7-H1 [43] or green fluorescence protein (GFP) cDNA was inserted into the *Bam*HI and *Not*I sites of the vector, which was transfected into 293GPG cells using Lipofectamine, according to the manufacturer's instructions (Gibco, BRL, Grand Island, NY). The supernatants containing recombinant retrovirus were harvested as described [42].

Undiluted virus supernatants were used to infect XS106 cells for 24 h. Stable, transfected cells were established, and the population expressing a high level of B7-H1 molecules or GFP was sorted using a Vantage flow cytometer (Becton Dickinson, San Jose, CA).

Assessment of CHS responses

The induction and elicitation of CHS responses in mice were carried out as described previously [44]. Briefly, mice were sensitized with 0.5% DNFB or 0.5% FITC. The sensitized mice were challenged 5 days later by application of 0.2% DNFB or 0.5% FITC on each side of both ears. Ear thickness was measured in a blinded manner prior to and then at 24-h intervals following challenge. Naïve mice, which were not sensitized but were hapten-challenged, served as negative controls. The magnitude of ear thickness is reported as the mean increase in each group of three mice (i.e., six ears).

To determine the effects of hapten-loaded DC on the elicitation of CHS responses, the XS106, B7-H1/DC, or GFP/DC cells were γ -irradiated by 2000 rads and then labeled with 5 μ M DNBS or FITC for 10 min at 37°C. The viability of the cells was >90%, as assessed by the Trypan blue exclusion. Mice were sensitized with DNFB, and 5 days later, the mice were injected twice subcutaneously (s.c.) on the abdomen (two sites) with DNBS-labeled or unlabeled control DC (2×10^6 cells/mouse) with a 48-h interval between injections. Seven days after the last injection, the mice were challenged, and the CHS response was measured.

Isolation of hapten-activated DC

Hapten-activated DC were isolated from hapten-sensitized skin tissues in explant cultures as described previously [45]. Briefly, the dorsal side of mouse ears was painted with 5 μ l 0.5% DNFB and harvested 2 h later. The hapten-treated dorsal skin was peeled off and placed on the top of Dulbecco's modified Eagle's medium with 10% fetal calf serum. The migratory cells that contained hapten-activated skin DC were harvested 48 h after initiation of the cultures.

Isolation of hapten carrying DC from the regional lymph nodes was performed as described previously [46]. Briefly, mice were sensitized by application of FITC or DNFB on the shaved abdominal skin. The draining lymph node cells were harvested 22–24 h later. The draining lymph node cells were centrifuged through a metrizamide gradient (14.5%) at 600 *g* for 10 min. The interface population containing DC was collected.

CD11c-positive DC from naïve mice was isolated by using a kit from Miltenyi Biotec (Auburn, CA) according to the manufacturer's instruction. Briefly, lymph node cells were harvested and incubated with rat anti-mouse CD11c antibody-coupled magnet beads. After washes, the cells were run through a column attached to a high-power magnet. The bound cells in the column were eluted and harvested, which contained 80–85% of CD11c-positive DC, as assessed by flow cytometry.

Flow cytometry analysis

Analysis of surface antigens of T cell or DC by flow cytometry was carried out as described previously [47]. Nonspecific binding was blocked by incubation with an anti-CD16/CD32 monoclonal antibody (2.4G2). The cells were then stained with fluorescence-labeled antibodies and collected using a FACSCaliber with CellQuest software (Becton Dickinson). The data were analyzed using WinMDI 2.8 software (Scripps Research Institute, San Diego, CA).

To determine the expression of PD-1 by activated T cells, mice were sensitized with DNFB, and the draining lymph node cells were harvested 5 days later. The cells were stained with FITC-labeled anti-CD4 or anti-CD8 antibody, PE-labeled anti-CD44, and biotin-labeled anti-PD-1 antibody, followed by Cy5-coupled streptavidin. CD4⁺ or CD8⁺ T cells were gated, and the expression of CD44 and PD-1 was analyzed.

To determine the expression of B7-H1 and B7-DC by hapten-activated, cutaneous DC, the migratory cells from skin explant cultures were double-

stained with FITC-labeled CD11c and PE-labeled anti-B7-H1 or -B7-DC antibody. CD11c-positive cells were gated, and the expression of CD11c, B7-H1, or B7-DC was evaluated in a flow cytometer.

To determine the expression of B7-H1 and B7-DC by hapten-carrying DC in the draining lymph nodes, the DC were enriched from the draining lymph node cells of FITC-sensitized mice and stained with PE-labeled CD11c, anti-B7-H1, or -B7-DC antibody. The FITC-positive cells, which represented hapten-carrying cells, were gated for analysis of CD11c, B7-H1, and B7-DC expression in a flow cytometer.

To detect the expression of B7-H1 and B7-DC by naïve DC in lymph nodes, CD11c-positive DC were isolated from naïve mice by CD11c antibody-coupled magnet beads and stained with PE-labeled CD11c, B7-H1, or B7-DC antibody. The expression of CD11c, B7-H1, and B7-DC was analyzed in a flow cytometer.

Measurement of cytokine production by T cells

In vitro stimulation of cytokine production by hapten-primed T cells has been described previously [44]. To detect cytokine production stimulated by hapten-labeled DC, hapten-carrying DC were isolated from the draining lymph nodes of DNFB-sensitized mice as described above. XS106, B7-H1/DC, or GFP/DC were labeled with 5 μ M DNBS at 37°C for 10 min. The DC were γ -irradiated (2000 rads) and placed in culture with DNFB-primed T cells in 96-well round-bottom plates (DC: 2×10^4 /well; T cells: 2×10^6 /well; culture volume: 200 μ l). Supernatants were harvested 48 h later for quantification of cytokines by enzyme-linked immunosorbent assay (ELISA). In the experiments examining the effects of B7-H1 or B7-DC molecules, the antibodies or control rat IgG were added to the wells at the initiation of the cocultures.

Quantification of interferon- γ (IFN- γ) and IL-2 by cytokine-specific ELISA has been described previously [44]. The antibodies and recombinant cytokines for the standard curve were purchased from PharMingen.

T cell proliferation

The T cell-proliferative response to hapten-labeled DC was evaluated as described previously [46]. Briefly, XS106 cells or B7-H1/DC were γ -irradiated using 2000 rads and labeled with DNBS. Cocultures of DNFB-primed T cells from A/J mice and DNBS-labeled DC were established in triplicate (DC: 2×10^4 /well; T cells: 2×10^6 /well; culture volume: 200 μ l). After 72 h, cultures were pulsed with [3 H]-thymidine (Amersham, Little Chalfont, UK; 1 μ Ci/well) and harvested 18–20 h later. The amount of [3 H]-thymidine incorporation was determined by liquid scintillation counting using a Microbeta counter (Wallac, Canada). In experiments examining the effect of B7-H1 molecules and exogenous IL-2 on hapten-specific T cell proliferation, antibody (30 μ g/ml) or rIL-2 (100–200 pg/ml) was added.

Depletion of T cell subpopulations

In vivo depletion of CD4 $^+$ T cells was performed by treatment of animals with specific antibodies as described previously [44]. Briefly, mice were injected

intraperitoneally with 100 μ g GK1.5 (anti-CD4) on 3 consecutive days. Control mice were treated with normal rat IgG. The antibody treatment resulted in greater than 90% depletion of the target cells (data not shown).

