

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor***COSTIMULATORY B7 MOLECULES IN THE PATHOGENESIS OF INFECTIOUS AND AUTOIMMUNE DISEASES**HANS REISER, M.D.,  
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**M**OST immune responses depend on the activation of T cells. This class of lymphocytes consists of functionally and phenotypically distinct populations, the best characterized of which are helper T cells and cytotoxic T cells. Distinctive molecules on the cell surface provide markers of these populations: CD4 for helper T cells and CD8 for cytotoxic T cells. CD4 and CD8 T cells recognize antigens through a T-cell antigen receptor composed of an  $\alpha$  chain and a  $\beta$  chain. Other T cells bearing  $\gamma$ - and  $\delta$ -chain receptors have been identified, but their function is less clear.

CD4 helper T cells can be divided into two subtypes. Type 1 helper T cells mainly synthesize interferon- $\gamma$  and interleukin-2; type 2 helper T cells principally secrete interleukin-4, interleukin-5, and interleukin-10.<sup>1</sup> These two types of mature CD4 T cells were originally identified in mice. Similar subgroups exist in humans, although the distinctions are less sharp than in mice and there are functionally overlapping populations of helper T cells.<sup>1</sup>

The two types of CD4 helper T cells appear to serve different functions. Type 1 cells mediate delayed hypersensitivity, activate macrophages, and in mice promote a switch in antibody class from IgM to IgG2a. In contrast, type 2 cells mainly provide help for B cells, by promoting class switching from IgM to IgG1 and IgE. Furthermore, the pathogenesis of certain diseases appears to be strongly influ-

enced by the type of helper T cells involved. For example, in leishmaniasis and leprosy the development of a response polarized toward type 1 helper T cells is important for a successful immune defense, but a response polarized toward type 2 helper T cells is detrimental. In the case of infection with nematodes, however, an immune response polarized toward type 2 helper T cells may aid in clearing the infection.

The physiologic stimulation of T lymphocytes involves at least two activation signals from antigen-presenting cells. The first signal imparts specificity to T-cell activation; it consists of the presentation to the T cell of an immunogenic peptide bound to a protein of the highly polymorphic major histocompatibility complex (MHC), which in humans is also known as the HLA complex. Immunogenic peptides can be generated from exogenous proteins, such as bacterial proteins, or from endogenous proteins, such as tumor-associated antigens. There are several pathways of antigen processing and presentation,<sup>2</sup> but each terminates with the display by the MHC protein of an antigenic peptide on the surface of the antigen-presenting cell. This peptide-protein complex is recognized specifically by a particular T-cell-antigen receptor, which transduces an activation signal into the T cell.

The stimulation of a T-cell-antigen receptor is necessary, but not sufficient, to induce complete T-cell activation, and it does not lead to cell proliferation or cytokine secretion. Complete T-cell activation requires a second signal. These so-called costimulatory signals depend on the interaction of nonpolymorphic proteins and serve to initiate, maintain, and regulate the activation cascade. Studies of costimulatory pathways have enhanced our knowledge of immunologic diseases and opened up new possibilities for prophylaxis and therapy. In this article, we will review T-cell costimulation and its importance in immunity. We will focus on what may be the most important of the costimulatory pathways, called the B7:CD28/CTLA-4 costimulatory pathway, and review its role in the pathogenesis of infectious and autoimmune diseases.

**THE FEATURES OF T-CELL COSTIMULATION**

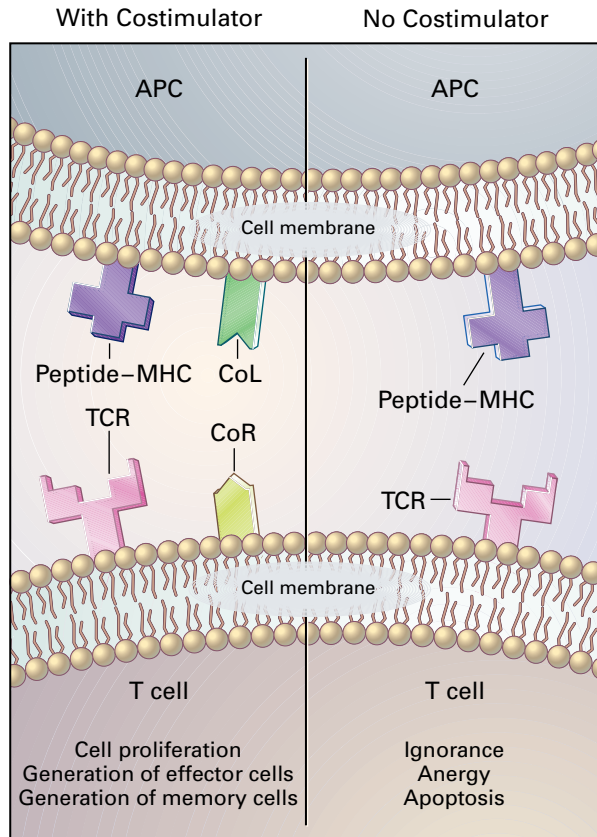
All "naive" T lymphocytes (cells in which an immune response has not yet been activated) require two signals to initiate an immune response. To what extent recently activated T cells or memory T cells depend on costimulation is unclear, however. Memory T cells are quantitatively less dependent on co-

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stimulatory signals than naive T cells,<sup>3</sup> but costimulator antagonists have a beneficial effect in mice with lupus erythematosus even if administered during an advanced stage of the disease.<sup>4</sup>

Signaling through the T-cell–antigen receptor in the absence of a costimulatory signal may or may not affect T cells (Fig. 1). Sometimes, the T cell simply ignores peptide–MHC complexes presented to it in the absence of costimulators. At other times, how-



**Figure 1.** Activation of Naive T Cells in the Presence or Absence of a Costimulatory Signal.

Physiologic activation of naive T cells requires two signals. The first signal is initiated by the binding of a peptide–MHC complex to the T-cell–antigen receptor (TCR); the second is initiated by the binding of a costimulatory ligand (CoL) to a costimulatory receptor (CoR). Both the peptide–MHC complex and the costimulatory ligand are presented to the T cell by an antigen-presenting cell (APC). Stimulation of naive T cells through the T-cell–antigen receptor in the presence of a costimulatory signal leads to cell proliferation, the generation of effector cells, and the generation of memory cells. In the absence of a costimulatory signal, activation of naive T cells is nonproductive. In some cases, the T cell remains oblivious to stimulation of the T-cell–antigen receptor (“ignorance”). In other situations, activation of the T-cell–antigen receptor renders the lymphocyte refractory to further stimulation (anergy) or leads to its death (apoptosis). The precise outcome of T-cell stimulation in the absence of a costimulatory signal appears to depend on its state of activation and differentiation.

