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*J Immunol* 1998; 161:192-199; [http://www.jimmunol.org/content/161/1/192](http://www.jimmunol.org/content/161/1/192)
Tissue-Specific Up-Regulation of B7-1 Expression and Function During the Course of Murine Relapsing Experimental Autoimmune Encephalomyelitis

Nitin J. Karandikar,* Carol L. Vanderlugt,* Todd Eagar,* Litjen Tan,* Jeffrey A. Bluestone,2† and Stephen D. Miller2,3*  

B7/CD28-mediated costimulation is a promising target for therapeutic intervention in autoimmune diseases. However, studies addressing the differential functional roles of B7-1 and B7-2 in several autoimmune models have resulted in conflicting data, perhaps due to the temporal dynamics of B7-1 and B7-2 surface expression on different cell types and/or at different sites during an autoimmune response. We examined the temporal expression of B7 costimulatory molecules in the CNS and in various lymphoid organs during the course of murine relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE). Following immunization of SJL mice with the immunodominant proteolipid protein epitope, PLP139–151, surface expression of B7-1 was up-regulated on B cells, T cells, and macrophages, relative to B7-2, on CNS-infiltrating cells and on splenocytes. Similar enhancement in splenic B7-1 expression could be induced in SJL mice by the adoptive transfer of PLP139–151-specific cells or by immunization with CFA alone. These changes were not observed on lymph node cells, including those isolated from lymph nodes draining the immunization site, which maintained the predominant B7-2 expression pattern seen in naive mice. These phenotypic expression patterns correlated with the functional predominance of B7-1 in costimulating T cell activation when employing APCs from the spleen or CNS of mice with ongoing R-EAE, while B7-2 remained functionally predominant on lymph node APCs. Variation of phenotypic expression and functional dominance of costimulatory molecule expression in different lymphoid compartments during an active inflammatory autoimmune response has important implications in immune regulation, autoimmune pathogenesis, and therapeutic strategies. The Journal of Immunology, 1998, 161: 192–199.

M urine relapsing experimental autoimmune encephalomyelitis (R-EAE) is a CD4+ Th1-mediated demyelinating disease and a well-established model for multiple sclerosis (MS) (1). It can be induced in SJL mice by immunization with the immunodominant proteolipid protein epitope, PLP139–151, or by the adoptive transfer of PLP139–151-specific T cells (2).  

T cells require at least two signals for activation. One signal is Ag specific and is delivered by the MHC peptide complex on the APC via the TCR. The second “costimulatory” signal is delivered via the CD28 molecule on the T cell surface by its interaction with the ligands, B7-1 (CD80) or B7-2 (CD86). The importance of B7/CD28-mediated signaling, in preventing T cell inactivation and tolerance, has been shown in multiple systems in vitro and in vivo (3–8). The presence of the two alternative costimulatory ligands, B7-1 and B7-2, both of which can bind CD28, led to studies addressing the individual functional role of these molecules. Some studies have suggested that B7-1 vs B7-2 costimulation leads to T cell differentiation along different pathways (9). It was proposed that B7-2-mediated costimulation induces IL-4 production in naive T cells pushing them toward Th2 development whereas B7-1 provides a more neutral signal resulting in high IL-2 production (10). However, other studies using B7-1 and B7-2 transfectants have not found any differences in the cytokine production following T cell costimulation (11,12). Thus, the differences in B7-1 and B7-2 activity may reflect either intrinsic differences in the molecules or differential expression on individual cell types or at distinct times during an immune response (13).