For in vitro depletion of T cell subsets, lymph node cells were incubated with rat anti-mouse CD4 (GK1.5) or CD8 (Lyt-2) antibodies at 5 μ g/ 10^6 cells. After washes, the cells were incubated with anti-rat IgG antibody-coupled magnetic beads (20 beads per target cells) according to the manufacturer's instructions (Biosource, Camarillo, CA). The bead-bound cells were removed using a high-power magnet (Dyna, Great Neck, NY), and suspended cells were harvested. In our hands, greater than 95% of the target cells are depleted as assessed by flow cytometry (data not shown).

Statistical analysis

The differences between experimental groups were analyzed using the Student's *t*-test, and *P* < 0.05 was considered statistically significant.

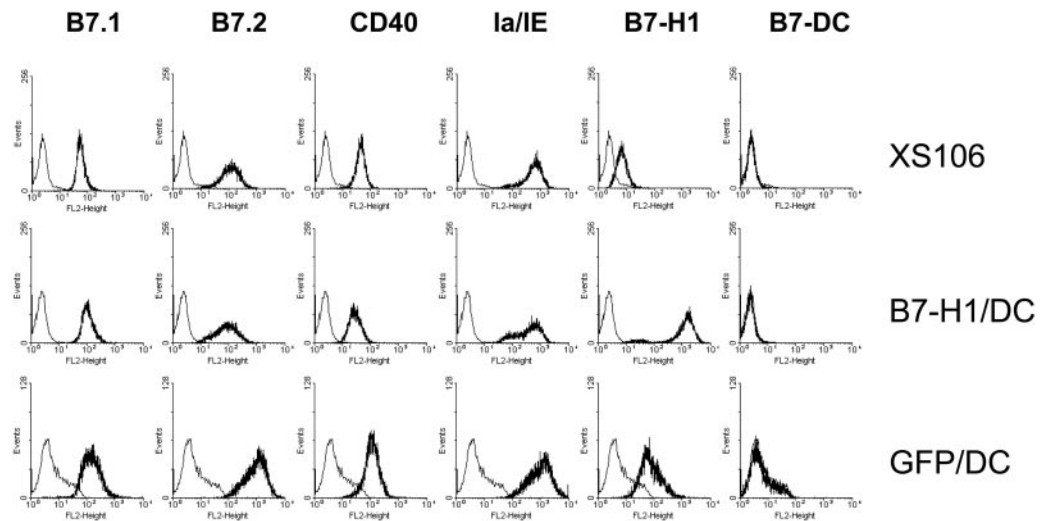
RESULTS

Generation and characterization of the B7-H1-transfected DC line

XS106, a Langerhans cell line derived from an A/Jax mouse, is well-established for the study of Langerhans cell function [41, 48, 49]. The infection with retrovirus did not have any noticeable effect on the growth of XS106. B7-H1/DC and GFP/DC were sorted, and stable transfectants were established. The high-level expression of B7-H1 molecules by the B7-H1/DC and GFP by the GFP/DC has not altered on long-term culture (>12 months). Neither the transfection process nor the high-level expression of B7-H1 molecules affects the migration of DC into the draining lymph nodes following s.c. injection in animals (data not shown).

Flow cytometry analyses indicated that the parental XS106 expressed a low level of B7-H1 molecules (Fig. 1). The expression of B7-H1 also was evaluated using PD-1-mouse IgG fusion proteins followed by FITC-labeled goat anti-mouse IgG (data not shown). The parental XS106 and the transfected DC did not express a detectable level of B7-DC antigens. The expression of B7-1, CD40, and major histocompatibility complex class II antigens was not noticeably different between the B7-H1/DC and parental XS106 cells (Fig. 1).

Fig. 1. Characterization of the B7-H1-transfected Langerhans cell line XS106. Cells were stained with PE-labeled antibodies or isotype controls and analyzed by flow cytometry. In histograms, thin lines represent isotype controls, and thick lines represent specific antibodies.



Detection of PD-1 and PD-1 ligands

It is known that activation induces the expression of PD-1 on T cells [8, 10]. To determine whether hapten sensitization induces the expression of PD-1 on T cells, C57BL/6 mice were sensitized with DNFB, and the draining lymph nodes were harvested 5 days later. Flow cytometry analysis confirmed the development of CD4⁺ and CD8⁺ T cell populations defined by high levels of CD44, a marker of T cell activation, in the sensitized mice (**Fig. 2A**). Our previous studies have demonstrated that these CD44 high subpopulations of T cells represent hapten-activated T cells, which are able to transfer CHS responses into naïve recipient mice, whereas the CD44 low population represents resting T cells, which are unable to transfer the response [46]. Further analysis indicated that 40–60% of the CD44 high CD4⁺ or CD8⁺ T cells expressed

PD-1 antigens, whereas only a few CD44 low T cells (<1%) expressed this molecule.

Previous studies reported that Langerhans cells and dermal DC from naïve mice did not express B7-H1 and B7-DC [31], which was confirmed in our experiments (data not shown). To examine whether hapten sensitization induced the expression of the molecules, hapten-treated ear skins of C57BL/6 mice were placed in explant cultures. The migratory cells contained DC, which expressed CD11c molecules. Gated, CD11c-positive cells expressed a significant level of B7-H1 and B7-DC antigens (**Fig. 2B**). Furthermore, hapten (FITC), carrying DC isolated from the draining lymph nodes of C57BL/6 mice, expressed CD11c molecules. These DC represented the migratory DC from the hapten-treated skin and expressed B7-H1 and B7-DC at higher levels than the migratory DC from skin