ever, recognition of peptide–MHC complexes in the absence of costimulators can induce apoptotic death of the T cell; it can also render the T cell anergic, a condition in which the T cell is refractory (that is, unable to respond to antigens even when they are presented by antigen-presenting cells that express a costimulatory molecule).<sup>5</sup> Precisely what determines the outcome of the stimulation of T-cell–antigen receptors in the absence of costimulation (“ignorance,” apoptosis, or anergy) is not known. Perhaps the state of differentiation of the T cell is important. For example, as compared with type 2 effector CD4 T cells, type 1 effector CD4 T cells are rendered anergic more easily.<sup>6</sup> The strength of the signal through the T-cell–antigen receptor and the overall avidity of the interaction between the antigen-presenting cell and the T cell may also influence the outcome.

### THE CLINICAL RELEVANCE OF T-CELL COSTIMULATION

Because microbial products can affect the expression of costimulatory molecules,<sup>7,8</sup> these molecules participate in the pathogenesis of infectious diseases. They also participate in the pathogenesis of autoimmune diseases, the rejection of grafted organs, and graft-versus-host disease, and antagonists of these molecules can prevent or ameliorate these conditions.<sup>6</sup> Another clinically relevant aspect of costimulation arises from the finding that tumor cells transfected with genes encoding costimulators can generate cytotoxic T-cell responses *in vivo*,<sup>9,10</sup> an indication that costimulators may have applications in antitumor and antiviral vaccines.

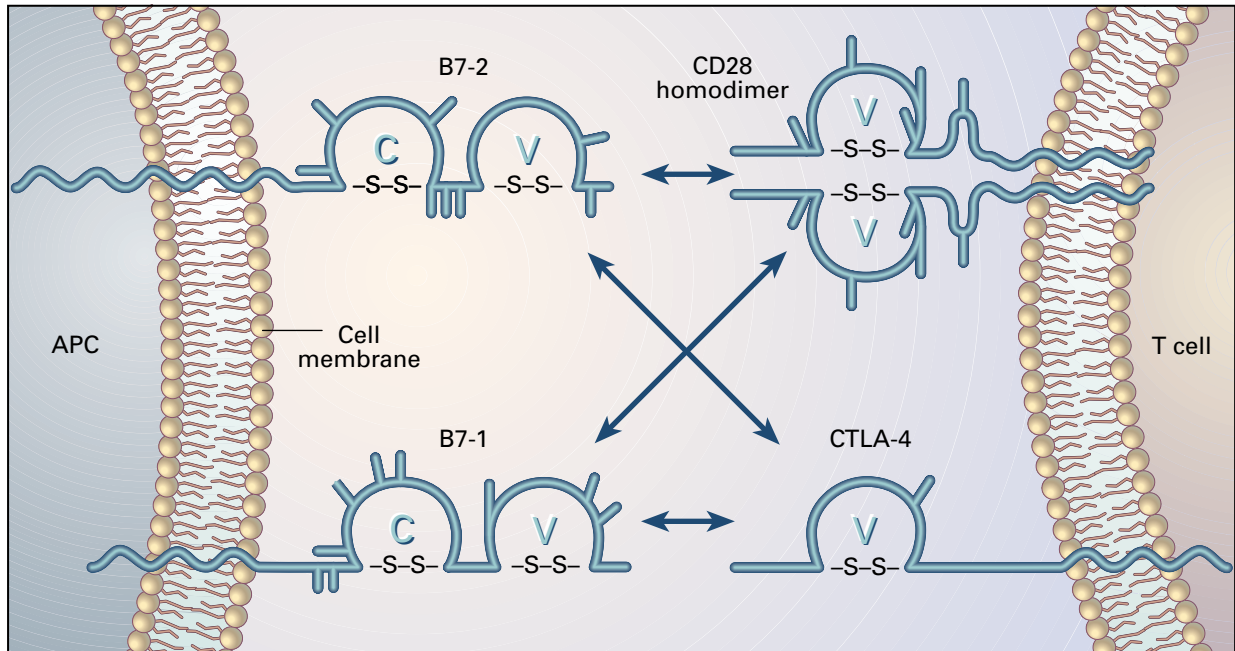
The manipulation of costimulatory pathways is by no means the only new avenue of therapeutic intervention. For example, the binding of MHC molecules containing altered peptide ligands to T-cell antigen receptors can inhibit T-cell function. Other possibilities for therapeutic intervention include the inhibition of adhesion receptors and the administration of cytokines or cytokine antagonists.<sup>11,12</sup>

### THE IMMUNOBIOLOGY OF THE B7:CD28/CTLA-4 COSTIMULATORY PATHWAY

Several ligand–receptor pairs function as costimulators.<sup>13</sup> Among these, the best characterized is the B7:CD28/CTLA-4 costimulatory pathway. It consists of two costimulatory ligands on antigen-presenting cells, B7-1 and B7-2, each of which binds to two receptors on T cells, called CD28 and CTLA-4 (Fig. 2).<sup>13</sup>

#### CD28 and CTLA-4

The CD28 and CTLA-4 proteins share amino acid sequences and have a similar overall structure, but they are expressed differently and serve different functions. CD28 is expressed by both resting and



**Figure 2.** Structure of B7-1 (CD80) and B7-2 (CD86) and Their Receptors, CD28 and CTLA-4.

B7-1 and B7-2, as well as CD28 and CTLA-4, are members of the immunoglobulin superfamily. Each of these four proteins consists of a single, amino-terminal, V-like domain (V) and contains sites for N-linked glycosylation. In B7-1 and B7-2, the V-like domain is followed by a C-like domain (C). B7-1 and B7-2 are present on the cell surface as monomers. The CD28 receptor is expressed predominantly in homodimeric form, although a small fraction of CD28 receptors exist as monomers (not shown). The CTLA-4 receptor exists in both homodimeric and monomeric configurations. The latter form is illustrated here. CD28 and CTLA-4 each binds both B7-1 and B7-2. CTLA-4, although expressed at much lower density than CD28, binds B7-1 and B7-2 with approximately 100 times more avidity than does CD28; B7-1 has a moderately higher avidity (2 to 3 times as high) for CD28 and CTLA-4 than does B7-2.<sup>14</sup> APC denotes antigen-presenting cell, and S-S disulfide bonds.

