CD154 Plays a Central Role in Regulating Dendritic Cell Activation During Infections That Induce Th1 or Th2 Responses

Amy D. Straw, Andrew S. MacDonald, Eric Y. Denkers and Edward J. Pearce

J Immunol 2003; 170:727-734; doi: 10.4049/jimmunol.170.2.727
http://www.jimmunol.org/content/170/2/727

References
This article cites 71 articles, 39 of which you can access for free at:
http://www.jimmunol.org/content/170/2/727.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
CD154 Plays a Central Role in Regulating Dendritic Cell Activation During Infections That Induce Th1 or Th2 Responses

Amy D. Straw,*† Andrew S. MacDonald,2* Eric Y. Denkers,† and Edward J. Pearce3*†

We compared splenic DC activation during infection with either the Th2 response-inducing parasite Schistosoma mansoni or with the Th1 response-inducing parasite Toxoplasma gondii. CD8α+ DC from schistosome-infected mice exhibited a 2- to 3-fold increase in the expression of MHC class II, CD80, and CD40 (but not CD86) compared with DC from uninfected control animals, while CD8α− DC exhibited a 2- to 3-fold increase in the expression of MHC class II and CD80 and no alteration, compared with DC from uninfected mice, in the expression of CD86 or CD40. Intracellular staining revealed that DC did not produce IL-12 during infection with S. mansoni. In contrast, infection with T. gondii resulted in a more pronounced increase in the expression of activation-associated molecules (MHC class II, CD80, CD86, and CD40) on both CD8α+ and CD8α− splenic DC and promoted elevated IL-12 production by DC. Analysis of MHC class I and of additional costimulatory molecules (ICOSL, ICAM-1, OX40L, 4-IBBL, and B7-DC) revealed a generally similar pattern, with greater indication of activation in T. gondii-infected mice compared with S. mansoni-infected animals. Strikingly, the activation of DC observed during infection with either parasite was not apparent in DC from infected CD154−/− mice, indicating that CD40/CD154 interactions are essential for maintaining DC activation during infection regardless of whether the outcome is a Th1 or a Th2 response. However, the ability of this activation pathway to induce IL-12 production by DC is restrained in S. mansoni-infected, but not T. gondii-infected, mice by Ag-responsive CD11c− cells. The Journal of Immunology, 2003, 170: 727–734.

Schistosomes are parasitic helminths that induce strong Th2 responses (1). Adult female Schistosoma mansoni worms begin to produce eggs 4–6 wk following initial cercarial invasion of the mammalian host; Ag secreted by the egg stage of the parasite are responsible for induction of the Th2 response that peaks at 6–8 wk postinfection (2–4). This polarized T cell response is dependent on the presence of IL-4 for development, is responsible for some of the more serious pathological consequences of infection, such as hepatic fibrosis, and yet is critical for host survival during infection (5–10). Toxoplasma gondii is an intracellular protozoan that induces a strong Th1 response during acute infection; the development of this response and the production and function of IFN-γ are required for host survival during both the acute and chronic stages of Toxoplasma infection (11).

Dendritic cells (DC) are APCs that efficiently induce T cell activation in the secondary lymphoid organs (12–14). Recent evidence indicates that DC also play an important role in determining the type of immune response that is generated against an Ag or pathogen; several factors that can influence the development of polarized responses include DC lineage, activation status, and the DC:T cell ratio (15–19). Two major DC subsets that differ in both CD8α expression and localization to different regions of the lymphoid organs have been reported to have different functions in the induction of T cell responses (20, 21). Although it has been proposed that CD8α− DC may be specialized for promoting Th1 responses and CD8α+ DC for Th2 responses (22, 23), subsequent studies have shown that distinct DC subsets are able to promote either type of response depending upon pathogen-derived signals and host-derived cytokines present in the microenvironment (24–27).

Previous work in our laboratory has shown that CD8α− bone marrow-derived DC exposed to schistosome egg Ag (SEA) in vitro do not up-regulate the expression of costimulatory molecules, IL-4, IL-10, or IL-13, and yet prime a strong Th2 response when injected into naive recipient animals (26). The development of this response in vivo depends on CD40 expression by transferred DC, in addition to the ability of recipient animals to make IL-4 (28, 29). Although it is clear from these previous studies that exposure to egg Ag does not strongly activate DC in a classical way, we do not yet understand how different DC subsets in vivo respond during infection with the parasite, or how other immune cells and cytokines might influence the behavior of these DC in infected animals.