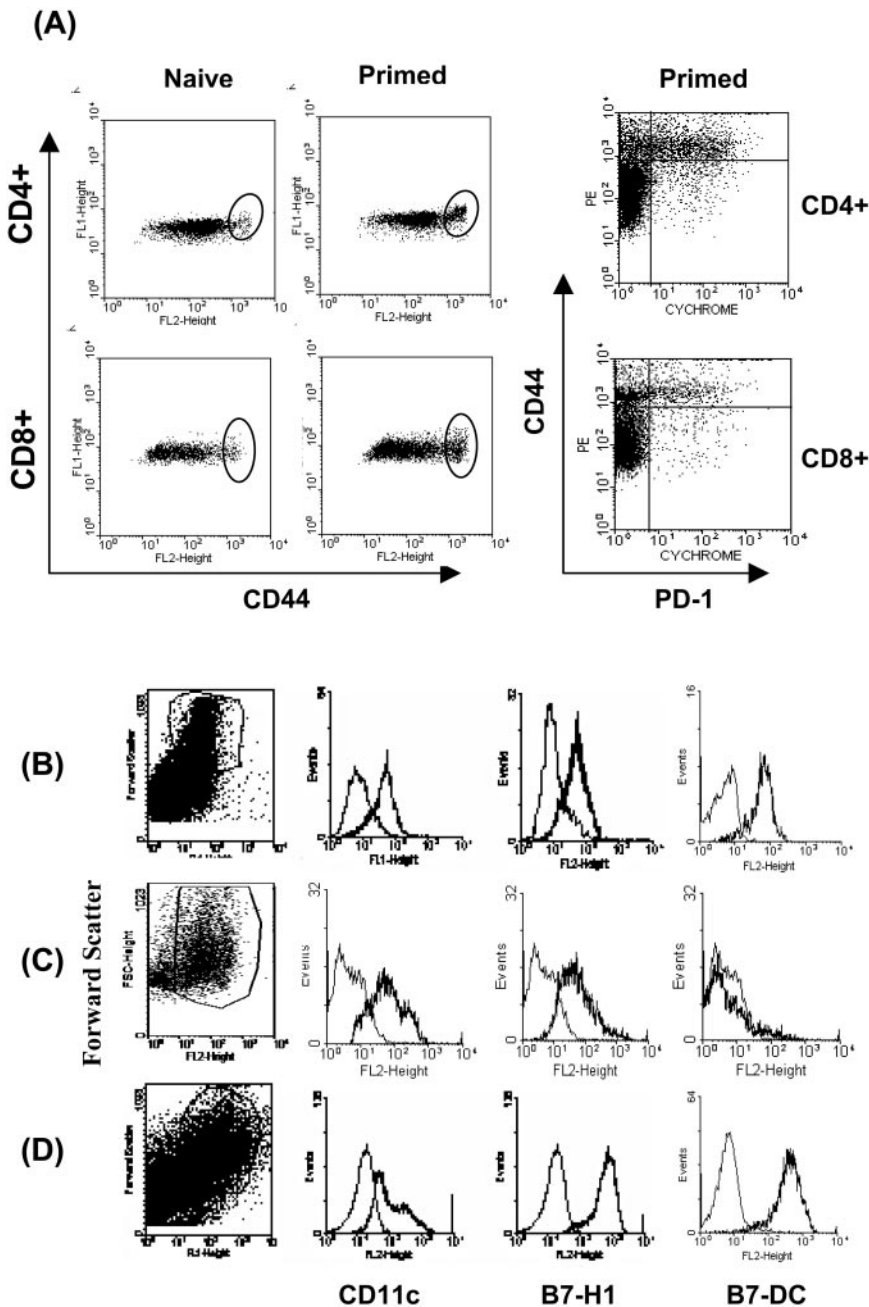


Fig. 2. The expression of PD-1 and PD-1 ligands by T cells and DC. (A) The expression of PD-1 by hapten-activated T cells isolated from the draining lymph nodes of hapten-sensitized mice. Left panel: CD4⁺ or CD8⁺ T cells are gated. Dot-plots show increased CD44 high population in primed CD4⁺ and CD8⁺ T cells versus naïve controls. Right panel: CD4⁺ and CD8⁺ T cells are pregated, and the expression level of PD-1 (Cychrome) by CD44 high (upper) and CD44 low (lower) populations is analyzed in quadrant. (B) The expression of B7-H1 and B7-DC by migratory DC, which were isolated from explant cultures of hapten-treated skin and stained with FITC-labeled CD11c and PE-labeled B7-H1 or B7-DC antibodies. CD11c-positive cells (circled in dot plots) are gated for histogram analysis. (C) The expression of B7-H1 and B7-DC by naïve DC. CD11c-positive cells were isolated from lymph nodes of naïve mice and stained with PE-labeled CD11c, B7-H1, or B7-DC antibodies. CD11c-positive cells (circled in dot plots) are gated for histogram analysis. (D) The expression of B7-H1 and B7-DC by hapten-carrying DC, which were isolated from the draining lymph nodes of FITC-sensitized mice and stained with PE-labeled CD11c, B7-H1, or B7-DC antibodies. FITC-positive cells (circled in dot plots), which represent hapten-labeled DC, are gated for histogram analysis, where thin lines represent isotype controls, and thick lines represent specific antibodies.

explant cultures (Fig. 2D). In contrast, CD11c-positive DC isolated from naïve mice expressed a lower level of B7-H1 than hapten-carrying DC and did not express a detectable level of B7-DC (Fig. 2C). It is not a surprise that CD11c-positive DC in lymph nodes are different from cutaneous DC in naïve mice, as they may come from different origins and may be at different maturation and activation stages. The current studies have demonstrated that B7-H1 and B7-DC molecules are induced on activation of cutaneous DC following hapten stimulation, and the expression level is increased further when hapten-carrying DC migrate into the draining lymph nodes.

Blockade of B7-H1 molecules enhances cytokine production by hapten-primed T cells

Tsushima et al. [31] reported that anti-B7-H1 but not -B7-DC antibody regulated the proliferation of hapten-primed T cells and the development of CHS responses. Our experiments examined further whether blockade of B7-H1 or B7-DC molecules regulates cytokine production by hapten-primed T cells in cultures with hapten-carrying DC. Anti-B7-H1- or -B7-DC-blocking antibodies or control, normal rat IgG were added to the cultures at 30 $\mu\text{g/ml}$, a concentration that had been predetermined as being effective in blocking the action of these molecules (data not shown). The addition of the anti-B7-H1 antibody enhanced the production of IL-2 and IFN- γ as compared with the addition of normal rat IgG (Fig. 3). The enhancement of production of IL-2 and IFN- γ was evident in the CD4⁺ and CD8⁺ T cell subpopulations. In contrast, the addition of the anti-B7-DC antibody did not cause any significant change in the production of any of these cytokines by the T cells. In control cultures that contained primed T cells alone or naïve T cells with hapten-labeled DC, the cytokine levels were below detectable limits. The results confirm that B7-H1 but not B7-DC molecules regulate the activities of hapten-primed T cells. Therefore, the following experiments were focused on B7-H1-mediated effects.

Enhanced B7-H1 costimulation inhibits the cytokine production by hapten-specific T cells

As blockade of B7-H1 molecules increased T cell activities, further studies were to determine whether enhanced B7-H1 costimulation could suppress the function of hapten-primed T cells in an antigen-dependent manner. To test it, we used the B7-H1-transfected DC cell line, which expressed high levels of B7-H1 molecules (B7-H1/DC). In the initial experiments, T cells isolated from the draining lymph nodes of DNFB-sensitized A/J mice were cocultured with DNBS-labeled DC, and cytokine production was evaluated. Compared with GFP/DC or XS106 cells, B7-H1/DC resulted in an approximate twofold reduction in the production of IFN- γ and IL-2, suggesting that the high level of B7-H1 expression inhibits the cytokine production by activated T cells (Fig. 4). In control cultures that contained naïve T cells with hapten-labeled DC or primed T cells with unlabeled GFP/DC, B7-H1/DC, or XS106, the production of the cytokines was below detection levels (data not shown).

Further analysis of the cytokine production by T cell subsets demonstrated that compared with GFP/DC or XS106 cells,

