Experimental Murine Schistosomiasis in the Absence of B7 Costimulatory Molecules: Reversal of Elicited T Cell Cytokine Profile and Partial Inhibition of Egg Granuloma Formation

Hector J. Hernandez, Arlene H. Sharpe and Miguel J. Stadecker

*J Immunol* 1999; 162:2884-2889; http://www.jimmunol.org/content/162/5/2884
Experimental Murine Schistosomiasis in the Absence of B7 Costimulatory Molecules: Reversal of Elicited T Cell Cytokine Profile and Partial Inhibition of Egg Granuloma Formation

Hector J. Hernandez,* Arlene H. Sharpe,† and Miguel J. Stadecker2*

The granulomatous inflammation in infection with the helminth Schistosoma mansoni represents a cellular hypersensitivity reaction mediated by, and dependent upon, MHC class II-restricted CD4+ Th cells sensitized to parasite Ags. The current work examines the role and significance of the B7:CD28/CTLA-4 pathway in providing the costimulation necessary for the activation of these pathogenic T cells. In vitro T cell responses in B7-1/−/− mice, 7–8 wk postinfection, were no different from wild-type controls, but the absence of B7-2 molecules resulted in a decrease in egg Ag-induced proliferation with increased IFN-γ production. Both B7-1/−/− and B7-2/−/− mice exhibited intact granuloma formation. In contrast, CD4+ Th cells from B7-1/2 double-deficient mice displayed a dramatic loss of proliferative capacity upon stimulation with egg Ag. Most strikingly, these T cells secreted only IFN-γ, but not IL-4 and IL-10, a pattern entirely opposite to that displayed by wild-type controls. Despite these major differences in T cell reactivity, B7-1/2−/− mice had only a limited reduction of granuloma size and fibrosis, without appreciable difference in cellular composition. These results show that substantial granuloma formation can occur under conditions of limited T cell expansion and restricted Th1-type cytokine production. They also support the notion that the combined effect of B7 signaling is not as critical for Th1 cell activation as it is for the development of the Th2 dominant environment characteristic of the evolving schistosome infection in H-2b mice. The Journal of Immunology, 1999, 162: 2884–2889.

Infection with the helminth Schistosoma mansoni results in hepatic and intestinal granulomatous inflammation around parasite eggs that can lead to severe hepatic fibrosis, portal hypertension, gastro-intestinal hemorrhage, and death (1, 2). Early studies in an experimental mouse model have shown that formation of egg granulomas is strictly dependent upon T cells, as these lesions do not form in athymic mice (3). More recently, it was demonstrated that the T cells mediating granuloma formation are egg Ag-specific CD4+ Th cells expressing the αβ heterodimeric receptor for Ag (4–6). CD4+ Th cells are divided into two functional subpopulations based on differential cytokine secretion (7). Th1 cells secrete IL-2 and IFN-γ and participate in cell-mediated immune reactions, while Th2 cells, which secrete IL-4, IL-5, and IL-10, provide help for humoral immune responses. There is evidence that both subtypes participate in the immunopathological response to schistosomes. A Th2-type dominant profile usually follows an initial Th1-type response (8–10).

CD4+ Th cells typically respond to exogenous Ags presented by accessory cells that express a primary signal consisting of complexed complexes of MHC class II molecules and specific Ag peptides, which engage the corresponding clonotypic TCR. However, optimal responses of CD4+ Th cells require a secondary signal, provided by costimulatory molecules. The B7 family of proteins, B7-1 (CD80) (11, 12) and B7-2 (CD86) (13–15), provide the major costimulatory signal for augmenting and sustaining a T cell response via interaction with the CD28 costimulatory receptor (16, 17). B7 costimulators also bind CTLA-4 (18), which can provide a negative signal down-regulating T cell activation (19, 20). The dual specificity of B7 molecules for CD28 and CTLA-4 have made it challenging to elucidate the function of this key costimulatory pathway. Recent studies have suggested that in some models of T cell anergy the unresponsive state is due to B7-CTLA-4 interaction (21) rather than induced by an absence of B7 costimulation (22–24).