In vivo studies, using reagents targeting B7-1 or B7-2, have led to similarly confusing results in different disease models. In nonobese diabetic (NOD) mice, treatment with anti-B7-2 prevented the development of diabetes, whereas anti-B7-1 mAb treatment resulted in exacerbated disease (14). In contrast to the NOD results, the same anti-B7-1 mAb administered to SJL mice during the induction phase of EAE blocked disease development, whereas anti-B7-2 treatment resulted in disease exacerbation (9). Recent studies in the murine model of Th2-dependent airway eosinophilia suggested that B7-1-mediated costimulation is not required for the induction of Th2 immune responses but rather for the amplification of lung inflammation (15). Though these results could be explained by the differences in the several models used, results from our lab using the same Abs in a similar model of R-EAE add to the intrigue. When these Abs were administered during the first remission after the acute phase of the disease, anti-B7-2 treatment had no effect, whereas blockade of B7-1/CD28 interactions using the
F(ab) fragments of anti-B7-1 resulted in the blockade of disease relapses (16). This correlated with the blockade of the process of epitope spreading that mediates clinical relapses in diseased animals (17). Thus, B7-1 appeared to be the predominant costimulatory molecule in animals with established disease.

In the present study, we analyzed the dynamics of surface expression of the costimulatory ligands, B7-1 and B7-2, in the context of R-EAE. Active immunization of SJL mice with PLP139–151 in CFA resulted in a temporal up-regulation of surface B7-1 expression, relative to B7-2, on splenic B cells, T cells, and macrophages, similar to our previous report demonstrating B7-1 predominance on cells infiltrating the CNS of mice with active disease (16). Similar enhancement of B7-1 expression was induced on spleen cells by the adoptive transfer of EAE or by immunization with CFA alone. Surprisingly, B7-1 expression did not change significantly on the lymph node cells from any of these treated mice. The selective up-regulation of B7-1 on splenic and CNS APCs in animals with active disease was reflected in the functional predominance of B7-1 in these organs. In contrast, B7-2 remained functionally predominant in lymph nodes, correlating with its expression. These results demonstrate selective up-regulation of splenic B7-1 with a dichotomy in the costimulatory phenotype of the peripheral lymphoid organs during ongoing autoimmune disease and support the notion that this differential expression of B7-1 and B7-2 at different sites in the animal may lead to distinct functional consequences.

Materials and Methods

Mice

Female SJL/J mice, 6 to 7 wk old, were purchased from Harlan Laboratories (Indianapolis, IN) housed in the Northwestern University animal care facility and maintained on standard laboratory food and water ad libitum.

Peptides

Peptides PLP139–151 (HSLGKWLGHPDKF) and VP2–70–86 (WTTSQEAFSHRIPLPH) were synthesized as described previously (16, 18).

Immunization and induction of active and adoptive R-EAE

For active R-EAE, mice were immunized with 100 μg of Mycobacterium tuberculosis H37Ra (Difco, Detroit, MI) and 80 μg of PLP139–151 (2). Mice immunized with CFA alone received similar emulsion without the addition of peptide or an emulsion containing a nonencephalitogenic peptide (Theiler’s virus protein VP2–70–86) in CFA (18). Adoptively transferred EAE was induced by the transfer of in vitro activated PLP139–151-primed lymph node cells (19). Clinical severity was assessed on a 0 to 5 scale (16).

mAb staining

Mice were sacrificed on the indicated day following immunization or transfer. Lymph node and spleen cells were pooled (two to three mice per time point) and prepared as previously described (16). FITC-conjugated mAbs to CD3 (T cells) and B220 (B cells) and biotin-conjugated hamster Ig control were purchased from Pharmingen (La Jolla, CA). In addition, the following mAbs were employed (16, 21): hamster control Ig (Cappel Research Products, NC), rat control Ig (111/10), anti-CD80 of the Ag. The following mAbs were employed (16, 21): hamster control Ig (Cappel Research Products, NC), rat control Ig (111/10), anti-CD80 (B7-1) mAb, 16–10A1 (Repligen Corp.); and anti-CD86 (B7-2) mAb GL-1. Cultures were pulsed with 1 μCi of [3H]TdR after 72 h and harvested at 96 h; [3H]TdR uptake was determined by scintillation counting. The actual counts of control cultures varied between groups, and, for comparison, results were expressed as mean percent proliferation (counts of test samples compared with relevant controls; hamster Ig for anti-B7-1; rat Ig for anti-B7-2, and a combination of hamster and rat Ig for both).