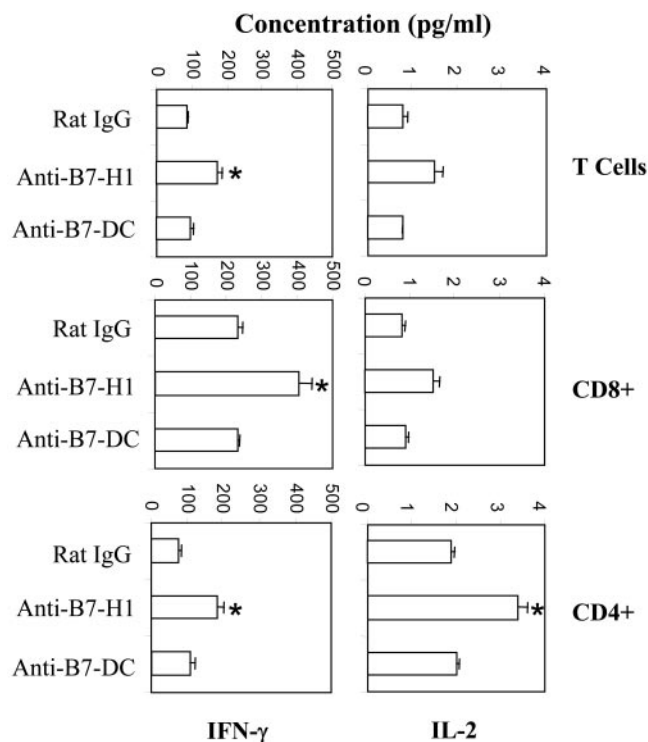


Fig. 3. The roles of B7-H1 and B7-DC in the regulation of T cell activation. DNFB-primed CD4⁺ and CD8⁺ T cell subpopulations and DNFB-carrying DC were isolated from the draining lymph nodes of DNFB-treated mice. The primed T cells were cultured with hapten-carrying DC in the presence of anti-B7-H1, -B7-DC, or normal rat IgG at 30 $\mu\text{g/ml}$. The supernatants were harvested after 48 h, and cytokine concentration was determined by cytokine-specific ELISA. The cytokine concentration in controls containing naïve T cells with hapten-labeled DC or primed T cells alone was below detectable levels. The data are representative of two independent experiments. *, $P < 0.05$.

B7-H1/DC cells reduced the level of IL-2 production by CD8⁺ T cells to below detectable levels, whereas IL-2 production by CD4⁺ T cells was reduced approximately twofold. The production of IFN- γ was greatly reduced (four- to fivefold) in CD4⁺ and CD8⁺ T cells. Similar results were observed in three independent experiments. Thus, a high level of expression of B7-H1 molecules by DC suppresses the function of activated CD4⁺ and CD8⁺ T cells.

Enhanced B7H1 costimulation inhibits the proliferation of hapten-specific T cells

Based on the findings that B7-H1/DC inhibited IL-2 production by activated T cells, further experiments were to examine whether T cell proliferation was regulated. On coculture of hapten-activated T cells (A/J mice) with hapten-labeled B7-H1/DC, the proliferative response of the T cells was significantly lower than on culture with hapten-labeled, parental XS106 and GFP/DC (Fig. 5A). On coculture with hapten-activated CD8⁺ T cells, the inhibition was more profound—up to three- to fourfold greater at the highest concentration of hapten-loaded B7-H1/DC than that observed on coculture with the control, hapten-loaded, parental XS106 and GFP/DC cells (Fig. 5B). This result provides further evidence of a preferential effect of the B7-H1-mediated inhibition on CD8⁺ T cells,

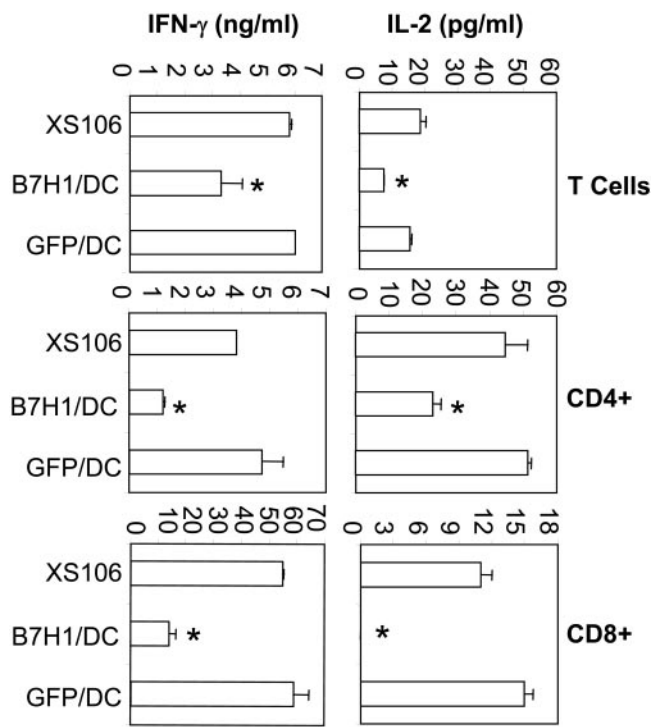


Fig. 4. The inhibitory effect of enhanced B7-H1 costimulation on cytokine production by activated T cells. The draining lymph node T cells were isolated from DNFB-sensitized A/J mice and were cultured with DNBS-loaded B7-H1/DC, GFP/DC, or XS106 cells for 48 h. The cytokines in the supernatants were measured by cytokine-specific ELISA. The cytokine concentration in controls containing naïve T cells with hapten-labeled DC or primed T cells with unlabeled DC was below detectable levels. The data are representative of three independent experiments. Please note the difference in the scale for cytokine concentration of the CD8⁺ T cells. *, $P < 0.05$.

which is consistent with the observation of greater reduction in IL-2 production in the CD8⁺ T cell subpopulation in the analysis of the effects of B7-H1/DC on cytokine production by the different T cell subpopulations (Fig. 4).

To confirm that the inhibition of T cell proliferation on coculture with B7-H1/DC is mediated by B7-H1 molecules, hapten-activated CD8⁺ T cells (A/J mice) were cocultured with B7-H1/DC in the presence of rat IgG or anti-B7-H1 antibody at various concentrations. The addition of anti-B7-H1 antibody to the cultures significantly enhanced the proliferative response of the T cells. This effect was dose-dependent, and control rat IgG had little effect at the same concentrations (Fig. 5C). Blockade of B7-H1 with the antibody completely restored the proliferation of T cells or CD8⁺ subpopulation on cocultures with B7-H1/DC, which reached a level equivalent to that observed on culture of the T cells with GFP/DC or parental XS106 cells (Fig. 5, D and E). Thus, the B7-H1 molecules are involved directly in the B7-H1/DC-mediated suppression of T cell proliferation. Addition of anti-B7-H1 antibody in cultures with GFP/DC slightly increased T cell proliferation, suggesting that a low level of endogenous B7-H1 expression might have minor effects (Fig. 5D). In separate experiments, addition of anti-PD-1 antibody enhanced the proliferative response of T cells on coculture with B7-H1/DC at a similar level to the addition of the anti-B7-H1 antibody (data not shown).

The observed down-regulation of IL-2 production could mediate the suppression of T cell proliferation. To test this hypothesis, we assessed the effect of addition of exogenous IL-2 on the T cell-proliferative response. Addition of IL-2 completely restored the proliferative response of the T cells on culture with B7-H1/DC (Fig. 5D). Although IL-2 production by CD8⁺ T cells was almost abrogated on coculture with B7-H1/DC, the addition of exogenous IL-2 was capable of enhancing the proliferative response of these cells by as much as fourfold (Fig. 5E). The addition of IL-2 into the T cell cultures in the absence of hapten-labeled DC did not result in significant T cell proliferation, suggesting that the effect of IL-2 on T cell proliferation is dependent on DC stimulation.