activated T cells and is the predominant receptor for B7 on resting T cells.<sup>6</sup> B7 binding to CD28 costimulates T-cell activation, leading to the expression of cytokines, cytokine receptors, and genes for cell survival.<sup>13,15,16</sup> In contrast to CD28, CTLA-4 is expressed only on activated T cells.<sup>13</sup> B7-1 and B7-2 have a higher affinity for binding with CTLA-4 than with CD28, which raises the possibility that CTLA-4 is the predominant B7 receptor on activated T cells. Unlike CD28, CTLA-4 can transduce an inhibitory signal, and its function may be to terminate T-cell activation.<sup>6,17-19</sup>

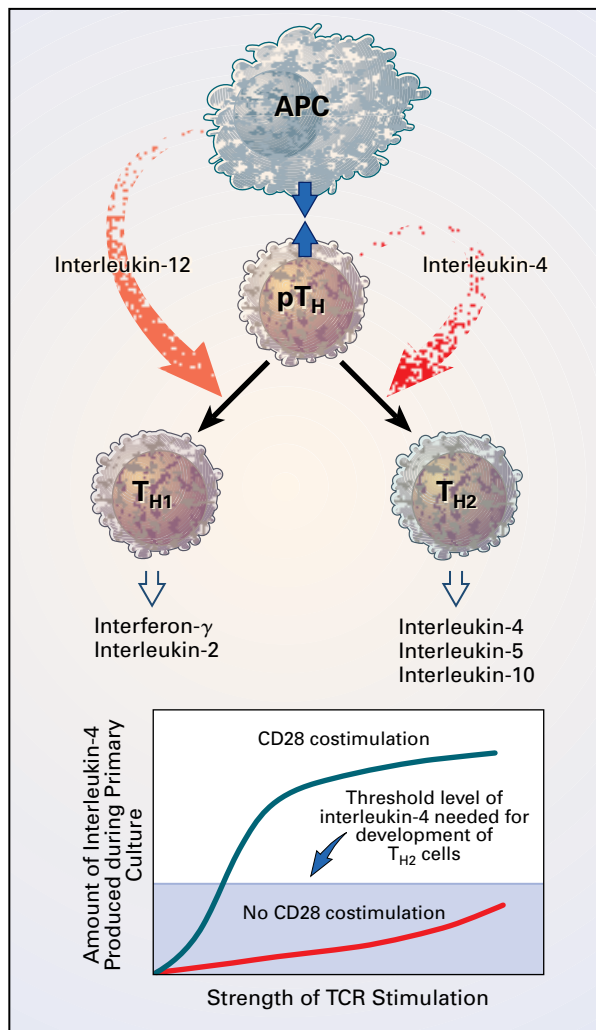
The biochemical basis of CD28-mediated signaling is not known.<sup>13</sup> Nevertheless, CD28-mediated signaling differs from signaling mediated by T-cell-antigen receptors and is not affected by the immunosuppressant drugs cyclosporine and tacrolimus.<sup>20,21</sup> The biochemical basis of CTLA-4-mediated signaling is largely unknown.<sup>22,23</sup>

#### The Regulation of B7-1 and B7-2 Expression

The expression of the B7 antigens, B7-1 (CD80) and B7-2 (CD86), is tightly regulated.<sup>14</sup> Unstimulated antigen-presenting cells are largely B7-2-negative and B7-1-negative, although B7-2 is expressed at

low levels by dendritic cells, on which it may participate in the priming of T cells.<sup>8,13,24</sup> After activation, dendritic and epidermal Langerhans' cells, B cells, and macrophages up-regulate the expression of B7-2 and B7-1. In addition to their expression on MHC class II-bearing cells, B7-2 is expressed on granulocytes,<sup>25</sup> B7-1 on fibroblasts,<sup>8</sup> and both molecules on activated murine and human T cells.<sup>13</sup> This pattern of expression raises the possibility that B7 molecules may have other functions in addition to the costimulation of T cells.

Several cytokines modulate the expression of B7-1 and B7-2, including interleukin-10, interleukin-4, granulocyte-macrophage colony-stimulating factor, and interferon- $\gamma$ .<sup>13</sup> The expression of B7 molecules is induced by bacterial lipopolysaccharides<sup>8,26</sup> or by neisserial porins.<sup>27</sup> In most acute or chronic immune responses, B7-2 is induced earlier than B7-1 and rises to higher levels.<sup>6</sup> An exception to this rule occurs in experimentally induced murine allergic encephalomyelitis, in which the surface expression and functional activity of B7-1 are preferentially increased on antigen-presenting cells.<sup>28,29</sup> This finding raises the possibility that B7-1 may be involved in the pathogenesis of multiple sclerosis, the apparent human



**Figure 3.** Possible Role of the B7:CD28 Pathway in the Development of Helper T Cells.

The upper part of the figure shows the differentiation pathways of murine CD4 T cells. On contact with antigen presented by antigen-presenting cells (APC), precursors of helper T cells ( $pT_H$ ) are induced to differentiate into effector cells ( $T_{H1}$ ) that produce either interferon- $\gamma$  and interleukin-2 or effector cells ( $T_{H2}$ ) that produce interleukin-4, interleukin-5, and interleukin-10. T-cell populations capable of producing both interleukin-4 and interferon- $\gamma$  exist; they are commonly designated type 0 (not shown). Interleukin-12 and interleukin-4 are the major cytokines responsible for inducing T cells along the  $T_{H1}$  and  $T_{H2}$  pathways, respectively. Interleukin-12 is mainly produced by antigen-presenting cells, whereas interleukin-4 is produced by the differentiating T cell, and possibly by third-party cells (not shown).<sup>1</sup> The graph, in the lower part of the figure, shows the requirement for CD28 stimulation in the differentiation of type 2 helper T cells. In the absence of CD28 costimulation, interleukin-4 production remains below the threshold required for the expansion of interleukin-4-secreting clones. TCR denotes T-cell receptor.

counterpart of experimentally induced murine allergic encephalomyelitis.

