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Role of B7 Signaling in the Differentiation of Naive CD4+ T Cells to Effector Interleukin-4-Producing T Helper Cells

Abstract
Signaling through the T cell receptor must be accompanied by costimulatory signals for the differentiation of naive T cells to cytokine-producing effector T helper cells. The costimulatory signal through CD28 is required for T cell activation resulting in increased interleukin (IL)-2 production in vitro, but its role in the production of IL-4 and in the in vivo response is still unclear. We have examined the effects of blocking CTLA-4 (the CD28 homologue) ligand interactions on the in vivo development of IL-4-producing T helper effector cells during a primary mucosal immune response to the nematode parasite *Heligmosomoides polygyrus* and during a primary systemic immune response to immunogenic anti-IgD antibodies. Our results demonstrate that CD28 and/or CTLA-4 signaling is required for T cell priming leading to IL-4 cytokine production, B cell activation, and IgE secretion during both immune responses, suggesting that other signaling molecules do not substitute for these molecules in either of these two different immune responses. Furthermore, the CD28 ligands, B7-1 and B7-2, can substitute for each other in providing the required T cell costimulatory ligand interactions during the primary immune response to *H. polygyrus*. In contrast, memory T cells during the challenge immune response do not require CD28/CTLA-4 ligand interactions for IL-4 production and T helper effector function.

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The costimulatory molecules, CD28 and CTLA4, may influence T cell differentiation quantitatively, determining the extent of T cell activation, and qualitatively influencing the cytokines that are produced by T helper (Th) cells once they become activated. Understanding the effects of costimulatory molecules on Th cell activation during the course of an in vivo immune response could aid the development of therapies that require the induction of T cell unresponsiveness, including autoimmune diseases and transplantation. If costimulatory molecules can promote immune deviation by influencing whether a type 1 [interferon (IFN)-γ-dominant] or a type 2 [interleukin (IL)-4-dominant] cytokine response develops, then targeting of these cell surface molecules may be useful for therapeutic manipulation of T cell cytokine production: an important capability for vaccine development. The T cell response may be dependent on whether CD28 or CTLA4 is predominantly expressed and which ligand, in particular CD80 or CD86, is predominantly bound. In this review, an interpretation of recent results involving the role of these molecules in Th cell differentiation towards effector function will be discussed.

The concept of T cell costimulation was developed from the two-signal model of T lymphocyte activation in which one signal is provided through the antigen-specific T cell receptor (TCR) and the second through a non-antigen-specific costimulatory signal [1–4]. The second signal is necessary to stimulate T cells to proliferate in vitro and its absence can induce a state of T cell unresponsiveness termed anergy [1, 5–7]. Several T cell surface molecules and cytokine receptors have been proposed to provide a second signal [8–12]. Perhaps the best defined is CD28 and its homologue, CTLA4, which interact with the B7 molecules including CD80 (B7-1) and CD86 (B7-2) on antigen presenting cells

(APCs). CD28 is constitutively expressed on T cells, while CTLA4 expression appears to be restricted to activated T cells [2–4]. CD86 and CD80 both bind CTLA4 with high avidity and CD28 with lower avidity but utilize different binding determinants and exhibit different binding kinetics to CD28 and CTLA4 [13].

The interaction of CD28/CTLA4 with CD80 and CD86 on APCs is necessary to induce T cells to produce IL-2 in vitro. Th1 clones and fresh T cells require signaling through CD28 in addition to signaling via the TCR for activation and IL-2 production [2, 14–16] and become anergic if they are stimulated through the TCR in the absence of costimulation via CD28 [17]. CD28 signaling stimulates T cell cytokine production both by regulating gene transcription and by stabilizing mRNAs, particularly IL-2 [2, 14, 16, 18]. In contrast, some Th2 clones that use IL-4 as their autocrine growth factor do not require CD28 costimulation and proliferate in response to TCR cross-linking in the presence of IL-1 [19–23]. Furthermore, although a chimeric fusion protein composed of CTLA4 and the Ig Fc region, called CTLA4Ig, inhibits alloantigen-specific responses and IL-2 and IFn-γ gene expression in mixed lymphocyte cultures, increased IL-4 gene expression and some proliferation persists [24]. These findings suggested that CTLA4-ligand interactions may be required for IL-2 production in vitro but that differentiation towards IL-4 production may be induced by other costimulatory signals. As will be discussed shortly, data obtained from our laboratory have shown that in vivo T cell differentiation to IL-4 production does require CTLA4 ligand interactions.