The role of the B7:CD28/CTLA-4 pathway has been extensively investigated in vivo in a variety of experimental autoimmune and infectious disease systems (25). In the schistosome infection, early expression of B7-2 molecules has been documented in hepatic egg granulomas by direct immunocytochemical analysis in situ (26). Moreover, up-regulation of B7 molecules on granuloma macrophages in vitro, following neutralization of IL-10, resulted in enhanced stimulation of egg Ag-specific monon- and polyclonal Th cell responses (27).

The critical dependence of granuloma formation on MHC class II Ags has been documented in vivo, as mice lacking class II expression, which do not develop CD4+ Th cells, altogether fail to mount immune or granulomatous responses to schistosome eggs (6). The recent development of mice lacking B7-1 molecules (28), B7-2 molecules, or both B7 molecules (29) has now made it possible to similarly scrutinize the role of the B7:CD28/CTLA-4 costimulatory pathway in experimental schistosomiasis. In particular, animals lacking both the B7-1 and B7-2 molecules provide a most advantageous and definitive model to study this disease under conditions of rigorous absence of known signaling through the B7-CD28/CTLA-4 pathway. Alternatively, the use of anti-B7-1 and anti-B7-2 mAbs may be associated with incomplete blockade, especially when examining a protracted immune response, because...
neutralizing Abs against the anti-B7 mAbs may be induced. In this paper, we report on the T cell response and immunopathology that develop during the schistosome infection in mice lacking either B7-1, B7-2, or both B7 molecules.

**Materials and Methods**

*Mice, parasites, and schistosomulum egg Ags (SEA)*

Mice deficient in B7-1 (B7-1−/−), B7-2 (B7-2−/−), or both (B7-1/2−/−) molecules were made by specific gene targeting in embryonic stem cells, as described previously (28, 29). The 129/SvJae (129) wild-type (WT) mice, used as controls, were bred in our breeding colony. Some mice were infected with 70 cercariae of *S. mansoni*, Puerto Rico strain. Cercariae were shed from infected *Biomphalaria glabrata* snails, provided by the Biomedical Research Institute (Rockville, MD). The SEA was obtained from the Center for Tropical Diseases, University of Massachusetts (Lowell, MA) in part subsidized by the United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases (Geneva, Switzerland).

**Lymphocyte populations**

Mesenteric lymph node cells were dissected from mice infected 7–8 wk previously with 70 cercariae of *S. mansoni*. The lymph node cells were used either intact, or purified CD4+ Th cells thereof were prepared by negative selection, as described previously (30). Briefly, the cells were passed through a nylon wool column and subjected to two cycles of incubation in the presence of mAb against I-Ab, heat stable Ag, and CD8, followed by rabbit complement. Dead cells were eliminated by density gradient centrifugation.

**Proliferation assay**

Bulk lymph node cells were cultured at a concentration of 2.5 × 10^6 cells/200 μl of medium in 96-microwell plastic plates in the presence of the indicated concentrations of SEA. The culture medium used in these experiments, the source of its components, and the culture conditions were identical to those described previously (30). CD4+ Th cells were cultured at a concentration of 1.5 × 10^5/well in the presence of SEA and 3 × 10^5 irradiated (3000 rad) splenocytes, serving as APC, as indicated. Cultures proceeded for 96 h. During the last 24 h of culture, cells were pulsed with 0.5 μCi of [3H]Tdr (Du Pont-New England Nuclear, Wilmington, DE), and incorporation into DNA was measured by liquid scintillation spectrometry.

**Cytokine assays**

Bulk lymph node cells (5 × 10^6/ml), or 1 × 10^6 CD4+ Th cells plus 4 × 10^5/ml irradiated splenic APC, were cultured together with 20 μg/ml SEA in 48-well plastic plates for 48 h. The cytokines IFN-γ, IL-4, and IL-10 were measured in culture supernatants by ELISA, using the corresponding cytokine-specific capture mAbs, detection mAbs, standard cytokines, and protocols from PharMingen (San Diego, CA).