Results

B7-1 expression is enhanced on spleen cells, but not on lymph node cells, following active induction of R-EAE

Previous studies have shown that B7-2, but not B7-1, is primarily expressed on naive spleen cells (22). However, we observed enhanced B7-1 expression during active EAE in SJL mice (16). Therefore, a temporal analysis of B7-1 and B7-2 expression was performed in SJL mice. As seen in Figure 1, the basal level of B7-2 expression was significantly greater than B7-1 on spleen cells and lymph node cells from naive SJL mice. In multiple experiments, naive animals at all ages showed similar findings and served as age-matched controls for other experiments in these studies. Figure 1A shows an example of a typical pattern of B7-1 and B7-2 expression in the naive SJL spleen. The relative expression of B7-1 to B7-2 was determined by the ratio of B7-1 fluorescence shift to that of B7-2 (16) and ranged from 0.1 to 0.6 in different sets of experiments (Fig. 1). Similarly, the percentage of total cells expressing B7-1 (4.6%) was lower than those expressing B7-2 (23.3%). Individual cell types, analyzed by gating for CD3+ T cells, B220+B cells, or F4/80+ macrophages, showed similar patterns of B7 expression with B7-1:B7-2 ratios of less than 1.0 (Fig.

Flow cytometry

Data collection and analysis were performed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Sufficient events were collected to assure that at least 5 × 10^4 live (propidium iodide-negative) events were analyzed per sample. Both B7-1 and B7-2 showed unimodal staining patterns, and expression was analyzed by mean fluorescence intensity shift over nonspecific background staining (ΔMFI). Nonspecific background staining was determined by incubating samples with isotype control mAbs. The actual value of ΔMFI in a given experiment was dependent on several factors, such as Ab affinities, voltage settings, biotinylation efficiency, etc. Thus, in any given set of experiments, all the settings and Ab stocks were kept constant. Values for B7-1 and B7-2 ΔMFI were used to calculate the relative B7-1:B7-2 ratios over time, as reported previously (16), and the patterns were consistent in multiple experiments. Percentage of cells staining positive for B7-1 or B7-2 were determined based on the histogram of cells obtained using relevant control Ig.

Isolation of CNS mononuclear cells

Mice in remission from the acute phase of adoptive R-EAE were anesthetized with methoxyflurane (Pittman-Moore) and perfused through the left ventricle with ~60 ml of PBS (20). Spinal cords were extruded by flushing the vertebral canal with PBS, and rinsed in PBS. Spinal cords were forced through 100-μm stainless steel screens to give a single cell suspension. CNS mononuclear cells were isolated by centrifugation (500 × g) at 24°C over 30%/70% discontinuous Percoll (Pharmacia) gradients. Cells collected from the 30%/70% interface were washed and resuspended in DMEM (19). F-α-expressing F4/80+ macrophages and microglia were enriched by a 2-h plastic adherence step at 37°C, harvested by scraping at 4°C, washed two times in DMEM, and assessed for their ability to enogenously activate a PLP139–151-specific T11 line.