Differences in T cell responses to CD28 versus CTLA4 signaling have also been detected. Whereas CD28 costimulation by anti-CD28 antibody, CD80, or CD86 induces cy-

tokine secretion and up-regulates IL-2 receptor expression on resting T cells [3, 25, 26], CTLA4 ligation in conjunction with TCR signaling can trigger apoptosis in vitro [27]. The ratio of CD28 to CTLA4 molecules on the surface of a T cell may influence whether T cell activation leads to cytokine production and helper function or deletion via apoptosis. The increased expression of CTLA4 relative to CD28 at later stages of the immune response may thus contribute to the down-regulation of the T cell response [27–29]. Alternatively, other findings suggest that CTLA4 can provide similar costimulatory signals to CD28 [25]. Whether these differences are due to the cell surface concentrations of CTLA4 or the different cell lines or culture conditions that have been used has not yet been determined. The possibility that CTLA4 may actually bind additional CTLA4 ligands besides CD80 and CD86 has also been suggested [27, 30].

These in vitro studies have provided an important basis for designing experiments to examine the role of B7-CD28/CTLA4 interactions in vivo. It should be emphasized that the in vivo immune response cannot be replicated in vitro and to understand the function of these molecules it is necessary to perform in vivo studies. Initial in vivo experiments primarily involved administration of the CTLA4Ig fusion protein with the hypothesis that this molecule would bind CD80, CD86 and perhaps other CTLA4 ligands thereby blocking their interactions with CD28 and CTLA4. Initially, a chimeric protein composed of the extracellular domain of human CTLA4 (a gene highly homologous to CD28) fused to a human IgG1 heavy chain was used. CTLA4Ig has a >20-fold higher affinity for CD80 than does CD28, and acts as a competitive inhibitor of CD28 binding to CD28 and CD86. More recently, a murine CTLA4/mouse IgG Fc chimeric protein has been shown to have higher affinity for murine B7 molecules and to be more effective in influencing in vivo murine immune responses [31]. Although cell depletion has not been observed [31], Fc-mediated effects such as complement-mediated cell killing are possible with this construct. Also, although B7 signaling has not yet been demonstrated, it is possible that binding or murine CTLA4Ig to B7 could initiate agonistic effects. CTLA4Ig administration in mice has inhibited the primary in vivo antibody response to sheep red blood cells [32], graft rejection [33] and autoimmune disease [34]. Our recent studies directly demonstrated that the in vivo development of IL-4-producing CD4+T cells could be blocked by CTLA4Ig administration [35, 36]. The immunization systems that we use include the mucosal immune response to oral inoculation with the live nematode parasite, *Heligmosomoides polygyrus*, and the systemic immune response to intravenous injection of a heterologous antimouse IgD antibody. Both systems elicit strong responses that are characterized by marked elevations in IL-4 expression at both the mRNA and protein levels. The nematode parasite elicits a mucosal immune response, while the anti-IgD antibody induces a systemic response.

The Immune Response to *H. polygyrus* and Heterologous Antimouse IgD

The nematode parasite, *H. polygyrus*, has a strictly enteral life cycle in which infective third-stage larvae (L3) invade the intestinal mucosa less than 24 h after ingestion and develop there into mature adults [37]. We mimic this by inoculating L3 larvae into the stomach with a ball-tipped feeding tube. The ensuing mucosal immune response is associated with marked elevations in mesenteric

lymph node (MLN) CD4+ T cell IL-4 cytokine gene expression and protein secretion, T-cell-dependent and independent elevations in IL-3, IL-5 and IL-9 mRNA, blood eosinophilia, mucosal mastocytosis (MMC) and also a vigorous B cell response that results by day 8–12 in marked increases in serum IgE and IgG1 [37, 38; unpubl. results]. The only source of MLN IL-4 that we have identified by cell sorting studies at early and late time points after *H. polygyrus* inoculation is CD4+, TCRα/β+ cells [38; unpubl. results].