Our initial studies indicated that CD8α− DC in schistosome-infected mice exhibit some up-regulation of MHC class II, but not of CD86 or CD40, and thus supported our findings from studies that used bone marrow-derived DC (26). However, we now know that CD40 expression by DC is essential for Th2 response induction driven by SEA (28), a finding that suggests that DC primed with SEA may become activated during their interaction with Th cells due to stimulation via CD40. Thus, the possibility existed that at some point during the course of the development of a Th2 response in vivo, DC do proceed through a phase of activation. To begin to examine these issues, we assessed the phenotype of splenic DC over the course of the S. mansoni infection, and compared

---

*Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19104; and †Department of Microbiology and Immunology, Cornell University College of Veterinary Medicine, Ithaca, NY 14853

Received for publication April 30, 2002. Accepted for publication November 8, 2002.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by National Institutes of Health Grants AI32573 and AI53825 (to E.J.P.) and AI47888 (to E.Y.D.). A.S.M. was supported by a Wellcome Trust International Prize Traveling Fellowship. E.J.P. is a Burroughs Wellcome Fund Scholar in Molecular Parasitology. Schistosome life cycle stages for this work were supplied through National Institute of Allergy and Infectious Disease, National Institutes of Health, Contract NOIAI455270.

2 Current address: University of Edinburgh, Institute of Cell, Animal, and Population Biology, Ashworth Laboratories, King’s Buildings, West Mains Road, Edinburgh, U.K. EH9 3JT.

3 Address correspondence and reprint requests to Dr. Edward J. Pearce, Department of Pathobiology, 203D Johnson Pavilion, 3610 Hamilton Walk, University of Pennsylvania, Philadelphia, PA 19104-6076. E-mail address: epierce@mail.med.upenn.edu

4 Abbreviations used in this paper; DC, dendritic cell; SEA, schistosome egg Ag; STAg, soluble tachyzoite Ag.
DC ACTIVATION DURING Th1 AND Th2 RESPONSES

To analyze DC percentages in the spleens of normal infected mice, splenocyte suspensions were first incubated with FC Block (BD PharMingen) for 15 min on ice. DC were then stained with FITC-conjugated anti-CD11c, PE-conjugated anti-IA, and CyChrome-conjugated anti-CD8 and at predetermined concentrations in fluid wash (Dulbecco’s PBS supplemented with 1% heat-inactivated FCS and 0.05% sodium azide) for 30 min on ice.

Surface marker expression was analyzed on purified DC with a combination of Abs to CD11c, MHC class II, CD80, CD86, CD40, ICOSL, B7-DC, OX40L, ICAM-1, and CD86. For detection of intracellular IL-12, DC were fixed in DPBS containing 3% paraformaldehyde (Sigma-Aldrich), 0.1 mM CaCl2, and 0.1 mM MgCl2 for 30 min on ice. DC were then washed twice in DPBS with 0.075% saponin (Sigma-Aldrich) and incubated with 5% NMS (Cedarlane Laboratories, Hornby, Canada) for 15 min. PE-conjugated anti-IL-12p40 mAb or rat control Ig was added at a predetermined saturating concentration for 30 min. CD11cDC were gated, and the expression of surface and intracellular proteins was analyzed on a FACSCalibur flow cytometer using CellQuest (BD Biosciences, Franklin Lakes, NJ) and FlowJo (Tree Star, San Carlos, CA) software. The geometric mean fluorescence intensity was used as a measure of expression for each marker on gated DC populations.

Results

The host immune response to schistosome infection is characterized by a strong Th2 response that is induced by parasite eggs, the production of which begins at ~5 wk of infection, resulting in peak Th2 responsiveness between wk 7 and 8 (2, 31). Because our primary interest was to understand the activation status of splenic DC during the inductive stage of the Th2 response, we analyzed the phenotype of DC before egg deposition and at key times during the development of the Th2 response (Fig. 1A). Splenic DC (CD11c+, MHC class II+ cells) were enriched to ~85% by MACS and analyzed by flow cytometry for surface proteins modulated during classical DC activation (MHC class II, CD80, CD86, and CD40). Comparison of surface marker fluorescence intensity between DC from normal and infected mice showed that overall, DC phenotype in the spleen was not significantly affected by infection with the parasite until wk 7–8, when there was a 2- to 3-fold increase in overall staining intensity for MHC class II, CD80, and CD40 (Fig. 1A). There was no shift at any time in the expression of CD86, a costimulatory molecule previously implicated in Th2 response development (32, 33). Similar findings were obtained when DC from hepatic and mesenteric lymph nodes were analyzed (data not shown). In addition, splenic DC maintained this partial state of activation until wk 15 of infection, a time point at which immunological responsiveness was subdued (2, 34).