#### The Role of B7-1 and B7-2 Molecules

The transfection of B7-1 or B7-2 genes into cell lines that lack costimulatory activity and the use of these artificial antigen-presenting cells in assays of T-cell activation have made possible the identification of the T-cell populations that can respond to B7-1 and B7-2 and the observation of the cellular responses stimulated by these molecules.<sup>30-33</sup> Thus, B7-1 or B7-2 transfectants can costimulate the proliferation of T cells,<sup>30,31</sup> the production of interleukin-2,<sup>30,31</sup> and the expression of interleukin-2 receptors on the cell surface.<sup>34,35</sup>

Antagonists of B7-1 and B7-2 can block immune responses both in vitro and in vivo. These antagonists consist of antibodies that act specifically against either B7-1 or B7-2, or chimeric fusion proteins that recognize both ligands. The most useful of these fusion proteins has been CTLA-4-Ig, which consists of the extracellular domain of CTLA-4 and the hinge- $C_{H2}$ - $C_{H3}$  region of IgG1. CTLA-4-Ig and anti-B7-2 antibodies are potent inhibitors of T-cell activation in vitro, and they are more effective than anti-B7-1 antibodies in most assays.<sup>36-39</sup> When administered in vivo, both CTLA-4-Ig and anti-B7-2 antibodies can block the production of antibodies.<sup>39,40</sup>

B7 transfectants can costimulate the production of both interferon- $\gamma$  and interleukin-4.<sup>33,41-43</sup> This strongly suggests that the B7:CD28 pathway can costimulate both type 1 and type 2 effector CD4 T cells (Fig. 3). The B7:CD28 pathway appears to be more important in the generation of type 2 helper T cells than in the generation of type 1 helper T cells, because mice lacking CD28 molecules have decreased levels of IgG1.<sup>44</sup> This may be explained by the requirement of type 2 precursor CD4 T cells for strong initial T-cell stimulation, because the magnitude of the initial T-cell stimulation determines the amount of interleukin-4 produced.<sup>1</sup> In the absence of CD28 costimulation, the production of interleukin-4 remains below the threshold required for optimal development of type 2 helper T cells (Fig. 3).<sup>1</sup> Although both B7-1 and B7-2 transfectants can costimulate the production of interleukin-4 in vitro,<sup>33,41-43</sup> studies in vivo indicate that the generation of type 2 helper T cells depends mainly on the interaction of CD28 with B7-2.<sup>28</sup> Presumably, B7-2 plays a dominant part in the production of interleukin-4 because of its early expression during T-cell priming.

By contrast, the need for B7 molecules in the generation of type 1 effector CD4 T cells is less clear. The development of type 1 helper T cells is induced by interleukin-12, a cytokine that is produced by macrophages but not by T cells (Fig. 3). Moreover, in the absence of CD28 costimulation, other costim-

ulatory receptor–ligand pairs may compensate to facilitate the generation of type 1 effector T cells.<sup>44</sup>

The costimulatory potential of B7 molecules is not limited to CD4 T lymphocytes. On the contrary, B7 molecules can costimulate CD8 T cells in the absence of exogenous help from CD4 T cells.<sup>45</sup> This effect has been exploited to generate experimental vaccines against melanoma in mice.<sup>9,10</sup> B7 molecules can also costimulate natural killer cells<sup>46</sup> and  $\gamma/\delta$  T cells,<sup>47</sup> further underscoring the possible importance of the B7:CD28 pathway in antitumor and antimicrobial immunity.<sup>48,49</sup>

It is likely that B7 molecules serve not only as ligands but also as signal-transducing receptors. This function as a receptor is made plausible by the molecular structure of the cytoplasmic domain of B7 molecules,<sup>13</sup> as well as by the observation that certain monoclonal anti-B7-1 antibodies can stimulate, rather than block, inflammatory reactions if administered in vivo.<sup>25,50</sup>

#### THE B7 COSTIMULATORY PATHWAY AND DISEASE

B7 molecules are important in the pathogenesis of graft rejection, graft-versus-host disease, infections, and autoimmune diseases; in addition, they participate in immunity against tumors.<sup>6</sup> We will focus on the role of B7-1 and B7-2 in infectious and autoimmune diseases.

##### B7 Molecules in Infectious Disease

The B7:CD28 costimulatory pathway participates in the pathogenesis of several bacterial, parasitic, and viral diseases. Certain bacteria or their products can increase the expression of B7. One of these bacterial products, lipopolysaccharide, increases the expression of B7-1 and B7-2 by antigen-presenting cells.<sup>8,39</sup> The incubation of macrophages with heat-killed *Listeria monocytogenes* also causes increased production of B7.<sup>51</sup> A series of proteins isolated from pathogenic strains of neisseria, *Neisseria meningitidis* and *N. gonorrhoeae*, up-regulate the expression of B7-2, which may explain the adjuvant effect of these neisserial proteins in antibody production.<sup>27</sup> In contrast, *Mycobacterium tuberculosis*<sup>52</sup> and the intracellular protozoan *Leishmania donovani*<sup>51</sup> inhibit the expression of B7-1 molecules after they infect macrophages in vitro.

The toxic shock syndrome and food poisoning are serious side effects of the immune system's defense against extracellular bacteria. These complications are caused by a class of microbial products, called superantigens, that can activate all T cells that express a particular family of T-cell antigen-receptor genes. The immune response to certain bacterial superantigens, such as the staphylococcal enterotoxins and the related toxic shock syndrome toxin (TSST-1), also depends on costimulation by B7-1 and B7-2.<sup>53</sup>

Only a few studies have addressed the role of the B7:CD28 pathway in viral infections. After infection with the human immunodeficiency virus, the expression of CD28 is reduced in both CD4 and CD8 T cells.<sup>54,55</sup> It is not known whether stimulation of the remaining CD28 molecules promotes<sup>54</sup> or inhibits<sup>56</sup> viral infection. In mice carrying a targeted mutation in the CD28 gene, the response of helper T cells to infection with vesicular-stomatitis virus is diminished, but cytotoxic T-cell responses to infection with lymphocytic-choriomeningitis virus are normal.<sup>44</sup> These findings suggest that the B7:CD28 pathway plays a more important part in viral infections in which viral clearance depends on antibody responses. Infection with the hepatitis B virus may be one of these, but this issue has not yet been examined.