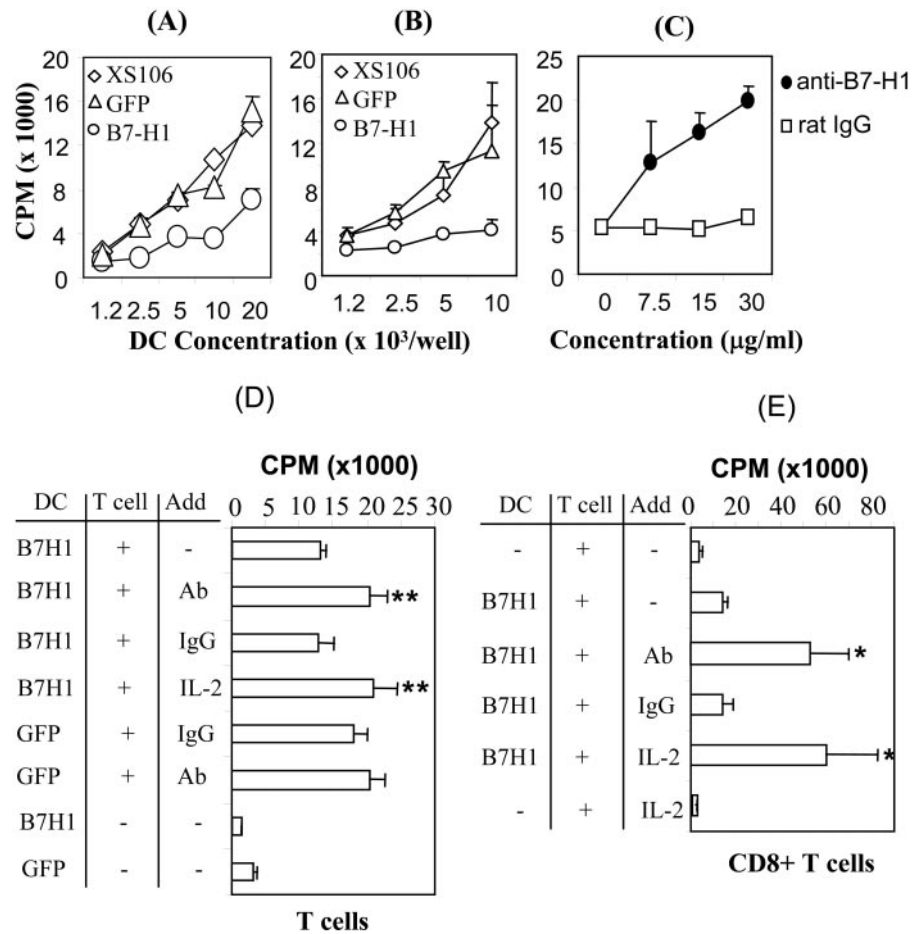
Enhanced B7-H1 costimulation suppresses the elicitation of CHS responses in sensitized animals

The B7-H1/DC-mediated, inhibitory effects on cytokine production and proliferation of hapten-primed T cells in cultures suggest that hapten-primed animals may be desensitized by administration of hapten-loaded B7-H1/DC cells. To test it, A/J mice were first sensitized with DNFB and then treated twice with DNBS-labeled B7-H1/DC. The treatment of the mice with hapten-labeled B7-H1/DC significantly reduced the magnitude of the CHS response following challenge compared with the responses of the control mice, which were treated with unlabeled B7-H1/DC or left untreated (Fig. 6A). The response in mice treated with unlabeled B7-H1/DC was identical to that in untreated mice, indicating that the regulation of immune responses by B7-H1/DC was hapten-dependent. Increasing the numbers of hapten-labeled B7-H1/DC cells (up to 5×10^6 /mouse) or increasing the number of treatments with hapten-labeled B7-H1/DC (up to four times) did not result in further suppression (data not shown).

The antigen specificity of the B7-H1/DC-mediated suppression of elicitation of CHS was tested further. A/J mice were sensitized with DNFB or FITC, then treated twice with DNBS- or FITC-labeled B7-H1/DC, and challenged with DNFB or FITC. The treatment of DNFB-sensitized mice with DNBS-labeled B7-H1/DC reduced the magnitude of CHS responses following challenge with DNFB compared with those treated with FITC-labeled cells. The CHS response in FITC-sensitized mice was reduced by treatment with FITC-labeled B7-H1/DC compared with those treated with DNBS-labeled cells (Fig. 6B). These data indicate that application of hapten-labeled B7-H1/DC is able to suppress the elicitation of CHS responses in an antigen-specific manner.

In further experiments, we examined whether B7-H1/DC could directly inactivate the effector CD8⁺ T cells of sensitized animals in the absence of CD4⁺ T cells. A/J mice were depleted of CD4⁺ T cells prior to sensitization with DNFB and then treated with DNBS-labeled B7-H1/DC, GFP/DC, or parental XS106 cells. The treatment with hapten-labeled B7-H1/DC significantly reduced the CHS responses in CD4⁺ T cell-depleted mice, whereas treatment with hapten-labeled GFP/DC or parental XS106 had little effect on the magnitude of the response compared with untreated, control mice (Fig. 6C). Thus, B7-H1/DC are able to directly inhibit the activity of hapten-specific CD8⁺ T cells at the elicitation of the CHS

Fig. 5. The inhibitory effect of enhanced B7-H1 costimulation on the proliferation of activated T cells. Draining lymph node T cells were isolated from DNFB-sensitized A/J mice and were cultured with DNBS-labeled B7-H1/DC, GFP/DC, or XS106 cells (in triplicate) for 4 days. The proliferation of cells was measured by [³H]-thymidine incorporation [counts per minute (CPM)] for the last 18 h. (A) Enhanced B7-H1 costimulation inhibits the proliferation of primed T cells on coculture with B7-H1/DC. The difference between B7-H1/DC and control XS106 cells is significant at all indicated DC concentrations ($P < 0.05-0.01$). (B) Enhanced B7-H1 costimulation inhibits the proliferation of CD8⁺ T cells on coculture with DC. The difference between B7-H1/DC with GFP/DC and parental XS106 cells is significant at all indicated DC concentrations ($P < 0.05-0.01$). (C) Blockade of B7-H1 molecules with a specific antibody increases the proliferation of T cells on coculture with B7-H1/DC in a dose-dependent manner. (D and E) The suppression of T cell proliferation on coculture with B7-H1/DC can be restored by anti-B7-H1 antibody (Ab; 30 $\mu\text{g/ml}$) or exogenous IL-2 (200 $\mu\text{g/ml}$). Normal rat IgG (30 $\mu\text{g/ml}$) served as a control. *, $P < 0.01$; **, $P < 0.05$.



response. It is to note that the kinetics of CHS responses in normal, undeleted and CD4⁺ T cell-depleted mice was different (Fig. 6, A and C). This phenomenon has been observed in our previous studies as well, which implicates that depletion of CD4⁺ T cells not only increases the level but also prolongs the duration of CHS responses [44].

DISCUSSION

The present studies demonstrate that although resting DC in the skin do not express B7-H1 and B7-DC, they are induced to express both molecules following antigen stimulation. Blockade of B7-H1 but not B7-DC molecules with specific antibodies enhances DC-mediated activation of hapten-specific T cells. In contrast, enhanced B7-H1 costimulation inhibits the activity of hapten-specific T cells. In vivo administration of hapten-carrying DC, which express a high level of B7-H1 molecules, suppresses the elicitation of CHS responses in sensitized animals in a hapten-specific manner. These data indicate that DC expressing high levels of B7-H1 molecules are able to desensitize preimmunized animals in an antigen-specific manner and reduce inflammatory reactions.