To compare and contrast the DC response during schistosome infection with that which occurs during evolution of a Th1 response, we infected mice with T. gondii and analyzed DC on days 3, 6, 11, and 34 after infection (Fig. 1B). Based on previous reports, these time points fall within the initial (day 3), peak (days 6–11), and subdied (day 34) phases of the systemic Th1 response that occurs in response to this parasite (35, 36). Analysis of DC showed peak activation on day 6 following infection, when all activation markers analyzed were highly elevated (Fig. 1B). At this time there was, overall, a 3-fold increase in MHC class II expression, a 6-fold increase in both CD80 and CD40 expression, and a
2-fold increase in CD86 expression. Similar levels of DC activation were evident in mesenteric LN (data not shown). DC activation waned by day 11 and was completely absent by 34 days of infection despite the fact that the animals remain infected at this time. Thus, DC in the spleen attain different degrees of activation during infection with Th2 and Th1 response-inducing pathogens; DC from schistosome-infected mice exhibited a relatively low level of activation during the peak of the Th2 response compared with the high level of activation found in DC at the peak of the Th1 response to T. gondii.

Infection is accompanied by increased numbers of splenic DC

DC motility and viability can be altered by some pathogens that enable immature DC in the periphery to rapidly migrate to the secondary lymphoid organs (37, 38). Schistosome Ag could potentially reach the spleen passively through the bloodstream or in association with migrating DC following encounter with egg Ag in the gut or liver (39). To determine whether there was a change in the total number of splenic DC during infection with S. mansoni, viable splenocytes were counted at the peak of DC activation, and the percentage of CD11c<sup>hi</sup>/MHC class II<sup>+</sup> DC was determined by flow cytometry. The calculated total number of splenic DC dramatically increased during S. mansoni infection (Fig. 2A). Furthermore, this increase was not merely a reflection of the splenomegaly that occurs in response to infection with this parasite, as the total percentage of DC in the spleen increased from 4 to 8% as a result of infection (Fig. 2A). In addition, analysis of CD8α<sup>+</sup> and CD8α<sup>−</sup> DC demonstrated that there was a significant increase in both subsets of DC during infection (Fig. 2C). Infection with T. gondii also resulted in an increase in the total number of DC and a similar 2-fold increase in the percentage of DC in the spleen at the peak of infection (Fig. 2B), but, interestingly, the increase in CD8α<sup>+</sup> DC was less pronounced during infection with this parasite at this time point (Fig. 2D). These results suggest that there is increased recruitment or retention of viable DC in the spleen during infection with both pathogens.

Analysis of CD8α<sup>+</sup> and CD8α<sup>−</sup> DC phenotype during infection

We and others have shown that CD8α<sup>−</sup> bone marrow-derived DC can promote both Th1 and Th2 responses following exposure to Ag derived from pathogens that inherently induce Th1 or Th2 responses (26, 40–43). However, others have proposed specialized roles for CD8α<sup>+</sup> DC in Th1 response induction and for CD8α<sup>−</sup> DC in Th2 response induction (22, 23, 44). To determine whether one or both of the DC subsets were preferentially activated in response to schistosome infection, we purified DC from the spleens of mice that had been infected with S. mansoni for 8 wk and analyzed activation of each subset by comparing staining intensity for MHC class II, CD80, CD86, and CD40 on CD8α<sup>−</sup> and CD8α<sup>+</sup> gated cells (Fig. 3A). In keeping with our observations for