In vitro proliferation assays

Mice were primed with PLP139–151 in CFA. On the indicated days postimmunization, lymph node and spleen cells were harvested from groups of two to three immunized mice as well as age-matched naive controls. For use as APCs, cells were irradiated at 3000 rad and plated at 4 × 10^5 cells/well in 96-well plates. Column-purified responder T cells (>98% CD4+ by flow cytometry) were obtained from lymph nodes of mice primed with the unrelated, nonencephalitogenic Theiler’s virus peptide, VP2–70–86 (3 × 10^5 T cells/well) (18). Cells were cultured in the presence or absence (background) of Ag (VP2–70–86, 25 μM) in a total volume of 200 μl complete DMEM-7. Cells were allowed to incubate with the indicated Abs (10 μg/well) for 30 to 45 min at 37°C before the addition of the Ag. The following mAbs were employed (16, 21): hamster control Ig (Cappel Research Products, NC), rat control Ig (111/10), anti-CD80 (B7-1) mAb, 16–10A1 (Repligen Corp.); and anti-CD86 (B7-2) mAb GL-1. Cultures were pulsed with 1 μCi of [3H]TdR after 72 h and harvested at 96 h; [3H]TdR uptake was determined by scintillation counting. The actual counts of control cultures varied between groups, and, for comparison, results were expressed as mean percent proliferation (counts of test samples compared with relevant controls; hamster Ig for anti-B7-1; rat Ig for anti-B7-2, and a combination of hamster and rat Ig for both).
2). Naive lymph nodes also exhibited low levels of B7-1 as compared with B7-2 (Fig. 1, C and D; B7-1:B7-2 ratio of 0.22), with a lower percentage of cells expressing B7-1 (2.8%) as compared with B7-2 (9.9%). The same pattern of low or undetectable B7-1 expression was seen on B cells and T cells. Significant numbers of F4/80<sup>+</sup> cells were not detectable in lymph nodes to assess B7 expression on macrophages in this tissue (data not shown).

Mice were sacrificed at varying time points following the active induction of R-EAE by immunization with PLP139–151 in CFA and analyzed for changes in the expression of B7-1 and B7-2 on the surface of individual cell types in the draining lymph nodes and spleen. Immunization of SJL mice resulted in a relative up-regulation of surface B7-1 expression and a decrease in B7-2 expression on spleen cells (Fig. 2, A and B). Thus, the B7-1:B7-2 ratio flipped to 3.38 (Fig. 2B) during preclinical disease, as compared with 0.21 in age-matched naive controls (Fig. 2A). These relative changes in B7-1/B7-2 expression were seen as early as 5 to 7 days after immunization of mice and were consistently found in the preclinical phase, acute phase, remission, and first relapse phases of R-EAE (Fig. 2, and data not shown). These changes were reproducible over several experiments and were similar to our previous observations in cells isolated from the central nervous system of diseased mice (16).

It was apparent from the flow cytometric profiles that B7-1 was significantly up-regulated on a subpopulation of splenic cells (4.6% B7-1<sup>+</sup> cells in naive spleen vs 14.2% in the preclinical phase). Therefore, individual subsets were examined, as shown in Figure 2C. The changes in both the levels of B7-1 expression and the percentage of B7-1<sup>+</sup> cells, observed in the overall splenic population (Fig. 2A), were also reflected in the individual cell types, including T cells, B cells, and macrophages (Fig. 2C), reaching highest levels on the surface of F4/80<sup>+</sup> macrophages.

Interestingly, these changes in surface expression patterns of B7-1 and B7-2 did not occur in the draining lymph node cell population (Fig. 3). Lymph node cells taken at various time points following immunization (as early as day 2 and as late as day 40; data not shown) continued to express relatively constant levels of surface B7-2 on both T cells and B cells, and at no time point was a detectable increase in B7-1 expression observed. This lack of change was evident in both the draining lymph nodes (Fig. 3) as well as cells isolated from remote (periaortic) lymph nodes (data not shown). Thus, there appears to be a selective enhancement of B7-1 in the central nervous system (16) and the spleen (Fig. 2), but not in the lymph nodes (Fig. 3), during this chronic autoimmune disease.