Tsushima et al. [31] reported that in vivo administration of anti-B7-H1 antibody during hapten sensitization enhanced the development of CHS responses, and addition of the antibody in cultures increased the proliferation of hapten-activated T cells. However, administration of the anti-B7-H1 antibody prior to

challenge did not have a significant effect on the elicitation of the CHS response. The current studies demonstrate that the treatment of immunized animals with hapten-labeled B7-H1/DC suppressed the elicitation of CHS responses in a hapten-specific manner, implicating that enhanced B7-H1 costimulation desensitizes primed animals to respond to challenge. This is further supported by in vitro findings that enhanced B7-H1 costimulation mediated by DC inhibits the production of IFN- γ by hapten-primed T cells, whereas blocking B7-H1 molecules increases the cytokine production. IFN- γ is an important, inflammatory cytokine for CHS responses [50, 51]. The down-regulation of IFN- γ production represents a mechanism for B7-H1-mediated inhibition of hapten-specific T cells and the immune response. It is unclear why the treatment of primed animals with anti-B7-H1 antibody did not have any effect on the elicitation of CHS responses in the previous report [31]. One possible explanation is that the antibody reacts not only with DC but also with other types of B7-H1-positive cells, which include activated T cells, keratinocytes, endothelial cells, myeloid cells, and other tissue cells [14, 15]. This wide spectrum of reactivity may affect the outcome of immune responses in the treated animals. Our studies are focused specifically on the role of B7-H1 in DC-mediated regulation of hapten-activated T cells, which express PD-1. Desensitization of immunized animals with B7-H1/DC opens potentials for therapeutic strategies for autoimmune disease and transplantation in an antigen-specific manner.

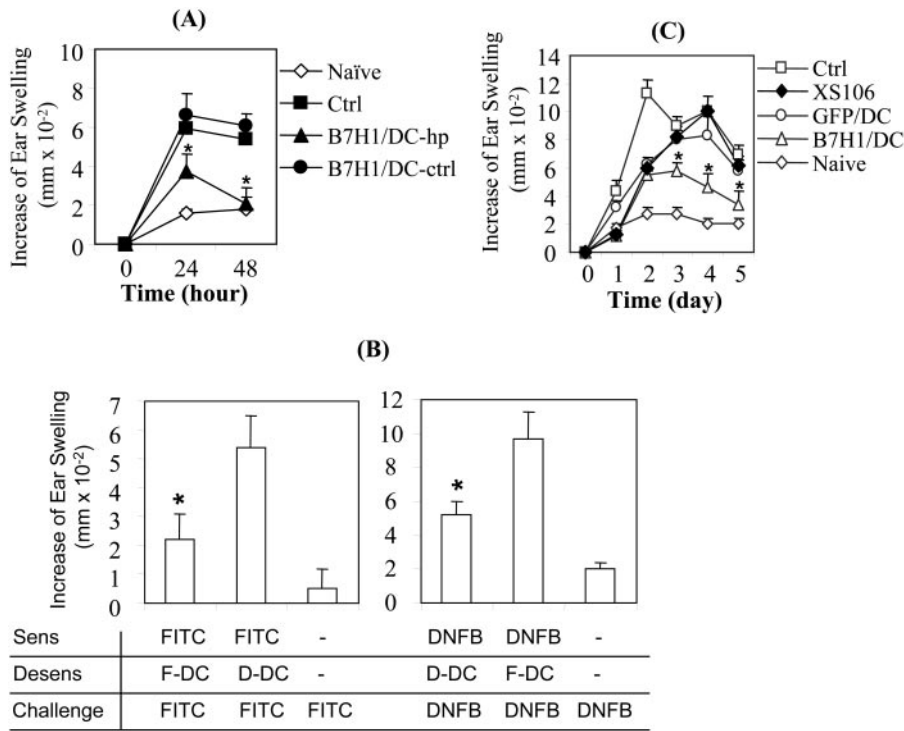


Fig. 6. Desensitization of immunized animals by hapten-labeled B7-H1/DC. A/J mice (three/group) were sensitized with DNFB and treated with DNBS-labeled DC. The CHS response was measured following challenge. (A) Desensitization of immunized animals with hapten-labeled B7-H1/DC. Mice were sensitized with DNFB and then treated with DNBS-labeled (B7-H1-hp) or unlabeled (B7-H1-ctrl) B7-H1/DC or left untreated (Ctrl). Naïve mice served as a negative control for challenge. (B) Hapten specificity of desensitization induced by treatment with hapten-loaded B7-H1/DC. Mice were sensitized (Sens) with DNFB or FITC and then treated (Desens) with DNBS (D-DC)- or FITC (F-DC)-labeled B7-H1/DC. CHS responses were determined following challenge with DNFB or FITC, respectively. (C) Desensitization of immunized animals deficient in CD4⁺ T cells. Mice were depleted of CD4⁺ T cells and sensitized with DNFB. The mice were then treated with DNBS-loaded B7-H1/DC, GFP/DC, or parental XS106 or were untreated (Ctrl). Naïve mice, which were not sensitized but challenged, served as a negative control. *, $P < 0.05$.

The down-regulation of IL-2 production appears to be a critical mechanism for B7-H1-mediated suppression of T cell activities in our model. Although IL-2 production by CD4⁺ T cells is reduced, application of B7-H1/DC in cultures almost abrogates the production of IL-2 by CD8⁺ T cells. Correspondingly, the inhibition of proliferation is more profound in CD8⁺ T cell cultures than in the presence of CD4⁺ T cells (Fig. 5, A and B). The ability of exogenous IL-2 to restore the proliferation of T cells in coculture with B7-H1/DC suggests that the T cells may retain the ability to proliferate in response to IL-2 and that compensation for the loss of IL-2 can overcome the inhibitory effect mediated by B7-H1 molecules. Similar results were reported in other studies, where T cells were stimulated with anti-CD3 and B7-H1 fusion protein [23]. A recent study has reported that B7-H1 can induce apoptosis of activated T cells, although this process occurs independently of the PD-1 receptors [22, 27]. As IL-2 is involved in the regulation of cell growth and death, more studies are required to further define the role of IL-2 in B7-H1-mediated, inhibitory mechanisms.

Given the fact that PD-1 is only detected on hapten-activated T cells, the interaction of B7-H1 with PD-1 may be involved primarily in the feedback regulation of activated T cells. In this scenario, B7-H1-mediated, negative signals control the intensity and duration of immune responses. We have found that the desensitized mice can be resensitized to develop an equivalent level of CHS responses, irrespective of whether they had been treated originally with B7-H1/DC or control DC (data not shown). This suggests that B7-H1-mediated inhibition may be directed to activated T cells and thus, does not affect the development of immune responses, which are mediated by newly developed, hapten-specific T cells following resensitization.

In some studies, B7-H1 has been shown to enhance T cell activation and up-regulate immune responses [20, 43, 52].

These studies primarily investigated the effect of B7-H1 costimulation on the activation of naïve T cells, which did not express a detectable level of PD-1 antigens. Evidence suggests that there may be other PD-1-like receptors, which could interact with B7-H1 and mediate stimulatory signals for T cell activation [22, 52]. In fact, our preliminary studies showed that B7-H1/DC increased the activation of T cell receptor transgenic T cells in primary cultures but inhibit the restimulation of preactivated T cells compared with control DC (unpublished data). Our ongoing studies are in the process of defining the mechanism for the differential effects of B7-H1 on naïve and activated T cells.

Although hapten-activated DC express a significant level of B7-DC, their role in the regulation of CD4⁺ or CD8⁺ T cells in hapten-induced CHS responses appear to be minor. Similarly, it has been reported that administration of the same anti-B7-DC antibody, TY25, had little effect on CHS responses [31]. The lack of a B7-DC-mediated effect is not unique to CHS responses, however, as it also has little effect in the development of intestinal mucosal inflammations [29]. In contrast, application of the TY25 anti-B7-DC antibody regulates the activation of T cells and development of asthmatic responses [53], and the role of B7-DC in the regulation of immune responses has been proven in other models [18, 54]. It is unknown why B7-DC has different functions in different experimental models.