**Assessment of hepatic pathology**

Hepatic pathology was evaluated on 5-μm tissue sections stained with hematoxylin/eosin. Granuloma formation was assessed quantitatively by computer-assisted morphometric analysis using Image Pro Plus software (Image Processing Solutions, North Reading, MA) by observers unaware of the experimental conditions. Data are expressed in surface units. Hepatic fibrosis was assessed by measuring hydroxyproline, as described previously (31).

**Results**

**Hepatic immunopathology and in vitro immune response in schistosome-infected B7-1−/− and B7-2−/− mice**

In a first set of experiments, we compared the outcome of the schistosome infection in mice lacking either B7-1 or B7-2 with WT controls. After 7–8 wk of infection, livers were subjected to quantitative histopathological analysis, and bulk cell populations or purified CD4+ Th cells from the mesenteric lymph nodes were used to assess proliferative and cytokine responses to SEA.

Examination of livers from B7-1−/− and B7-2−/− mice after 7 to 8-wk schistosome infections revealed egg granuloma formation comparable with that seen in WT mice. This was confirmed by computer-assisted morphometric analysis, which reflected no significant difference between the groups, as shown in Table I. The composition of the granulomas in terms of inflammatory cells was similar as well.

A significantly diminished proliferative response to SEA was found in bulk mesenteric lymph node cells from B7-2−/−, but not B7-1−/− mice (Fig. 1). Further analysis of this impaired response using purified CD4+ Th cells disclosed that APC from uninfected B7-2−/− mice were incapable of providing normal stimulation to CD4+ Th cells regardless of whether these were of WT, B7-1−/−, or B7-2−/− origin (Fig. 2). The data also demonstrate that the

<table>
<thead>
<tr>
<th>Table I. Analysis of liver pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>13</td>
</tr>
<tr>
<td>14</td>
</tr>
</tbody>
</table>

<sup>a</sup> Measured by computer-assisted morphometric analysis. A total of 8–20 granulomas were scored per liver.

<sup>b</sup> Compared with WT control.

**FIGURE 1.** Mesenteric lymph node cell proliferative responses in B7-1−/−, B7-2−/−, and WT mice. Cells were obtained from 7 to 8-wk infected mice, plated at a concentration of 2.5 × 10^5/microwell, and cultured for 96 h in the presence of SEA. During the last 24 h of the incubation period, 0.5 μCi of [3H]Tdr was added to each well. 3H incorporation into DNA was assessed by liquid scintillation spectrometry. Points represent mean cpm ± SEM of triplicate determinations. B7-2−/− values are statistically significantly different from WT controls by the Student t test (p < 0.05).

All experiments shown were repeated two to four times with comparable results. Only statistically significant differences are given.

<sup>1</sup> Abbreviations used in this paper: SEA, schistosomal egg Ag; WT, wild type.
B7-2−/− CD4+ Th cells themselves are intrinsically capable of giving normal proliferative responses when stimulated with competent WT or B7-1−/− APC.

When culture supernatants from SEA-stimulated bulk lymph node cells were analyzed for cytokine secretion, a nearly 3-fold increase in IFN-γ production, together with a decreased production of IL-4 and IL-10, was observed in B7-2−/− cells, as compared with WT cells (Fig. 3). IL-4 and IL-10 production were also reduced in B7-1−/− cultures, but to a lesser extent than in B7-2−/− cultures. Purified B7-2−/− CD4+ Th cells also gave a markedly stronger IFN-γ response than those from B7-1−/− or WT nodes under conditions where the T cells were stimulated with SEA in the presence of WT APC (Fig. 4). This difference between the B7-2−/− and the B7-1−/− or WT CD4+ Th cells was less manifest in the case of IL-4 and IL-10 secretion, even though B7-2−/− cells made less IL-4 and IL-10 than those from WT controls.