**Relative up-regulation of B7-1 expression on spleen cells as a consequence of an inflammatory response**

The basis of the selective up-regulation of B7-1 in the CNS and the spleen might be the active CNS inflammation induced subsequent to the peptide injection or the inflammatory response mediated by the CFA immunization. Therefore, we examined
the expression of B7-1 and B7-2 following the induction of disease by the adoptive transfer of in vitro-activated PLP139–151-specific cells. This protocol establishes demyelinating inflammation in the CNS without peripheral CFA-induced inflammation. Mice were analyzed for B7-1 and B7-2 expression at varying time points following adoptive transfer. As seen in Figure 4, there was consistent temporal up-regulation of B7-1 expression in the spleen, with a B7-1:B7-2 ratio of greater than 1.0 during the acute phase of disease (day 10–14). Thus, the pattern of B7-1/B7-2 expression was similar to that seen in active disease (Fig. 2A), although the kinetics were slightly delayed. Analysis of individual cell subsets, including T cells, B cells, and macrophages, showed similar up-regulation of B7-1 expression as seen in the whole population (data not shown). In contrast, lymph node cells from these animals showed no enhancement in B7-1 expression (data not shown), similar to our observations in active disease (Fig. 3). Thus, Th1-mediated CNS inflammation was sufficient to induce changes in B7-1 expression in the spleen.

In another set of experiments, we examined the effect of immunization on the B7-1 expression pattern. Mice were primed with either CFA alone or with a nonencephalitogenic Theiler’s virus peptide in CFA (Materials and Methods). At the indicated times following immunization (See Fig. 2A), mice were sacrificed and analyzed for B7 expression patterns on draining lymph node cells. The data are plotted as ΔMFI units, and the figures in parentheses indicate the relative B7-1:B7-2 expression ratios of the indicated cells. The data for naive controls are representative of age-matched naive SJL mice. Results are representative of four separate experiments.
These findings suggest that inflammation, induced by either active immunization or adoptive transfer of encephalitogenic T cells, results in the selective up-regulation of B7-1 expression in the spleen, whereas lymph nodes consistently maintain a predominantly B7-2-expressing phenotype.

Functional relevance of B7-1 and B7-2 expression patterns

We next analyzed the functional consequences of changes in the relative expression of B7-1 and B7-2 in the lymph nodes, spleens, and of mice immunized with PLP139–151/CFA. Draining lymph node cells and spleen cells from immunized mice and age-matched naive controls were harvested at varying time points and used as APCs in proliferation assays. Ag-specific responder T cells were isolated from the lymph nodes of mice immunized with a non-cross-reactive, I-A<sup>+</sup>-binding peptide from the Theiler’s virus protein, VP2–70–86 (18). Proliferation assays were conducted in the presence of CTLA4-Ig, anti-B7-1 mAb, anti-B7-2 mAb, or a combination of these mAbs (or their relevant controls). Figure 6 shows the results of a representative assay using APCs from mice 10 days postimmunization. Correlating with the relative expression of B7-1 and B7-2 in the different cell populations, anti-B7-2 mAb preferentially inhibited proliferation when naive splenocytes (Fig. 6C) or...
hamster Ig induced a vigorous proliferative response indicating with the peptide-specific T cell line in the presence of control in the absence of added peptide. In contrast, direct culture of CNS APCs seen in Figure 7, splenic APCs from naive SJL/J mice failed to of PLP139–151-induced R-EAE. CNS APCs were irradiated and purified from the spinal cords of mice remitting from acute phase immunized mice.

Ronald w. Determined the costimulatory dependence of Ag en- donogenously presented by CNS APCs from SJL mice with R-EAE is predominantly B7-1 dependent. CNS plastic adherent APCs were purified from the spinal cords of mice remitting from the acute phase of adoptive PLP139–151-specific R-EAE (approximately 14 days post T cell transfer). To assess the endogenous ability of the CNS APCs to present myelin epitopes in comparison to naive splenocytes (Spl. APCs) from age-matched controls, graded numbers (10^4–5 × 10^5/well) were cocultured with a PLP139–151-specific T cell line (5 × 10^5 cells/well) for 72 h in the absence of any added peptide. CNS APCs were allowed to incubate with the indicated mAb reagents for 30 min before the addition of the T cell line. Results are expressed as cpm of [3H]thymidine incorporated and are representative of numerous experiments. Figures in parentheses indicate percent inhibition.