Infection with *Leishmania major* perhaps best exemplifies the consequences of the dominance of a particular subgroup of helper T cells on the immune response. Strains of mice that use type 1 helper T cells to respond to *L. major* produce large amounts of interferon- $\gamma$ , and thus resist the parasite. By contrast, strains of mice that use type 2 helper T cells, and consequently produce interleukin-4 and interleukin-10, succumb to the organism. CTLA-4-Ig, if administered early after infection, reduces the production of interleukin-4 and halts the progression of disease in susceptible BALB/c mice but has no effect in the genetically resistant C57BL/6 strain of mice.<sup>57</sup> These different effects in susceptible and resistant mice indicate that the priming of type 2 helper T cells is more dependent on the B7:CD28 pathway than is the priming of type 1 cells<sup>57</sup> and provides evidence for the interpretation of analogous results in mice deficient in CD28.<sup>44</sup>

Infection with the trematode helminth *Schistosoma mansoni* may be an instance in which the levels of B7 expression on antigen-presenting cells correlate with, if they do not dictate, the severity of disease. *S. mansoni* antigens sensitize type 1 and type 2 helper T cells to form large granulomas in the liver around eggs of the parasite. However, the response of the type 1 helper T cells and the intensity of granulomatous inflammation spontaneously subside after the acute stage of the infection. There is substantial expression of B7-2 in cells of granulomas during the acute schistosomal disease; it rapidly diminishes thereafter.<sup>58</sup> It is likely that the down-regulation of B7 expression and of the granulomatous reaction are precipitated by interleukin-10,<sup>59,60</sup> given that macrophages from egg granulomas secrete interleukin-10 and induce unresponsiveness in type 1 helper T cells.<sup>59</sup> Neutralization of interleukin-10 by specific antibodies increases the expression of B7-2 by these macrophages and thus restores costimulatory activity.<sup>60</sup> A similar process has been observed in humans infected with *S. haematobium*.<sup>61</sup> Taken togeth-



er, these findings suggest that the inhibition of B7 expression may permit therapeutic control of schistosomal-egg granulomas. In fact, injection of interleukin-10 at a time when its endogenous expression is low or absent results in decreased formation of granulomas and decreased cytokine production by type 1 helper T cells.<sup>62</sup>

The observation that, as compared with type 2 effector CD4 T cells, type 1 effectors depend more on costimulation and are more susceptible to anergy has implications for chronic infections in which immune defenses depend on type 1 helper T cells. In tuberculosis, leprosy, schistosomiasis, filariasis, and leishmaniasis, the waning of the B7:CD28 costimulatory pathway may change the clinical form of the disease by depressing cell-mediated immunity and inducing T-cell anergy. To what extent this downregulation of T-cell immunity is caused by the organisms themselves<sup>52</sup> or mediated by cytokines<sup>60</sup> is not known.

#### The B7:CD28/CTLA-4 Pathway in Autoimmunity

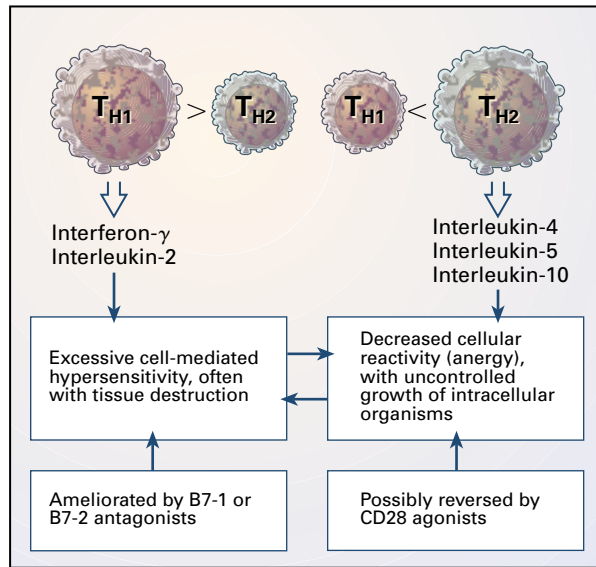
The expression and function of B7-1 and B7-2 have been studied in mice with systemic lupus erythematosus, diabetes mellitus, and experimentally induced allergic encephalomyelitis. Systemic lupus erythematosus, the prototype of a multisystem autoimmune disorder, occurs spontaneously in several strains of inbred or cross-bred mice. NZB/W F1 mice have not only chronic glomerulonephritis, caused by the deposition of immunoglobulin molecules in the kidney, but also anti-DNA antibodies in the serum. Injection of NZB/W F1 mice with CTLA-4-Ig, which neutralizes both B7-1 and B7-2, blocked the production of anti-DNA antibodies and prolonged life, even when the treatment was delayed until the most advanced stage of the illness.<sup>4</sup> To examine the individual roles of B7-1 and B7-2, mice were injected with specific antibodies against B7-1 or B7-2.<sup>63</sup> Injection of anti-B7-1 antibodies alone had little effect on the serum levels of anti-DNA antibodies or on the development of nephritis. By contrast, the injection of anti-B7-2 antibodies inhibited the production of anti-DNA antibodies, especially those of the IgG1 subclass, but did not affect nephritis. Injections of both anti-B7-1 and anti-B7-2 antibodies prevented the production of anti-DNA antibodies of all subclasses as well as the development of nephritis, and resulted in prolonged survival.<sup>63</sup>

B7 antagonists inhibit the development of insulinitis and diabetes mellitus in nonobese diabetic mice,<sup>50</sup> an animal model of insulin-dependent diabetes mellitus. In these mice, islet-cell lesions appear as early as three to four weeks after birth. Both CD8 T cells and CD4 type 1 helper T cells infiltrate the pancreas and participate in the destruction of the insulin-producing beta cells.<sup>64</sup> Both CTLA-4-Ig and anti-B7-2 antibodies blocked the development of diabetes

when administered in mice two to four weeks of age, whereas anti-B7-1 antibodies had no protective effect.<sup>50</sup>

These studies suggest that B7-2 is more important than B7-1 in the pathogenesis of autoimmune and inflammatory disorders. By contrast, in experimentally induced murine allergic encephalomyelitis, B7-1 seems more important than B7-2. Allergic encephalomyelitis can be induced in susceptible strains of mice by active immunization with the dominant encephalitogenic epitope of myelin proteolipid protein. The disease is characterized by an initial acute phase of paralysis followed by recovery and subsequent recurrences. During these recurrences, T cells emerge that are reactive with epitopes of myelin proteolipid protein other than the one used in the initial immunization, a phenomenon called epitope spreading. Injections with anti-B7-1 antibodies, but not with anti-B7-2 antibodies, reduced the severity of disease.<sup>28,29</sup> Blockade of the B7-1:CD28 interaction also prevents epitope spreading.<sup>29</sup> These two studies of mice therefore suggest that B7-1 plays a major part in the pathogenesis of experimentally induced allergic encephalomyelitis.