We have found a highly consistent sequential cytokine expression pattern during the course of the immune response to this nematode parasite. Non-T cells are initially the primary cytokine source and CD4+, TCRα/β+ cells become a major source later. During the first 24–48 h after *H. polygyrus* inoculation, the major cytokine mRNA elevations that we have detected include IL-5, IL-3 and IL-9. As much as a 1,000-fold increase in IL-9 gene expression has been detected in the gut mucosa and the Peyer's patch by 12 h after *H. polygyrus* inoculation [38]. These cytokines remain elevated at these time points in T-cell-deficient nude and B/T-cell-deficient severe combined immunodeficiency (SCID) mice, suggesting that non-B/non-T cells are the primary source [38]. At day 2 after *H. polygyrus* inoculation, CD4+, TCRα/β+ cells contribute increasingly to the elevations in these cytokines and also produce IL-4, which is detectable at the protein and mRNA level in the mesenteric lymph node by day 6 after inoculation. By day 8–10, IL-4 mRNA and protein levels have peaked and the IL-4 levels and other cytokine elevations decrease thereafter [38].

The other immunogen, heterologous antimouse IgD antibody, binds to and cross-links mouse B cell membrane IgD in the spleen within several hours after intravenous injection, activating the B cells [39]. It is internalized and processed, and presented by the large number of activated B cells to IgG-specific naive T cells that are specific for the heterologous IgG, with the result that a wave of T cell activation is observed [39–41]. Increased CD4+ T cell size and IL-2 receptor (IL-2R) expression are detected by 3 days [42] as are elevations in CD4+, TCRα/β+ T cell IL-2, IL-4 and IL-9 gene expression. By day 6, marked elevations in IL-4 and IL-10 mRNA are also detected along with a smaller increase in IFN-γ mRNA [43]. During this response, T cells mediate B cell Ig class switching: elevations in serum IgE, IgG1, IgG2a and IgG3 are detected by 8–10 days after immunization [39, 44]. Thus the immune response to heterologous antimouse IgD antibody elicits effector cells characteristic of both type 1 and type 2 patterns, a response sometimes referred to as a Th0 response, although type 2 cytokines predominate. This response is susceptible to intervention as we have shown that induction of endogenous IFN-α production [45] or exogenous IL-12 administration [46] can shift the in vivo immune response from a predominantly type 2 pattern to a predominantly type 1 pattern, while anti-IFN-γ monoclonal antibody administration favors a predominantly type 2 pattern [44].

The highly consistent, short time course of these two strong immune responses makes them particularly useful models to study T cell differentiation to cytokine production in vivo. Also, since they are very different immune responses, they are useful for identifying common pathways of T cell activation and differentiation to cytokine production and for discerning pathways that vary between different immune responses.

We have also analyzed other effector cells (including eosinophils, mast cells and B cells) that are characteristic of each response and in recent studies we also examined the influence

of blocking CTLA4 ligand interactions on their development. Analyzing these non-T cell populations not only provides an additional corroboration of our measurements of T cell cytokine production, since we have previously shown that certain T-cell-derived cytokines are required for the activation and/or proliferation of specific non-T cell populations [44, 47], but it also provides additional insights into the effects of blocking CTLA4 ligands on a T-cell-dependent response to a live pathogen, in the case of *H. polygyrus*, or a potent systemic immunogen, in the case of heterologous antimouse IgD antibody.

CTLA4Ig Blocks an in vivo Mucosal T Cell Response

We used the stimulus, *H. polygyrus*, to examine the effect of CTLA4Ig administration on the development of the in vivo mucosal immune response. The CTLA4Ig fusion protein (100 μg) was administered on day 0 and 1 after oral inoculation of live L3 *H. polygyrus* larvae, and mice were subsequently analyzed for T and B cell activation at day 8 and 14 after immunization. Summarizing our recently published results [38], our data suggest that CTLA4-ligand interactions are required for (1) increased IL-3, IL-4 and IL-9 gene expression in the Peyer's patch and mesenteric lymph node, as measured by RT-PCR at day 8 after inoculation, (2) increased IL-4 cytokine protein secretion by CD4+, TCRα/β+ cells, as determined by ELISPOT of FACS-sorted cells, (3) increased B cell MHC class II expression and size and (4) increased serum IgE levels. We only observed a partial decrease in blood eosinophilia and correspondingly little change in IL-5 gene expression and secretion. Non-T cells are a major source of IL-5 during this response, suggesting that other populations that are not dependent on T