Primed lymph node cells (Fig. 6A) were used as APCs. In contrast, when splenocytes from immunized animals were used, the proliferation was preferentially inhibited by anti-B7-1 mAb (Fig. 6B). In all cases, the addition of a combination of both Abs had the most inhibitory effect, similar to CTLA-4Ig, a molecule that binds both all cases, the addition of a combination of both Abs had the most inhibitory effect, similar to CTLA-4Ig, a molecule that binds both all Ag-specific signals via the TCR and a second costimulatory signal, provided via CD28 on the T cell surface by its interaction with the B7 family members, B7-1 (CD80) or B7-2 (CD86) (23, 24). Studies addressing the roles of B7-1 and B7-2 in autoimmune settings have resulted in conflicting reports. Early treatment of NOD mice with anti-B7-2 inhibited diabetogenesis, whereas anti-B7-1 mAb treatment exacerbated the onset of diabetes (14). In contrast, studies in EAE showed that anti-B7-1 mAb blocked disease induction, whereas anti-B7-2 resulted in exacerbation of disease (9). The mechanism was proposed to involve the preferential differentiation of T helper cells along Th1 or Th2 pathways. However, our group subsequently showed that anti-B7-2 mAb treatment had no effect on the progression of R-EAE when administered during remission after the acute phase of disease (16). During this phase, treatment with the F(ab) fragments of anti-B7-1 blocked disease relapses (16), whereas treatment with the intact anti-B7-1 mAb exacerbated disease (25), indicating that intact anti-costimulatory Abs may actually signal through the target molecules rather than merely act as blocking agents.

One likely explanation for these conflicting results is that costimulatory ligands may be differentially expressed at different sites in the body, on different cell types, and/or at different times during the autoimmune response. In vitro data suggests that B7-1 and B7-2 are differentially regulated on APCs as well as T cells (13, 22, 26–28). However, little information is available on the in vivo expression patterns of these costimulatory molecules on different cell types during an immune response.

The present study was conducted to examine the surface expression of B7-1 and B7-2 in different organs during the course of a Th1-mediated demyelinating disease, R-EAE. As described previously in various systems, naive SJL mice preferentially expressed surface B7-2 in both the lymph nodes and the spleen (Fig. 1) (13, 22). We have shown previously that during the course of R-EAE there was heightened B7-1 expression, relative to B7-2, on CNS-infiltrating T cells, B cells, and macrophages (16). In this study, similar up-regulation of B7-1 expression with concomitant B7-2 down-regulation was observed on spleen cells following active disease induction. These changes in B7-1/B7-2 expression were very consistent over multiple experiments. Moreover, the changes in the expression patterns in both the spleen and on CNS-infiltrating F4/80+ cells had important functional consequences as B7-1 became the predominant costimulatory ligand in proliferation assays (Figs. 6 and 7). Interestingly, our results indicate that, in the inflammatory demyelinating milieu of the CNS of mice with active disease, resident F4/80+ macrophages/microglia endogenously display a significant amount of surface I-Aα-associated myelin epitopes, including the immunodominant PLP139–151 determinants (Fig. 7) and other less dominant epitopes such as PLP178–191 and MBP84–104 (data not shown), associated with epitope spreading and disease relapses (17). Thus, following induction of EAE in SJL mice, B7-1 becomes the dominant costimulatory molecule in the spleen and in the CNS. This could explain our findings that anti-B7-2 treatment of mice during disease remission had no detectable effect on R-EAE progression, whereas blockade of B7-1-mediated interactions inhibited disease relapses (16). Up-regulation of B7-1 may be a normal consequence of chronic inflammation as treatment with anti-B7-2 mAb also failed to affect the progression of diabetes when administered to NOD mice at 10 wk of age or later (14).