Hepatic immunopathology and in vitro immune response in schistosome-infected B7-1/2−/− mice

A similar set of experiments was conducted to compare the immunopathology as well as the proliferative and cytokine T cell responses between schistosome-infected B7-1/2−/− and control WT mice. Histological analysis of the B7-1/2−/− livers after a 7 to 8-wk infection revealed a granulomatous reaction that was smaller and less fibrotic than that seen in WT controls (Table I). However, this reduction was marginally significant, and the granulomas were qualitatively indistinguishable in terms of cellular composition, including macrophages, eosinophils, and occasional multinucleated giant cells. Of interest was the unexpected presence in some B7-1/2−/− livers of variable cytoplasmic vacuolization of hepatocytes associated with an interstitial infiltration of mono- and polymorphonuclear inflammatory cells. After 8 wk of infection, increasing hepatocellular damage was evident, causing the death of several animals. For this reason, it was not possible to examine the progression of egg granulomas and related T cell parameters at later time points.

The in vitro proliferative response to SEA by bulk B7-1/2−/− lymph node cells was profoundly inhibited when compared with WT control cells (Fig. 5). Purified WT CD4+ Th cells were less effectively stimulated by B7-1/2−/− splenic APC than by the homologous WT counterparts (Fig. 6), but, most strikingly, B7-1/2−/− CD4+ Th cells altogether failed to respond to SEA in the presence of homologous B7-1/2−/− splenic APC and only gave marginal responses in the presence of WT APC. These results demonstrate that splenic APC lacking B7-1 and B7-2 molecules are suboptimal in stimulating the SEA-specific WT CD4+ Th cells and that there is defective CD4+ Th cell priming to SEA in schistosome-infected mice lacking both B7-1 and B7-2 molecules.

Analysis of supernatants from SEA-stimulated lymph node cells demonstrated a dramatic contrast in cytokine profile between B7-1/2−/− and control WT mice. Strikingly, SEA-stimulated B7-1/2−/− lymph node cells produced substantial amounts of IFN-γ, but negligible IL-4 and IL-10, whereas in WT cells, the secretion pattern was entirely reversed (Fig. 7). Similarly, purified B7-1/2−/− CD4+ Th cells stimulated with SEA in the presence of WT APC secreted large amounts of IFN-γ, but not IL-4 and IL-10, while the opposite was true with WT CD4+ Th cells, which under the same circumstances secreted IL-4 and IL-10, but not IFN-γ.

FIGURE 2. Proliferative responses of CD4+ Th cells from B7-1−/−, B7-2−/−, and WT mice. Cells were obtained from 7 to 8-wk infected mice, purified as described in Materials and Methods, and cultured at a concentration of 1.5 × 10^6 together with SEA and 3 × 10^5 irradiated normal splenic APC from the indicated sources. Culture conditions and assessment of proliferation were identical to those described in Fig. 1. Points represent mean cpm ± SEM of triplicate determinations. B7-2−/− values are significantly different from WT controls (p < 0.005). Values for all T cell cultures in the absence of APC were < 610 cpm.

FIGURE 3. Cytokine production by mesenteric lymph node cells from B7-1−/−, B7-2−/−, and WT mice. Cells were obtained from 7 to 8-wk infected mice and plated in 48-well plates at a concentration of 5 × 10^6/ml in the presence of 20 μg/ml of SEA. After 48 h, IFN-γ, IL-4, and IL-10 present in the culture supernatants were measured by ELISA. Bars represent means ± SEM of triplicate determinations. For IFN-γ, B7-2−/− value is significantly different from WT control (p < 0.005); for IL-4, B7-1−/− and B7-2−/− values are significantly different from WT controls (p < 0.005 and p < 0.0005, respectively); and for IL-10, B7-1−/− and B7-2−/− values are significantly different from WT controls (p < 0.05 and p < 0.005, respectively). IFN-γ values in the absence of SEA were < 0.024 ng/ml. IL-4 and IL-10 were not detected in the absence of SEA.
vitro T cell responses from infected B7-1 Th1 subset upon challenge with SEA in vitro. Interestingly, in respond with an altered cytokine secretion pattern in favor of the during the course of the schistosome infection in vivo, but that they fully stimulate either WT or B7-2