In summary, the current studies indicate that B7-H1 molecules expressed by activated DC provide inhibitory signals for activated T cells. The high-level expression of B7-H1 molecules can render the DC immunosuppressive, which can result in antigen-specific immunosuppression in hapten-sensitized animals. This finding is of special importance in application of DC-based immunotherapies for treatment of patients with autoimmune diseases or graft rejection, as the immune system in

these patients is already activated. Further studies are certainly required to validate the strategy.

ACKNOWLEDGMENTS

The study was supported by grants from the National Institutes of Health and the Department of Defense (H. X). This investigation was conducted in a facility constructed with support from Research Facilities Improvement Program Grant No. C06 RR 15490 from the National Center for Research Resources, National Institutes of Health. Our special thanks is given to Dr. Fiona Hunter for her critical comments on the manuscript.

REFERENCES

1. Van Parijs, L., Ibraghimov, A., Abbas, A. K. (1996) The roles of costimulation and Fas in T cell apoptosis and peripheral tolerance. *Immunity* **4**, 321–328.
2. Khoury, S. J., Sayegh, M. H. (2004) The roles of the new negative T cell costimulatory pathways in regulating autoimmunity. *Immunity* **20**, 529–538.
3. Liu, Y. (1997) Is CTLA-4 a negative regulator for T-cell activation? *Immunol. Today* **18**, 569–572.
4. Walker, L. S., Abbas, A. K. (2002) The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat. Rev. Immunol.* **2**, 11–19.
5. Maher, S., Toomey, D., Condron, C., Bouchier-Hayes, D. (2002) Activation-induced cell death: the controversial role of Fas and Fas ligand in immune privilege and tumor counterattack. *Immunol. Cell Biol.* **80**, 131–137.
6. Hamad, A. R., Schneck, J. P. (2001) Antigen-induced T cell death is regulated by CD4 expression. *Int. Rev. Immunol.* **20**, 535–546.
7. Lenardo, M., Chan, F. K.-M., Hornung, F., McFarland, H., Siegel, R., Wang, J., Zheng, L. (1999) Mature T lymphocyte apoptosis—immune regulation in a dynamic and unpredictable antigenic environment. *Annu. Rev. Immunol.* **17**, 221–253.
8. Ishida, Y., Agata, Y., Shibahara, K., Honjo, T. (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J.* **11**, 3887–3895.
9. Carreno, B. M., Collins, M. (2002) The B7 family of ligands and its receptors: new pathways for costimulation and inhibition of immune responses. *Annu. Rev. Immunol.* **20**, 29–53.
10. Agata, Y., Kawasaki, A., Nishimura, H., Ishida, Y., Tsubata, T., Yagita, H., Honjo, T. (1996) Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int. Immunol.* **8**, 765–772.
11. Greenwald, R. J., Latchman, Y. E., Sharpe, A. H. (2002) Negative co-receptors on lymphocytes. *Curr. Opin. Immunol.* **14**, 391–396.
12. Nishimura, H., Okazaki, T., Tanaka, Y., Nakatani, K., Hara, M., Matsumori, A., Sasayama, S., Mizoguchi, A., Hiai, H., Minato, N., Honjo, T. (2001) Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* **291**, 319–322.
13. Nishimura, H., Nose, M., Hiai, H., Minato, N., Honjo, T. (1999) Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **11**, 141–151.
14. Mazanet, M. M., Hughes, C. C. (2002) B7–H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. *J. Immunol.* **169**, 3581–3588.
15. Yamazaki, T., Akiba, H., Iwai, H., Matsuda, H., Aoki, M., Tanno, Y., Shin, T., Tsuchiya, H., Pardoll, D. M., Okumura, K., Azuma, M., Yagita, H. (2002) Expression of programmed death 1 ligands by murine T cells and APC. *J. Immunol.* **169**, 5538–5545.
16. Freeman, G. J., Long, A. J., Iwai, Y., Bourque, K., Chernova, T., Nishimura, H., Fitz, L. J., Malenkovich, N., Okazaki, T., Byrne, M. C., Horton, H. F., Fouser, L., Carter, L., Ling, V., Bowman, M. R., Carreno, B. M., Collins, M., Wood, C. R., Honjo, T. (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J. Exp. Med.* **192**, 1027–1034.
17. Liu, X., Gao, J. X., Wen, J., Yin, L., Li, O., Zuo, T., Gajewski, T. F., Fu, Y. X., Zheng, P., Liu, Y. (2003) B7DC/PDL2 promotes tumor immunity by a PD-1-independent mechanism. *J. Exp. Med.* **197**, 1721–1730.