Interestingly, while relative B7-1 up-regulation was seen in the CNS (16) and in the spleen (Fig. 2), B7-1 expression was low or undetectable in the lymph nodes throughout the disease course (Fig. 3). The costimulatory phenotype of the lymph node environment also remained B7-2 predominant (Fig. 6). Several previous reports have shown that B7-2 is the predominant costimulatory
molecule in the induction of immune responses (14, 29). Interestingly, these studies employed immunization protocols that induced the initial immune response in the draining lymph nodes or used naïve spleen cells as MLR stimulators, sites where B7-2 provides the predominant costimulatory signal based on both expression (Fig. 1) and function (Fig. 6C). However, responses that arise in an inflammatory environment, such as in a relapsing autoimmune disease, may well be B7-1 dependent. B7-1-mediated costimulation was recently shown to be important in the induction of local lung eosinophilia, but not in the generation of systemic eosinophilia or IgE responses in a TH2-dependent model of airway eosinophilia (15). In a relapsing Th1-mediated autoimmune disease like R-EAE, ongoing tissue destruction is dependent on the continued recruitment of new T cell responses to endogenously presented tissue epitopes, a phenomenon termed epitope spreading (17, 30). In PLP139–151-induced disease, the first clinical relapse is predominantly mediated by T cells specific for a secondary PLP epitope, PLP178–191 (17). The two most likely sites in the animal where the priming and spreading of such immune responses may occur are the CNS, the site of destruction, and the spleen, where tissue breakdown products may be concentrated and presented. Both these environments are high in B7-1 expression and function. This explains why blockade of B7-1-mediated interactions, using F(ab) fragments of anti-B7-1 mAb (16), results in the blockade of epitope spreading and an inhibition of ongoing disease. Similar enhancement of B7-1 expression has been shown in multiple sclerosis (31); thus, B7-1 could serve as an important target for therapeutic intervention in established disease.

One of the interesting issues that remains to be resolved is the molecular basis of selective up-regulation of B7-1 in these tissues. To date, in vitro studies of cellular expression of B7-1 and B7-2, employing mitogens, Abs, or cytokines to activate the cells, have not found selective B7-1 up-regulation (13, 22). However, our in vivo studies suggest that relative B7-1 up-regulation is a generalized response to a Th1-type inflammation, whether initiated in the periphery or the CNS. Destructive inflammatory stimuli, such as immunization of mice with CFA alone (Fig. 5) or induction of R-EAE by adoptive transfer of encephalitogenic T cells (Fig. 4), are sufficient to induce these changes in the splenic cell populations. The kinetics of B7-1 up-regulation is slightly delayed in the case of adoptive disease, as compared with CFA-immunization, probably due to the time required to induce peripheral effects following initiation of inflammation within the CNS following adoptive transfer of encephalitogenic cells. Also, the patterns of changes in B7-2 expression are different in CFA-immunized mice (constant expression) vs the disease-inducing models (decreased expression). The changes in B7-1/B7-2 expression patterns are most striking in active disease (Fig. 2) and are probably an effect of both the CFA-induced inflammation (early) as well as the CNS inflammation (late). The mechanism of this up-regulation of B7-1, relative to B7-2, still remains to be understood. It is likely that local cellular interactions or cytokines and chemokines secreted in the inflammatory milieu may be responsible for the selective up-regulation of B7-1. Cytokines, such as IFN-γ, TNF-α, IL-4, IL-5, and IL-10, which are present in the CNS of diseased animals (Ref. 32, and our unpublished observations), have been shown to regulate the surface expression of costimulatory molecules on several cell types (22, 33, 34). These factors, alone or in combination, could result in the selective up-regulation of B7-1 in vivo. However, the interplay of these factors and the exact molecular events involved in these processes need to be elucidated.