The effects of B7 antagonists on autoimmune disorders may be explained in two ways. In one model, the interaction of B7-1 and B7-2 with the same receptor on a precursor helper T cell results in the generation of different subgroups of helper T cells. According to this model, B7-1 preferentially acts as a costimulator for type 1 helper T cells and B7-2 induces the production of type 2 helper T cells.<sup>28</sup> The difficulty with this explanation is that studies of transfection have not established a clear difference between B7-1 and B7-2 in their capacity to induce the production of cytokines or second messengers.<sup>33,41-43</sup> Furthermore, hapten-induced contact-sensitivity reactions and the development of diabetes in nonobese diabetic mice (both of which appear to be mediated by CD4 type 1 helper T cells and CD8 T cells<sup>64,65</sup>) are inhibited by the administration of anti-B7-2 antibodies but not anti-B7-1 antibodies.<sup>25,50</sup> We prefer the second model, according to which B7-1 and B7-2 induce similar signals in precursor helper T cells but can have distinct roles in immune responses because of their different patterns of expression. As discussed above, B7-2 is typically expressed earlier than B7-1, and at higher levels, during an immune response. One would therefore predict that anti-B7-1 antibodies would inhibit only cytokines, such as interferon- $\gamma$ , associated with type 1 helper T cells, whereas anti-B7-2 antibodies would block the production of interleukin-4 as well as of interferon- $\gamma$ . In fact, this pattern was reported in one study.<sup>63</sup> Furthermore, in the case of experimentally induced encephalomyelitis, in which anti-B7-1 but not anti-B7-2 antibodies can block the progression of disease, B7-1 is expressed at higher levels than B7-2.<sup>29</sup>



**Figure 4.** Possible Role of B7 Costimulatory Molecules in the Pathogenesis of Inflammatory Diseases.

The left-hand side of the figure shows one end of a spectrum of immune response, characterized by the overstimulation of type 1 helper T cells ( $T_{H1}$ ) relative to type 2 helper T cells ( $T_{H2}$ ). This leads to pronounced cell-mediated immunity, which may be associated with granuloma formation or destructive inflammation, including that leading to autoimmune diseases. In animals, the progression of disease can be slowed with B7-1 or B7-2 antagonists, although it remains to be determined to what extent the timing of administration influences the development of helper T-cell subgroups and disease outcome. The right-hand side of the figure illustrates the opposite end of the spectrum, characterized by a reduced ratio of  $T_{H1}$  to  $T_{H2}$  cells. Several chronic infectious diseases, such as lepromatous leprosy, microfilaric filariasis, anergic leishmaniasis, exudative tuberculosis, and mucocutaneous candidiasis, may belong in this category. In some of these diseases, the clinical outcome may be improved by an enhancement of costimulation.

To what extent B7-1 and B7-2 molecules have a role in the pathogenesis of other autoimmune and inflammatory diseases is not known. CTLA-4-Ig can prevent the onset of collagen-induced arthritis in BB rats, but the relative roles of B7-1 and B7-2 in these animals have not yet been explored.<sup>66</sup> Recently, inflammatory bowel disease was identified in several strains of mice.<sup>67</sup> T cells from one of these strains, interleukin-2-deficient mice, are costimulated by B7-1 or B7-2 transfectants<sup>35</sup> — a finding that raises the possibility that B7 antagonists could be used in the prevention or treatment of inflammatory bowel disease.

The role of the CTLA-4 receptor in the pathogenesis of autoimmunity is not known. A lethal lymphoproliferative disease develops in CTLA-4-deficient mice that is characterized by T-lymphocytic infiltration of multiple organs and destruction of tissue.<sup>18,19</sup> This phenotype is evidence of a suppressive

role for the CTLA-4 receptor and raises the possibility that mutations in the CTLA-4 gene can lead to autoimmune disease in humans.

## CONCLUSIONS

The role of the B7:CD28/CTLA-4 costimulatory pathway in the pathogenesis of chronic diseases described in this article may be understood by considering these disorders as lying along a spectrum of immune response (Fig. 4). At one end are autoimmune and granuloma-forming diseases in which the predominant stimulation of type 1 helper T cells is a key mechanism. Under these conditions, B7 molecules might induce or sustain the generation of pathogenic type 1 helper T cells, even if the up-regulation of B7 in inflammatory tissues is a secondary event. At the other end of the spectrum are infectious diseases such as lepromatous leprosy<sup>68</sup> and microfilaric filariasis.<sup>69</sup> In these diseases, reduced ratios of type 1 to type 2 helper T cells can impair cell-mediated immunity, which may result in anergy. However, to what extent these conditions coexist with an overall reduction in costimulation, due either to reduced expression or function of B7 or CD28 molecules or to increased expression or function of the CTLA-4 receptor, remains to be established.

During the past decade, the study of T-cell costimulation has emerged as one of the most exciting areas of immunology. The functional characterization of the B7:CD28/CTLA-4 pathway has greatly enhanced our understanding of the pathogenesis of infectious and autoimmune diseases. Further investigations of this pathway should lead to the design of novel immunosuppressive compounds and to the generation of new vaccines. However, not every condition at the ends of this spectrum of immune response will require the therapeutic modification of T-cell costimulation, because the state of anergy may have advantages or disadvantages that vary from disease to disease.

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## REFERENCES

1. Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4+ T cells. *Annu Rev Immunol* 1994;12:635-73.
2. York IA, Rock KL. Antigen processing and presentation by the class I major histocompatibility complex. *Annu Rev Immunol* 1996;14:369-96.
3. Croft M, Bradley LM, Swain SL. Naive versus memory CD4 T cell response to antigen: memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J Immunol* 1994;152:2675-85.