18. Tseng, S. Y., Otsuji, M., Gorski, K., Huang, X., Slansky, J. E., Pai, S. I., Shalabi, A., Shin, T., Pardoll, D. M., Tsuchiya, H. (2001) B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J. Exp. Med.* **193**, 839–846.
19. Dong, H., Zhu, G., Tamada, K., Chen, L. (1999) B7–H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat. Med.* **5**, 1365–1369.
20. Tamura, H., Dong, H., Zhu, G., Sica, G. L., Flies, D. B., Tamada, K., Chen, L. (2001) B7–H1 costimulation preferentially enhances CD28-independent T-helper cell function. *Blood* **97**, 1809–1816.
21. Latchman, Y., Wood, C. R., Chernova, T., Chaudhary, D., Borde, M., Chernova, I., Iwai, Y., Long, A. J., Brown, J. A., Nunes, R., Greenfield, E. A., Bourque, K., Boussiotis, V. A., Carter, L. L., Carreno, B. M., Malenkovich, N., Nishimura, H., Okazaki, T., Honjo, T., Sharpe, A. H., Freeman, G. J. (2001) PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat. Immunol.* **2**, 261–268.
22. Wang, S., Bajorath, J., Flies, D. B., Dong, H., Honjo, T., Chen, L. (2003) Molecular modeling and functional mapping of B7–H1 and B7-DC uncouple costimulatory function from PD-1 interaction. *J. Exp. Med.* **197**, 1083–1091.
23. Carter, L., Fouser, L. A., Jussif, J., Fitz, L., Deng, B., Wood, C. R., Collins, M., Honjo, T., Freeman, G. J., Carreno, B. M. (2002) PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2. *Eur. J. Immunol.* **32**, 634–643.
24. Ansari, M. J. I., Salama, A. D., Chitnis, T., Smith, R. N., Yagita, H., Akiba, H., Yamazaki, T., Azuma, M., Iwai, H., Khoury, S. J., Auchincloss Jr., H., Sayegh, M. H. (2003) The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *J. Exp. Med.* **198**, 63–69.
25. Dong, H., Strome, S. E., Matteson, E. L., Moder, K. G., Flies, D. B., Zhu, G., Tamura, H., Driscoll, C. L., Chen, L. (2003) Costimulating aberrant T cell responses by B7–H1 autoantibodies in rheumatoid arthritis. *J. Clin. Invest.* **111**, 363–370.
26. Iwai, Y., Ishida, M., Tanaka, Y., Okazaki, T., Honjo, T., Minato, N. (2002) Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc. Natl. Acad. Sci. USA* **99**, 12293–12297.
27. Dong, H., Strome, S. E., Salomao, D. R., Tamura, H., Hirano, F., Flies, D. B., Roche, P. C., Lu, J., Zhu, G., Tamada, K., Lennon, V. A., Celis, E., Chen, L. (2002) Tumor-associated B7–H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat. Med.* **8**, 793–800.
28. Iwai, Y., Terawaki, S., Ikegawa, M., Okazaki, T., Honjo, T. (2003) PD-1 inhibits antiviral immunity at the effector phase in the liver. *J. Exp. Med.* **198**, 39–50.
29. Kanai, T., Totsuka, T., Uraushihara, K., Makita, S., Nakamura, T., Koga-nei, K., Fukushima, T., Akiba, H., Yagita, H., Okumura, K., Machida, U., Iwai, H., Azuma, M., Chen, L., Watanabe, M. (2003) Blockade of B7–H1 suppresses the development of chronic intestinal inflammation. *J. Immunol.* **171**, 4156–4163.
30. Trabattini, D., Saresella, M., Biasin, M., Boasso, A., Piacentini, L., Ferrante, P., Dong, H., Maserati, R., Shearer, G. M., Chen, L., Clerici, M. (2003) B7–H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression. *Blood* **101**, 2514–2520.
31. Tushima, F., Iwai, H., Otsuki, N., Abe, M., Hirose, S., Yamazaki, T., Akiba, H., Yagita, H., Takahashi, Y., Omura, K., Azuma, M. (2003) Preferential contribution of B7–H1 to programmed death-1-mediated regulation of hapten-specific allergic inflammatory responses. *Eur. J. Immunol.* **33**, 2773–2782.
32. Steinman, R. M., Hawiger, D., Nussenzweig, M. C. (2003) Tolerogenic dendritic cells. *Annu. Rev. Immunol.* **21**, 685–711.
33. Liu, Y. J. (2001) Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell* **106**, 259–262.
34. Lanzavecchia, A., Sallusto, F. (2001) Regulation of T cell immunity by dendritic cells. *Cell* **106**, 263–266.
35. Banchereau, J., Briere, F., Caux, C., Davoust, J., Lebecque, S., Liu, Y. J., Pulendran, B., Palucka, K. (2000) Immunobiology of dendritic cells. *Annu. Rev. Immunol.* **18**, 767–811.
36. Heath, W. R., Carbone, F. R. (2001) Cross-presentation, dendritic cells, tolerance and immunity. *Annu. Rev. Immunol.* **19**, 47–64.
37. Grohmann, U., Fallarino, F., Puccetti, P. (2003) Tolerance, DCs and tryptophan: much ado about IDO. *Trends Immunol.* **24**, 242–248.
38. Lutz, M. B., Schuler, G. (2002) Immature, semi-mature and fully mature dendritic cells: which signals induce tolerance or immunity? *Trends Immunol.* **23**, 445–449.
39. Curiel, T. J., Wei, S., Dong, H., Alvarez, X., Cheng, P., Mottram, P., Krzysiek, R., Knutson, K. L., Daniel, B., Zimmermann, M. C., David, O., Burow, M., Gordon, A., Dhurandhar, N., Myers, L., Berggren, R., Hemminki, A., Alvarez, R. D., Emilie, D., Curiel, D. T., Chen, L., Zou, W.

- (2003) Blockade of B7–H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nat. Med.* **9**, 562–567.
40. Brown, J. A., Dorfman, D. M., Ma, F. R., Sullivan, E. L., Munoz, O., Wood, C. R., Greenfield, E. A., Freeman, G. J. (2003) Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J. Immunol.* **170**, 1257–1266.
 41. Matsue, H., Matsue, K., Walters, M., Okumura, K., Yagita, H., Takashima, A. (1999) Induction of antigen-specific immunosuppression by CD95L cDNA-transfected “killer” dendritic cells. *Nat. Med.* **5**, 930–937.
 42. Ory, D. S., Neugeboren, B. A., Mulligan, R. C. (1996) A stable human-derived packaging cell line for production of high titer retrovirus/vesicular stomatitis virus G pseudotypes. *Proc. Natl. Acad. Sci. USA* **93**, 11400–11406.
 43. Subudhi, S. K., Zhou, P., Yerian, L. M., Chin, R. K., Lo, J. C., Anders, R. A., Sun, Y., Chen, L., Wang, Y., Alegre, M-L., Fu, Y-X. (2004) Local expression of B7–H1 promotes organ-specific autoimmunity and transplant rejection. *J. Clin. Invest.* **113**, 694–700.
 44. Xu, H., Dilulio, N. A., Fairchild, R. L. (1996) T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon γ -producing (Tc1) effector CD8+ T cells and interleukin 4/IL-10-producing (Th2) negative regulatory CD4+ T cells. *J. Exp. Med.* **183**, 1001–1012.
 45. Xu, H., Guan, H., Zu, G., Bullard, D., Hanson, J., Slater, M., Elmetts, C. A. (2001) The role of ICAM-1 molecule in the migration of Langerhans cells in the skin and regional lymph node. *Eur. J. Immunol.* **31**, 3085–3093.
 46. Xu, H., Banerjee, A., Dilulio, N. A., Fairchild, R. L. (1997) Development of effector CD8+ T cells in contact hypersensitivity occurs independently of CD4+ T cells. *J. Immunol.* **158**, 4721–4728.
 47. Xu, H., Heeger, P. S., Fairchild, R. L. (1997) Distinct roles of B7–1 and B7–2 determinants during priming of effector CD8+ Tc1 and regulatory CD4+ Th2 cells for contact hypersensitivity. *J. Immunol.* **159**, 4217–4226.
 48. Timares, L., Takashima, A., Johnston, S. A. (1998) Quantitative analysis of the immunopotency of genetically transfected dendritic cells. *Proc. Natl. Acad. Sci. USA* **95**, 13147–13152.
 49. Xu, S., Ariizumi, K., Caceres-Dittmar, G., Edelbaum, D., Hashimoto, K., Bergstresser, P. R., Takashima, A. (1995) Successive generation of antigen-presenting, dendritic cell lines from murine epidermis. *J. Immunol.* **154**, 2697–2705.
 50. Grabbe, S., Schwarz, T. (1998) Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol. Today* **19**, 37–44.
 51. Xu, H., Bjarnason, B., Elmetts, C. A. (2000) Sensitization versus elicitation in allergic contact dermatitis: potential differences at cellular and molecular levels. *Am. J. Contact Dermat.* **11**, 228–234.
 52. Chen, L. (2004) Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat. Rev. Immunol.* **4**, 336–347.
 53. Matsumoto, K., Inoue, H., Nakano, T., Tsuda, M., Yoshiura, Y., Fukuyama, S., Tsushima, F., Hoshino, T., Aizawa, H., Akiba, H., Pardoll, D., Hara, N., Yagita, H., Azuma, M., Nakanishi, Y. (2004) B7-DC regulates asthmatic response by an IFN- γ -dependent mechanism. *J. Immunol.* **172**, 2530–2541.
 54. Shin, T., Kennedy, G., Gorski, K., Tsuchiya, H., Koseki, H., Azuma, M., Yagita, H., Chen, L., Powell, J., Pardoll, D., Housseau, F. (2003) Cooperative B7–1/2 (CD80/CD86) and B7-DC costimulation of CD4+ T cells independent of the PD-1 receptor. *J. Exp. Med.* **198**, 31–38.