cell help can remain active during this response. Since IL-5 can directly induce blood eosinophilia [47], it is not surprising that this effector cell activity persists in the absence of CD28 signaling. The complete absence of detectable B cell activation, as measured by increased B cell size and MHC II expression as well as increased serum Ig levels, suggests that B cells cannot be initially activated by the antigens and other molecular structures characteristic of this live pathogen without the help of T cells activated by CD28/CTLA4-B7 interactions.

Although CTLA4Ig administration had been previously shown to block antibody production in vivo, our results were the first demonstrating that CTLA4Ig administration could block T cell cytokine production in vivo. Thus, in this immunization system, other putative costimulatory signals, such as IL-1 [48–50], the heat-stable antigen [51], or LFA-1 and CD2 [52–55] do not substitute for CTLA4-ligand interactions. In contrast to our observations, mice transgenic for soluble murine CTLA4-Hγ1 exhibit normal T cell priming and cytokine production after immunization with a T-cell-dependent antigen [56]. One possible reason for this difference from our results is that these transgenic mice lack CD28/CTLA4 costimulatory signals from birth and may have compensated during ontogeny by using other signals. Alternatively, mCTLA4-Hγ1 may not be produced at sufficient levels to block CTLA4-ligand interactions required for the induction of T cell responses. Our results clearly show that blocking CTLA4 ligands by administration of CTLA4Ig in normal mature animals completely inhibits elevations in T-cell-derived cytokine gene expression and IL-4 protein secretion. Our observation was also seemingly in conflict with in vitro studies suggesting that T cells differentiating to IL-4 production did not require CD28 interactions. One possibili-

ty was that the inhibition of IL-4 was an indirect effect resulting from the inhibition of T cell IL-2 production by CTLA4Ig administration; numerous studies have shown a requirement of CD28/CTLA4-B7 interactions for T cell IL-2 production [14, 17, 57–59]. If IL-2 is required for IL-4 production, a possibility for which there is considerable evidence primarily based on in vitro studies [60], then blocking B7 interactions may inhibit IL-2, preventing T cell differentiation to IL-4 production. However, despite the elevations in IL-4, no elevations in IL-2 gene expression are detected during the immune response to *H. polygyrus* [38] and, furthermore, treatment of mice with anti-IL-2/anti-IL-2R antibodies, at concentrations that block IL-2 activity in other in vivo systems [61, 62], does not affect T-cell-dependent elevations in serum IgE production [Finkelman, unpubl. data]. In addition, we have recently observed marked elevations in IL-4 production in *H. polygyrus*-inoculated IL-2 gene knockout mice, further suggesting that IL-2 is not required for T cell IL-4 production in this response [Gause, in preparation]. Our findings thus suggest that B7-ligand interactions are important for the generation of an IL-4 response even in an IL-2-independent system.

We believe a more likely explanation for the discrepancy observed between in vivo and in vitro T cell costimulatory requirements for IL-4 production is the state of activation of the responding T cells. Th1 or Th2 cell clones are continually restimulated and are probably more similar to in vivo activated T effector or memory cells than naive T cells. In our system, since CTLA4Ig administration completely inhibits T cell cytokine production, the cell populations involved in the immune response to *H. polygyrus* are probably naive Th cells, a population which has been shown to be more dependent on costimulatory signals than effector or memory Th cells [63]. We

have tested this hypothesis by administering CTLA4Ig during the initiation of a memory response. Fourteen days after *H. polygyrus* inoculation, BALB/c mice (5/treatment group) were treated with an antihelminthic drug (pyrantel pamoate), which causes rapid worm expulsion, and 60 days later challenged with *H. polygyrus* plus either CTLA4Ig or the control fusion protein, L6. In these experiments, CTLA4Ig administration did not down-regulate elevations in either T cell IL-4 production or serum IgE levels [Gause et al., in preparation]. We have repeated this experiment and found the results to be highly consistent. Furthermore, we have found that administration of CTLA4Ig at day 3 and 4 after primary *H. polygyrus* inoculation does not inhibit elevations in T cell IL-4 production at day 8 after immunization [35]. Taken together, these data suggest that naive T cells requiring CD28/CTLA4-B7 interactions initially respond to *H. polygyrus* during the development of the mucosal type 2 response. Memory Th cells that are activated during the challenge response and activated Th cells that develop shortly after primary *H. polygyrus* inoculation no longer require these costimulatory signals.