CD8α− bone marrow-derived DC exposed to SEA (26), CD8α− splenic DC from schistosome-infected mice displayed a very low activation status, exhibiting only a modest increase in the expression of MHC class II on all DC and enhanced expression of CD80 on a small proportion of cells. In contrast, the CD8α+ population exhibited a more distinct pattern of activation, displaying a more pronounced elevation of MHC class II, CD80, and CD40 expression in response to *S. mansoni*. Additional analysis of other DC surface molecules that play a role in T cell response induction and are regulated by DC activation (45–49) revealed a similar picture. Specifically, surface expression of ICOSL, ICAM-1, 4-1BBL, OX40L, and B7-DC as well as MHC class II, CD80, and CD40 were increased compared with normal (Table I). These findings contrast with the effect on DC of *T. gondii* infection on day 6, which induced marked increases in the expression of all analyzed surface proteins (except ICAM-1, the expression of which did not change during infection) within each DC subset (Fig. 3B and data not shown). In addition there was a higher proportion of activated DC within each subset (Fig. 3B and data not shown) during *T. gondii* infection. These results indicate that both DC subsets remain in a relatively low level of activation during *S. mansoni* infection compared with the high activation state that splenic DC achieve during the response to *T. gondii* infection. However, in contrast to bone marrow-derived DC exposed to SEA in vitro, splenic DC change phenotypically during schistosome infection and splenic CD8α+ DC generally exhibit greater activation-associated changes than do CD8α− DC.

### Table I. Expression of MHC class I and other DC surface molecules

<table>
<thead>
<tr>
<th></th>
<th>ICOSL</th>
<th>ICAM1</th>
<th>4-1BBL</th>
<th>OX40L</th>
<th>B7-DC</th>
<th>MHC Class I</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8α− normal</td>
<td>8.4 ± 2.3</td>
<td>181 ± 69</td>
<td>3.5 ± 1.9</td>
<td>3.2 ± 1.8</td>
<td>3.1 ± 1.7</td>
<td>96.9 ± 29.2</td>
</tr>
<tr>
<td>CD8α− infected</td>
<td>8.8 ± 3.1</td>
<td>186 ± 71</td>
<td>7.4 ± 2.4</td>
<td>6.2 ± 2.1</td>
<td>9.2 ± 3.4</td>
<td>184 ± 38</td>
</tr>
<tr>
<td>CD8α+ normal</td>
<td>10.4 ± 3.6</td>
<td>332 ± 106</td>
<td>3.6 ± 1.2</td>
<td>4.1 ± 1.8</td>
<td>7.2 ± 2.8</td>
<td>120 ± 27</td>
</tr>
<tr>
<td>CD8α+ infected</td>
<td>12.0 ± 4.2</td>
<td>299 ± 95</td>
<td>4.4 ± 2.0</td>
<td>5.6 ± 2.0</td>
<td>12.9 ± 3.5</td>
<td>141 ± 29</td>
</tr>
</tbody>
</table>

* DC from normal or schistosome-infected mice were enriched using MACs, stained with Ab against CD11c, CD8α, and other costimulatory molecules as described in Materials and Methods, and analyzed by flow cytometry. The table indicates the average geometric mean fluorescence intensity ± SE for five individual mice.

### DC IL-12 production during infection

IL-12 is a major growth factor and stimulator of IFN-γ production that promotes Th1 response development and can be produced by DC in response to both pathogen stimuli and CD40 ligation (50–52). Although SEA does not directly induce the production of IL-12 by DC in vitro (29), other inflammatory signals or ligation of CD40 (expressed at low levels on DC from infected animals) could presumably activate DC to produce this cytokine during infection. To examine this possibility, DC were purified and stained for intracellular IL-12. DC from mice carrying 8-wk schistosomiasis did not exhibit elevated levels of IL-12 staining compared with DC from uninfected control animals (Fig. 4). In contrast, a proportion of DC from mice infected with *T. gondii* for 6 days produced higher levels of IL-12 (Fig. 4).