FIGURE 4. Cytokine production by CD4+ Th cells from B7-1+/−, B7-2+/−, and WT mice. CD4+ Th cells were obtained from 7 to 8-wk infected mice, purified as described in Materials and Methods, and cultured at a concentration of 1 × 10⁶ together with 20 μg/ml of SEA and 4 × 10⁶ irradiated splenic APC from normal WT mice. Cytokines were determined as described in Fig. 3. Values for all T cell cultures in the absence of APC were identical to those described in Fig. 2. Points represent mean SEM of triplicate determinations. Values for all T cell cultures in the absence of APC were equal to 37020 cpm.

Discussion

This paper examines the outcome of the murine schistosome infection in the absence of B7-1, B7-2, or both B7 costimulatory molecules. The absence of either B7 molecule alone had no apparent effect on the development of the hepatic immunopathology. B7-2−/− lymph node cells, however, displayed a sharply reduced proliferative response to SEA in vitro. This defect appeared to reside at the APC level because B7-2−/− splenic APC failed to fully stimulate either WT or B7−/− CD4+ Th cells, and CD4+ Th cells from B7-2−/− mice, themselves, were capable of unabridged responses when stimulated with competent APC. These findings suggest that in B7-2−/− mice, Th cells are normally primed to SEA during the course of the schistosome infection in vivo, but that they respond with an altered cytokine secretion pattern in favor of the Th1 subset upon challenge with SEA in vitro. Interestingly, in vitro T cell responses from infected B7-1−/− mice were in all instances comparable to those from WT controls.

In contrast to the mice lacking either B7-1 or B7-2, infected mice lacking both B7 molecules displayed dramatic changes in their lymphoid cell responses to SEA in vitro, as well as significant differences in the hepatic pathology in vivo. Our results showed that in the absence of both B7 molecules, SEA-stimulated CD4+ Th cells are incapable of mounting proliferative responses and that, strikingly, their cytokine response is predominately, if not entirely, of the Th1 type. These observations suggest that the combined effect of both B7-1 plus B7-2 signaling is required for the development of the Th2-dominant/Th1-poor response characteristically seen in week 7–8 of infection in H-2b mice (8–10).

An impaired Th2 response, as well as the development of significant hepatic immunopathology in a Th1 cytokine dominant milieu, agrees with a study of King et al. (32), who examined the schistosome infection in CD28-deficient mice and also reported an inhibition of the Th2-associated IgG1, but not of the Th1-associated IgG2a response in these mice. However, proliferative and IFN-γ responses to SEA were found to be normal, and there was no demonstrable effect on hepatic egg granuloma formation associated with the natural infection. These differences may be attributable to residual signaling through the B7:CD28/CTLA-4 pathway in the CD28-deficient mouse, as recently demonstrated (33, 34).

The Journal of Immunology

2887

FIGURE 5. Mesenteric lymph node cell proliferative responses in B7-1−/− and WT mice. Cell culture conditions and assessment of proliferation were identical to those described in Fig. 1. Points represent mean cpm ± SEM of triplicate determinations. B7-1−/− values are significantly different from WT controls (p < 0.0005).

FIGURE 6. Proliferative responses of CD4+ Th cells from B7-1−/− and WT mice. Cell culture conditions and assessment of proliferation were identical to those described in Fig. 2. Points represent mean cpm ± SEM of triplicate determinations. Values for B7-1−/− CD4+ Th cells are significantly different from corresponding WT controls, both with WT APC and B7-1−/− APC (p < 0.005). Values for all T cell cultures in the absence of APC were <600 cpm. Value for B7-1−/− CD4+T cells stimulated with 20 U/ml of IL-2 in the presence of B7-1−/− APC was equal to 37020 cpm.
Significantly different from WT controls (p < 0.005). IFN-γ values in the absence of SEA were <0.62 ng/ml. IL-4 and IL-10 values in the absence of SEA were <0.0013 ng/ml.