The observation that Th1 clones are more B7 dependent for their activation than Th2 clones suggests that in vivo differentiated effector Th cells may differ in their requirements for B7-mediated costimulation, depending on whether they produce IL-4 or IFN-γ. Although T cells producing Th1 cytokines have not yet been directly examined for their B7 dependence during an in vivo immune response to an antigen, the type 1 response to *Leishmania* in C57BL/6 mice is refractory to CTLA4Ig administration, although the type 2 response in BALB/c mice is B7 CTLA4-ligand dependent [64]. Furthermore, in studies of donor-specific tolerance of MHC-incompatible rat renal allografts in

inbred strains, induction of immunosuppression was optimized when CTLA4Ig administration was delayed [65]. With this regimen, IL-4 but not IFN-γ remained elevated in the renal allografts, a reversal of the cytokine pattern observed in control antibody-treated mice. These findings that blocking B7 interactions after initial T cell activation favors the development of a type 2 T cell cytokine pattern are consistent with the model that after initial activation T cell differentiation towards IL-4 production is less B7 dependent than differentiation towards IFN-γ production. It will be important in future studies to directly examine the role of B7 interactions in T cell differentiation during a type 1 response.

Although our findings suggest that an ongoing or memory type 2 T cell response does not require CTLA4 ligand interactions, the results may still be important from a therapeutic viewpoint since they suggest that the T cell development pathway leading to IL-4 production in the mucosal region can be inhibited by blocking B7 ligand interactions. It is also possible that chronic CTLA4Ig treatment after immunization may eventually inhibit the Th2 response by blocking renewal of primed cells. One potential therapeutic approach relies on the premise that blocking CTLA4-ligand interactions during an immune response to an allergen or other antigen might induce tolerance, resulting in unresponsiveness to subsequent challenges. Our findings that the primary T cell response to *H. polygyrus* is dependent on B7 costimulation suggests an experimental in vivo model for future studies directed towards determining whether T cell anergy will occur in the absence of CTLA4-ligand costimulatory signals during a Th2-like response.

CTLA4Ig Blocks an in vivo Systemic T Cell Response

Although T cell differentiation towards IL-4 production during a mucosal immune response is inhibitable by CTLA4Ig administration, a systemic T cell response may have different costimulatory requirements including signaling. Hence, T cell differentiation towards IL-4 production, or cytokine production in general, may not require B7 costimulatory signals during the systemic immune response. We have addressed this possibility by examining the effect of blocking B7 ligands on the immune response to heterologous antimouse IgD antibodies. We determined that B7 ligand interactions are required for (1) increased IL-2, IL-4 and IL-9 but not IL-10 gene expression, (2) elevations in the number of IL-4- but not IL-10-secreting cells, as measured by ELISPOT, in both unsorted splenic cells and sorted CD4+, TCRα/β+ T cells, (3) in situ IL-4 protein expression in spleen sections as measured by immunohistological staining and (4) elevated serum IgG1, IgE, IgG3 and IgG2a [36]. These effects were dependent on early administration at day 3–4 after immunization, no effects on elevations in cytokine production or serum Ig levels were detected. These results established that B7 plays a key role in costimulating naive CD4+ T cells to differentiate to IL-4-producing cells during a systemic as well as a mucosal immune response.

We used three different assay systems to measure IL-4 expression, all of which provided corroborating data. Each assay has distinct advantages and disadvantages. The RT-PCR technique measures changes in gene expression in situ with essentially no manipulation of tissue required; however, it does not provide direct information on protein production. The ELISPOT assay provides protein secretion data and in many ways is a

marked improvement over other in vitro assays which require restimulation or extended cultures, but still requires some in vitro culture (3 h) which might induce production of cytokines not being made in vivo. Finally, immunohistochemistry can measure cytokine protein in situ, but because cytokine proteins are rapidly made, secreted, and utilized, sufficient quantities may not be present at a given time point to be detected.