4. Finck BK, Linsley PS, Wofsy D. Treatment of murine lupus with CTLA4Ig. *Science* 1994;265:1225-7.
5. Janeway CA Jr, Bottomly K. Signals and signs for lymphocyte responses. *Cell* 1994;76:275-85.
6. Bluestone JA. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* 1995;2:555-9.
7. Liu Y, Janeway CA Jr. Microbial induction of co-stimulatory activity for CD4 T-cell growth. *Int Immunol* 1991;3:323-32.
8. Razi-Wolf Z, Freeman GJ, Galvin F, Benacerraf B, Nadler L, Reiser H. Expression and function of the murine B7 antigen, the major costimulatory molecule expressed by peritoneal exudate cells. *Proc Natl Acad Sci U S A* 1992;89:4210-4.
9. Chen L, Ashe S, Brady WA, et al. Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. *Cell* 1992;71:1093-102.
10. Townsend SE, Allison JP. Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. *Science* 1993;259:368-70.
11. Sloan-Lancaster J, Allen PM. Altered peptide ligand-induced partial T cell activation: molecular mechanisms and role in T cell biology. *Annu Rev Immunol* 1996;14:1-27.
12. Röcken M, Racke M, Shevach EM. IL-4 induced immune deviation as antigen-specific therapy for inflammatory autoimmune disease. *Immunol Today* 1996;17:225-31.
13. Guinan EC, Gribben JG, Boussiotis VA, Freeman GJ, Nadler LM. Pivotal role of the B7:CD28 pathway in transplantation tolerance and tumor immunity. *Blood* 1994;84:3261-82.
14. Linsley PS, Greene JL, Brady W, Bajorath J, Ledbetter JA, Peach R. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. *Immunity* 1994;1:793-801. [Erratum, *Immunity* 1995;2:203a.]
15. Boise LH, Minn AJ, Noel PJ, et al. CD28 costimulation can promote T cell survival by enhancing the expression of Bcl-X<sub>L</sub>. *Immunity* 1995;3:87-98.
16. Lucas PJ, Negishi I, Nakayama K, Fields LE, Loh DY. Naive CD28-deficient T cells can initiate but not sustain an in vitro antigen-specific immune response. *J Immunol* 1995;154:5757-68.
17. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994;1:405-13.
18. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctl-4. *Science* 1995;270:985-8.
19. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995;3:541-7.
20. June CH, Ledbetter JA, Gillespie MM, Lindsten T, Thompson CB. T-cell proliferation involving the CD28 pathway is associated with cyclosporine-resistant interleukin 2 gene expression. *Mol Cell Biol* 1987;7:4472-81.
21. Galvin F, Freeman GJ, Razi-Wolf Z, Benacerraf B, Nadler L, Reiser H. Effects of cyclosporin A, FK 506, and mycalamide A on the activation of murine CD4+ T cells by the murine B7 antigen. *Eur J Immunol* 1993;23:283-6.
22. Schneider H, Prasad KV, Shoelson SE, Rudd CE. CTLA-4 binding to the lipid kinase phosphatidylinositol 3-kinase in T cells. *J Exp Med* 1995;181:351-5.
23. Marengère LEM, Waterhouse P, Duncan GS, Mittrücker H-W, Feng G-S, Mak TW. Regulation of T cell receptor signaling by tyrosine phosphatase SYP association with CTLA-4. *Science* 1996;272:1170-3.
24. Inaba K, Witmer-Pack M, Inaba M, et al. The tissue distribution of the B7-2 costimulator in mice: abundant expression on dendritic cells in situ and during maturation in vitro. *J Exp Med* 1994;180:1849-60.
25. Reiser H, Schneeberger EE. Expression and function of B7-1 and B7-2 in hapten-induced contact sensitivity. *Eur J Immunol* 1996;26:880-5.
26. Hathcock KS, Laszlo G, Pucillo C, Linsley P, Hodes RJ. Comparative analysis of B7-1 and B7-2 costimulatory ligands: expression and function. *J Exp Med* 1994;180:631-40.
27. Wetzler LW, Ho Y, Reiser H. Neisserial porins induce B lymphocytes to express costimulatory B7-2 molecules and to proliferate. *J Exp Med* 1996;183:1151-9.
28. Kuchroo VK, Das MP, Brown JA, et al. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell* 1995;80:707-18.
29. Miller SD, Vanderlugt CL, Lenschow DJ, et al. Blockage of CD28/B7-1 interaction prevents epitope spreading and clinical relapses of murine EAE. *Immunity* 1995;3:739-45.
30. Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. *J Exp Med* 1991;173:721-30.
31. Freeman GJ, Borriello F, Hodes RJ, et al. Murine B7-2, an alternative CTLA4 counter-receptor that costimulates T cell proliferation and interleukin 2 production. *J Exp Med* 1993;178:2185-92.
32. Azuma M, Cayabyab M, Buck D, Phillips JH, Lanier LL. CD28 interaction with B7 costimulates primary allogeneic proliferative responses and cytotoxicity mediated by small, resting T lymphocytes. *J Exp Med* 1992;175:353-60.
33. Galvin F, Freeman GJ, Razi-Wolf Z, et al. The murine B7 antigen provides a sufficient costimulatory signal for antigen-specific and MHC-restricted T cell activation. *J Immunol* 1992;149:3802-8.
34. Reiser H, Freeman GJ, Razi-Wolf Z, Gimmi CD, Benacerraf B, Nadler LM. The murine B7 antigen provides an efficient costimulatory signal for activation of murine T lymphocytes via the T-cell receptor/CD3 complex. *Proc Natl Acad Sci U S A* 1992;89:271-5.
35. Razi-Wolf Z, Holländer GA, Reiser H. Activation of CD4+ T lymphocytes from interleukin 2-deficient mice by costimulatory B7 molecules. *Proc Natl Acad Sci U S A* 1996;93:2903-8.
36. Wu Y, Guo Y, Liu Y. A major costimulatory molecule on antigen-presenting cells, CTLA4 ligand A, is distinct from B7. *J Exp Med* 1993;178:1789-93.
37. Razi-Wolf Z, Galvin F, Gray G, Reiser H. Evidence for an additional ligand, distinct from B7, for the CTLA-4 receptor. *Proc Natl Acad Sci U S A* 1993;90:11182-6.
38. Lenschow DJ, Su GH-T, Zuckerman LA, et al. Expression and functional significance of an additional ligand for CTLA-4. *Proc Natl Acad Sci U S A* 1993;90:11054-8.
39. Hathcock KS, Laszlo G, Dickler HB, Bradshaw J, Linsley P, Hodes RJ. Identification of an alternative CTLA-4 ligand costimulatory for T cell activation. *Science* 1993;262:905-7.
40. Linsley PS, Wallace PM, Johnson J, et al. Immunosuppression in vivo by a soluble form of CTLA-4 T cell activation molecule. *Science* 1992;257:792-5.
41. de Boer M, Kasran A, Kwekkeboom J, Walter H, Vandenberghe P, Ceuppens JL. Ligation of B7 with CD28/CTLA-4 on T cells results in CD40 ligand expression, interleukin-4 secretion and efficient help for antibody production by B cells. *Eur J Immunol* 1993;23:3120-5.
42. Levine BL, Ueda Y, Craighead N, Huang ML, June CH. CD28 ligands CD80 (B7-1) and CD86 (B7-2) induce long-term autocrine growth of CD4+ T cells and induce similar patterns of cytokine secretion in vitro. *Int Immunol* 1995;7:891-904.
43. Natesan M, Razi-Wolf Z, Reiser H. Costimulation of IL-4 production by murine B7-1 and B7-2 molecules. *J Immunol* 1996;156:2783-91.
44. Shahinian A, Pfeffer K, Lee KP, et al. Differential T cell costimulatory requirements in CD28-deficient mice. *Science* 1993;261:609-12.
45. Harding FA, Allison JP. CD28-B7 interactions allow the induction of CD8+ cytotoxic T lymphocytes in the absence of exogenous help. *J Exp Med* 1993;177:1791-6.
46. Nandi D, Gross JA, Allison JP. CD28-mediated costimulation is necessary for optimal proliferation of murine NK cells. *J Immunol* 1994;152:3361-9.
47. Sperling AI, Linsley PS, Barrett TA, Bluestone JA. CD28-mediated costimulation is necessary for the activation of T cell receptor-gamma delta+ T lymphocytes. *J Immunol* 1993;151:6043-50.
48. Moretta A, Bottino C, Vitale M, et al. Receptors for HLA class-I molecules in human natural killer cells. *Annu Rev Immunol* 1996;14:619-48.
49. Haas W, Pereira P, Tonegawa S. Gamma/delta cells. *Annu Rev Immunol* 1993;11:637-85.
50. Lenschow DJ, Ho SC, Sattar H, et al. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 1995;181:1145-55.
51. Kaye PM, Rogers NJ, Curry AJ, Scott JC. Deficient expression of costimulatory molecules on *Leishmania*-infected macrophages. *Eur J Immunol* 1994;24:2850-4.
52. Saha B, Das G, Vohra H, Ganguly NK, Mishra G. Macrophage-T cell interaction in experimental mycobacterial infection: selective regulation of co-stimulatory molecules on *Mycobacterium*-infected macrophages and its implication in the suppression of cell-mediated immune response. *Eur J Immunol* 1994;24:2618-24.
53. Muraile E, De Smedt T, Thielemans K, Urbain J, Moser M, Leo O. Activation of murine T cells by bacterial superantigens requires B7-mediated costimulation. *Cell Immunol* 1995;162:315-20.
54. Haffar OK, Smithgall MD, Bradshaw J, Brady B, Damle NK, Linsley PS. Costimulation of T-cell activation and virus production by B7 antigen on activated CD4+ T cells from human immunodeficiency virus type 1-infected donors. *Proc Natl Acad Sci U S A* 1993;90:11094-8.