In a related study, the same depression of Th-2 cytokines was achieved with anti-B7-2 Abs, but, in contrast to the natural infection, this treatment inhibited the formation of granulomas around lung-embolized eggs injected i.v. (35). In our hands, treatment with anti-B7-1 and/or anti-B7-2 mAbs had no effect on hepatic egg granulomas associated with the natural infection (data not shown).

Our studies indicate that CD4+ Th cells, abnormally primed to SEA in the absence of B7 costimulation, proliferate poorly, but secrete IFN-γ when stimulated with Ag, a situation similarly described in C57BL/6 invariant chain-deficient mice, which have an impaired ability to present class II-restricted Ags, and develop reduced numbers of CD4+ Th cells that secrete IFN-γ but do not proliferate normally (36). Our findings, together with those of King et al. (32), lend support to the notion that, taken together, the B7:CD28/CTLA-4 pathway is necessary for the development of Th2 cells and normal B cell function (29). Indeed, in B7-1/2−/−-deficient mice, there is absent germinal center formation and impaired Ig isotype switching (29). Interestingly, the schistosome infection in Igα-deficient mice, which have no B cells or Abs, shares many of the characteristics seen in the B7 knockout mice and is marked by normal granuloma formation in a Th1-dominant environment (30).

There appear to be complementary functions of B7-1 and B7-2 in which the absence of B7-2 is most consequential, but only the combined defect seems to lead to sufficient immunological changes that alter the hepatic immunopathology. Even though somewhat reduced in size and fibrous matrix, granuloma formation in B7-1/2−/− mice is qualitatively indistinguishable from WT controls and far greater than the scanty periportal cell aggregates observed in nude (3), MHC class II (6), TCR αβ (5), or Rag-1-deficient mice (5), all of which have no CD4+ Th cells. If their in vitro attributes were to be similar in vivo, it appears that CD4+ Th from B7-1/2−/− mice are capable of mediating substantial granuloma formation under circumstances of impaired clonal expansion and altered cytokine production. These findings suggest that relatively few SEA-specific CD4+ Th cells suffice to spark granuloma formation. They also imply that the B7:CD28/CTLA-4 costimulatory pathway is either not necessary or is largely compensated by another system, to provide the second signal for the observed schistosome-specific Th1-type response. It is possible that in the absence of B7 costimulation, the generated Th1 response is suboptimal and accounts for the somewhat diminished granuloma formation, although it could be argued that this additional granulomatous inflammation is normally mediated by Th2 cells.

The results in the schistosome model complement our studies using DO11.10 TCR transgenic mice, in which we demonstrated that if TCR stimulation is intense enough to induce some IL-2, IFN-γ production will occur in the absence of B7 costimulation. We have found that B7-mediated costimulation mainly affects IL-4 and IL-2 production by naive DO11.10 T cells and influences IL-2 production in activated DO11.10 T cells (43). Furthermore, when naive DO11.10 TCR transgenic T cells were stimulated with APC lacking either B7-1 or B7-2 alone, we observed that B7-2 had a major influence on, but was not obligatory for, IL-4 production (44).

Finally, the basis of the observed liver cell changes in infected B7-1/2−/− mice is not clear. An increase in IL-12 and nitric oxide, along with IFN-γ, and the absence of regulatory Th2 cytokines, such as IL-4 and IL-10, may be a possible explanation, although a direct effect of egg secretions cannot be excluded. However, these changes may also be the consequence of the loss of a yet unknown function controlled by the B7:CD28/CTLA-4 pathway.

**Acknowledgments**

We thank Drs. Yong Wang and Gerardo A. Mirkin for performing the morphometric analysis of the granulomatous lesions, Dr. Allen W. Cheever...
for measuring the fibrosis in the liver samples, and Diana Pierce for expert editorial assistance.

References