The nature of the immune response to immunogenic antimouse IgD may favor a critical role for CD28/CTLA4 costimulation, since B cells are the principal APC in this system [66]. If B cells rely on B7 as the principal costimulatory ligand for naive T cell activation, then immunization favoring B cell antigen presentation may be particularly sensitive to CTLA4Ig. In vivo, other APCs, such as dendritic cells or activated macrophages, may be more permissive to an absence of CD28/CTLA4 signaling because they express a greater repertoire of cell surface or secreted costimulatory molecules. However, this possibility seems unlikely as CTLA4Ig also blocked the mucosal T cell response to *H. polygyrus* where non-B cells are probably the major APCs. Furthermore, Wallace et al. [31] has recently blocked elevations in T cell IL-4 production in the spleen during the systemic immune response to sheep red blood cells.

The possibility existed in the anti-IgD antibody system that blocking CD28/CTLA4-B7 interactions inhibited IL-2 production that was required for later IL-4 production as has been demonstrated in vitro [67, 68]. However, as in the mucosal immune response to *H. polygyrus*, we have not been able to block elevations in IL-4 gene expression by treating immunogenic anti-IgD-immunized mice with anti-IL-2 and anti-IL-2Rα monoclonal antibodies [Gause, unpubl. data]. Taken together, these results suggest that during a systemic immune response, a strong immunogen favor-

ing a Th2-like response can rapidly induce previously resting T cells by a CD28-dependent pathway to produce IL-4 in the absence of initial increases in IL-2 or the appearance of a Th0-like pattern.

The observation that CTLA4Ig did not block the T cell response when given 2–3 days after immunization suggests that unlike naive T cells, effector T cells do not require CD28/CTLA4 signaling and that T cell-B cell interactions occurring at later stages of the immune response may not require such signaling. The effectiveness of CTLA4Ig treatments only at the onset of the immune response also suggests that in this system resting T cells and not already activated cells are initially stimulated by antigen-presenting B cells, consistent with increasing evidence that activated B cells can present antigen to and activate resting T cells [66, 69].

A notable exception to the inhibition of T cell cytokines by CTLA4Ig was the sustained elevation of IL-10. Our results suggest that IL-10 gene expression and protein secretion is regulated differently than other Th2 cytokines in vivo. In other immunization systems, we have also found that IL-10 gene expression is not coordinately elevated with other Th2 cytokines [38, 43, 70]. Differential regulation of IL-4 and IL-10 has also been shown in activated T cell clones where elevations in IL-4 gene expression are cyclosporin sensitive, while IL-10 elevations are cyclosporin resistant [71]. The sustained elevation of IL-10 in the absence of the CD28 costimulatory signal may suggest that either other costimulatory molecules can substitute for CD28 in vivo or that signaling through the TCR is sufficient for up-regulating IL-10 gene expression and protein production. IL-10 has been shown to affect a variety of cell types including B cells, macrophages and T cells [72–74]. Moreover, IL-10 can selectively inhibit the up-regulation of B7 on macrophages thereby inhibiting

macrophage costimulatory activity [75]. Recent studies from our laboratories suggest that administration of CTLA4Ig during primary anti-IgD immunization tolerizes T cells such that they become unresponsive in challenge immunizations [Gause and Finkelman, unpubl. data]. Whether elevated IL-10 may contribute to the induction of T cell tolerance observed in vivo is currently being examined.

Signaling through CD80 versus CD86

Although in vivo experiments involving CTLA4Ig administration have documented the importance of B7 ligands in the development of effector T cells, the role of the individual B7 molecules, CD80 and CD86, is uncertain. It has also been proposed that CD86 and CD80 may be more important at early and later stages of the immune response, respectively, and that this difference may explain the differential effects of anti-CD80 and anti-CD86 antibodies on the development of diabetes in the nonobese diabetic mouse [27, 28, 76–78]. Consistent with this hypothesis, lipopolysaccharide-stimulated human B cells express CD86 within 24 h of activation, whereas CD80 cells surface expression peaks several days later [79]. Other studies suggest that monocytes constitutively express CD86 whereas CD80 is induced after culture with IFN-γ and that CD86 is expressed at low levels on unstimulated dendritic cells and expression of both CD80 and CD86 is up-regulated by granulocyte/macrophage-colony-stimulating factor [80]. However, these in vitro analyses, particularly of dendritic cells, may not reflect in vivo conditions and recent findings suggest that a lymph node dendritic cell population, not easily released into suspension, constitutively expresses high levels of CD86 [81]. If CD80 is expressed at later stages than CD86 on the relevant APC popu-

lations, then since CTLA4 is not expressed until after the initiation of the response, it may interact more with CD80 functioning to down-regulate immune responsiveness [28, 76, 77].