Another consistent finding in these studies was that lymph node cells from these animals did not significantly up-regulate surface B7-1 expression, despite the fact that under some conditions these nodes were draining the sites of CFA-induced inflammation. Despite the failure to up-regulate B7-1 in vivo, we (data not shown) and others (26) have shown that lymph node T cells from PLP139–151-primed mice up-regulate B7-1 expression in vitro following peptide-specific activation. There are several possible explanations for these findings. Some cell type or soluble factor in the lymph node environment in vivo may actively suppress B7-1 up-regulation. Alternatively, activated T cells may traffic out of the lymph node before they up-regulate significant levels of B7-1. This latter hypothesis is supported by the finding that T cells expressing high levels of B7-1 are found in the CNS-infiltrating population from diseased mice (16). However, trafficking of activated cells may be only part of the explanation for differential B7-1 expression in peripheral lymphoid compartments, considering that all the cell types tested (T cells, B cells, and macrophages) showed similar lack of B7-1 up-regulation. Finally, the spleen may contain certain cell types, such as granulocytes, that may produce factors that up-regulate B7-1 expression during the inflammatory response. Thus, our future studies will focus on the poorly understood factors intrinsic to the spleen and lymph nodes that are likely to affect the microenvironments and influence B7 expression.

The implications of the dichotomy in B7-1 and B7-2 expression in the two lymphoid compartments are multifold. B7-1 interacts with the CD28 homologue, CTLA-4, with higher avidity and differential kinetics as compared with B7-2 (35, 36). CTLA-4 has been shown to function as a negative regulator of T cell activation (37, 38) and is an important down-regulator of R-EAE (19). Based on the higher avidity of CTLA-4/B7-1 interactions and their similar delayed kinetics of expression, B7-1 may be the preferred ligand for CTLA-4 during an immune response (23). In fact, following in vitro activation of PLP139–151-specific T cells, CTLA-4 and B7-1 show very similar delayed expression kinetics (Ref. 19, and data not shown). It is tempting to speculate that the enhancement of B7-1 could be involved in the down-regulation of an ongoing immune response. Thus, the absence of B7-1 up-regulation in lymph nodes may preserve these sites for promoting immune responses to incoming Ags, whereas local sites of inflammation and the spleen may be involved, not only in the initial promotion of inflammation, but also the active modulation of an ongoing response. The exacerbating effect of anti-B7-1 mAb treatment on NOD mice and on ongoing EAE (23, 25) could be partially due to inhibition of B7-1-mediated down-regulation. However, it is clear that B7-1 can also serve as a positive costimulator of CD28-mediated signals. For example, intact anti-B7-1 inhibited EAE induction (9), while anti-B7-1 F(ab) treatment was shown to prevent disease relapses (16). In addition, B7-1 is the dominant costimulatory ligand for in vitro activation of primed PLP139–151-specific T cells, which express low levels of CTLA-4 (Figs. 6 and 7). Thus, B7-1/CTLA-4-mediated down-regulation probably depends on the site and timing of expression of these molecules. Additional studies in other disease models where B7-1 up-regulation is not sustained throughout the disease course may be helpful in resolving the mechanisms behind these opposing functions. It is also possible that B7-1-expressing T cells may be responsible for regulatory T cell-T cell interactions as there is evidence that engagement of this costimulatory molecule may lead to direct signaling of these cells (25, 39).

In summary, during the course of R-EAE, an inflammatory Th1-mediated autoimmune disease, there is an enhancement of B7-1 expression (and function), relative to B7-2, at the site of inflammation and in the splenic environment. In contrast, the lymph node environment maintains a B7-2-predominant phenotype. This dichotomy in the B7-1/B7-2 costimulatory phenotype of different
lymphoid microenvironments may reflect their diverging roles during the course of an immune response. Further insight into the functional role of differential regulation of costimulatory ligands in the modulation of immune responses would be a very important step toward the development of rational therapeutic strategies for autoimmune diseases.

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