55. Lewis DE, Tang DS, Adu-Oppong A, Schober W, Rodgers JR. Anergy and apoptosis in CD8+ T cells from HIV-infected persons. *J Immunol* 1994;153:412-20.
56. Levine BL, Mosca JD, Riley JL, et al. Antiviral effect and ex vivo CD4+ T cell proliferation in HIV-positive patients as a result of CD28 costimulation. *Science* 1996;272:1939-43.
57. Corry DB, Reiner SL, Linsley PS, Locksley RM. Differential effects of blockade of CD28-B7 on the development of Th1 or Th2 effector cells in experimental leishmaniasis. *J Immunol* 1994;153:4142-8.
58. Rathore A, Sacristán C, Ricklan DE, Flores Villanueva PO, Stadecker MJ. *In situ* analysis of B7-2 costimulatory, major histocompatibility complex class II, and adhesion molecule expression in schistosomal egg granulomas. *Am J Pathol* 1996;149:187-94.
59. Flores Villanueva PO, Harris TS, Ricklan DE, Stadecker MJ. Macrophages from schistosomal egg granulomas induce unresponsiveness in specific cloned Th-1 lymphocytes in vitro and down-regulate granulomatous disease in vivo. *J Immunol* 1994;152:1847-55.
60. Flores Villanueva PO, Reiser H, Stadecker MJ. Regulation of T helper cell responses in experimental schistosomiasis by IL-10: effect on expression of B7 and B7-2 costimulatory molecules by macrophages. *J Immunol* 1994;153:5190-9.
61. King CL, Medhat A, Malhotra I, et al. Cytokine control of parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. *J Immunol* 1996;156:4715-21.
62. Flores-Villanueva PO, Zheng XX, Strom TB, Stadecker MJ. Recombinant IL-10 and IL-10/Fc treatment down-regulate egg antigen-specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis. *J Immunol* 1996;156:3315-20.
63. Nakajima A, Azuma M, Koderá S, et al. Preferential dependence of autoantibody production in murine lupus on CD86 costimulatory molecule. *Eur J Immunol* 1995;25:3060-9.
64. Wicker LS, Todd JA, Peterson LB. Genetic control of autoimmune diabetes in the NOD mouse. *Annu Rev Immunol* 1995;13:179-200.
65. Gocinski BL, Tigelaar RE. Roles of CD4+ and CD8+ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. *J Immunol* 1990;144:4121-8.
66. Knoerzer DB, Karr RW, Schwartz BD, Mengle-Gaw LJ. Collagen-induced arthritis in the BB rat: prevention of disease by treatment with CTLA-4-Ig. *J Clin Invest* 1995;96:987-93.
67. Powrie F. T cells in inflammatory bowel disease: protective and pathogenic roles. *Immunity* 1995;3:171-4.
68. Sieling RA, Modlin RL. Cytokine patterns at the site of mycobacterial infection. *Immunobiology* 1994;191:378-87.
69. Mahanty S, Nutman TB. Immunoregulation in human lymphatic filariasis: the role of interleukin 10. *Parasite Immunol* 1995;17:385-92.

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