Although some studies suggest that CD80 and CD86 can equivalently mediate costimulation in vitro [26], recent in vivo studies show that quite different results can be obtained following administration of anti-CD80 monoclonal antibody, anti-CD86 monoclonal antibody or the combination of both during an in vivo immune response, with one report suggesting that CD80 costimulation favors a type 1 and CD86 favors a type 2 response [82]. In this report, T cell clones from proteolipid-protein-immunized mice, in which CD80 interactions were blocked, produced primarily IL-4, whereas anti-CD86 treatment favored experimental allergic encephalomyelitis severity and a type 1 response [82]. Understanding the developmental processes that govern the cytokines that are secreted by effector Th cells is required to promote optimal host-protective immune responses to pathogens and is becoming increasingly utilized in vaccine development [83]. This finding is thus of considerable interest from an applied as well as a basic research viewpoint. However, whether the observation that CD80 favors Th1 and CD86 Th2 cytokine production is generalizable to other immunization systems is unclear and since T cell clones were used, the contribution of artifacts resulting from their long-term in vitro culture is also uncertain. Recent in vitro studies of anti-CD3-stimulated T cells provided with either CD80 or CD86 costimulation showed that although CD86 promoted the production of the Th2 cytokine, IL-4, it also favored the Th1 cytokine tumor necrosis factor-β, while CD80 favored IFN-γ and IL-2 production but also promoted some IL-4 production [84]. In contrast to these two studies, we have recently

Fig. 1. B7 costimulation (either CD80 or CD86) is required for initial T cell triggering for the IL-2-independent development of IL-4-producing CD4+, TCRα/β+ Th cells during the primary mucosal immune response to *H. polygyrus*.
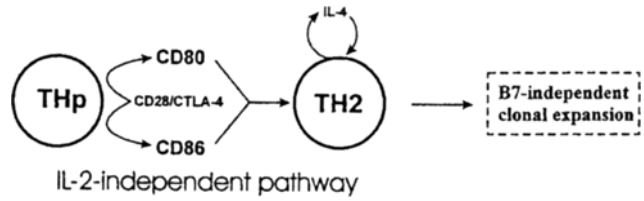
found that treatment of mice with either anti-CD80 or anti-CD86 antibodies did not block elevations in IL-4 production or serum IgG1 levels during the primary immune response to *H. polygyrus*, but treatment with the combination of both antibodies blocked elevations in both IL-4 and serum IgG1 levels [Gause, in preparation]. These results suggest that in this mucosal immune response to a live pathogen, the CD80 and CD86 molecules can substitute for each other. It also shows that either CD80 or CD86 is required for the development of the T cell response and that, if there are additional B7 molecules, they cannot substitute for the combined absence of these two signals.

Summary

In summary, our studies of the in vivo type 2 immune response suggest a model for the development of IL-4-producing T cells during the mucosal immune response to a nematode parasite (fig. 1). Although we have performed some parallel studies of the anti-IgD response, our findings are not yet complete enough to fully extend this model to a systemic immune response and it is possible that T cells differentiating in response to other immunogens or following immunization by other routes may utilize B7 signaling differently. Following *H. polygyrus* inoculation, IL-4-producing effector CD4+ Th cells rapidly develop from naive CD4+ T cells via an IL-2-independent pathway. T cell differentiation during this response initially requires B7 interactions and either CD80 or CD86 can provide this signaling. Shortly after T cell activation, the maturing T cells lose their requirement for B7 signaling and can progress to IL-4 production in its absence. Memory Th cells similarly do not require B7 costimulatory signals for their activation to IL-4 production during the in vivo challenge response.

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