### CD154 is required for DC activation during infection

SEA does not provide strong signals for classical activation of bone marrow-derived DC in vitro (26). In contrast, CD8α− and especially CD8α+ DC from spleens of schistosome-infected mice did exhibit up-regulation of cell surface activation markers, albeit to a limited degree. These differences suggested that during schistosomiasis, DC activation status is under the control of factors additional to those signals delivered directly by the pathogen. TNF family members, such as CD154, and TNF-α itself provide strong immune system-derived activation signals for DC (53–56). Since DC CD40 expression is crucial for Th2 responses initiated by SEA-pulsed, bone marrow-derived DC (28), we assessed whether DC activation in schistosome-infected mice is dependent upon CD40/CD154 interactions (Fig. 5A). We found that, unlike the situation in infected wild-type mice, there was no apparent increase in the expression of MHC class II, CD80, CD86, or CD40 on DC from infected CD154−/− animals (Fig. 5A). Having found...
that CD154 is essential for the observed low level activation of DC during schistosome infection, we next assessed whether a similar level of control was exerted during *T. gondii* infection. To our surprise, given the frequently reported ability of this parasite to directly activate DC (40, 57), we found that splenic DC activation in the CD154−/− mice, in that Ag-activated CD11c− cells promoted, rather than inhibited, IL-12 production by Ag-responsive CD11c− cells in our study is uniquely a feature of schistosome infection. In these experiments, consistent with previous reports (40), STAg alone was able to induce IL-12 production by spleen cells from normal mice (Fig. 6B). Specific analysis for IL-12p70 rather than p40 revealed this bioactive form of the cytokine only in the culture supernatants of DC from infected with *Toxoplasma* and CD8α− cells in our study that CD154 is essential for the observed low level activation of DC during schistosome infection, we next assessed whether a similar level of control was exerted during *T. gondii* infection. To our surprise, given the frequently reported ability of this parasite to directly activate DC (40, 57), we found that splenic DC activation in the CD154−/− mice, in that Ag-activated CD11c− cells promoted, rather than inhibited, IL-12 production by Ag-responsive CD11c− cells in our study is uniquely a feature of schistosome infection. In these experiments, consistent with previous reports (40), STAg alone was able to induce IL-12 production by spleen cells from normal mice (Fig. 6B). Specific analysis for IL-12p70 rather than p40 revealed this bioactive form of the cytokine only in the culture supernatants of DC from infected with *Toxoplasma* and CD8α− cells in our study.
OX40L, and B7-DC. In contrast, of the markers examined, only MHC class II, CD80, CD40, and B7-DC were clearly increased in CD8α+ DC from infected mice. Nevertheless, overall levels of DC activation in the schistosome-infected mice, as measured by the expression levels of this series of surface molecules, were notably low compared with those in DC recovered from the spleens of mice mounting a Th1 response during infection with T. gondii (Figs. 1 and 3). Moreover, DC from schistosome-infected mice were not found to be making IL-12, whereas a significant percentage of those from T. gondii-infected mice were (Fig. 4). During the acute phases of both infections, DC activation was found to be largely dependent upon CD154 (Fig. 5). Based on our findings we conclude that Th1 and Th2 response development proceeds in environments in which DC activation, as measured by increased MHC class II and costimulatory molecule expression, is promoted and maintained by CD40/CD154 interactions.

During infection, schistosome eggs are deposited into the portal bloodstream and either cross the endothelium and gut wall or become lodged within the liver sinusoids; granulomatous pathology ensues in both hepatic and intestinal sites (1, 5). Ag released from eggs are likely to be acquired by immature DC residing in the vasculature, lamina propria of the gut, and parenchyma of the liver. Additionally, egg Ag may pass directly to resident DC in the marginal zones of the spleen via the bloodstream. The observed increase in the splenic DC population following the onset of egg production by the parasite (Fig. 2) suggests either an accelerated migration of peripheral DC from the affected tissues into the spleen and/or an augmented survival of resident splenic DC subsequent to exposure to egg Ag. The exposure of DC to pathogen-derived signals or to CD154 can promote DC migration and the ability to stimulate T cell responses; CD40 ligation has also been shown to be an important survival signal for DC (58, 59).

Although CD86 was not up-regulated on DC during schistosome infection, it was expressed at significant basal levels on DC of both subsets in control and infected mice. Ligation of T cell CD28 by DC CD80/CD86 is the prototypical costimulatory signal to promote IL-2 production and up-regulation of the survival factor Bcl-xL in naive Th cells (60, 61). Experiments using CTLA-Ig fusion proteins or Abs to block CD28 costimulation have shown that signaling through this molecule appears to be critical for induction of Th2 responses, and that Th1 response development during many diseases and infections is less dependent upon this interaction, perhaps due to the up-regulation of alternative costimulatory molecules (62–64). Consistent with these observations, mice deficient in both CD80 and CD86 mount a Th1 instead of a Th2 response to egg Ag during schistosomiasis infection (65). It is interesting that although Th2 responses may rely more heavily on CD28 costimulation, we found that DC that induce Th2 responses in vivo express lower levels of these costimulatory molecules in response to parasite Ag than do DC that promote Th1 responses. However, CD8α+ and CD8α− DC from CD154−/− animals, which exhibit a defective Th2 response during infection (66), do not display increased CD80 expression. Together, these observations suggest that the constitutive expression of CD86 and the CD154-dependent up-regulation of DC CD80 expression that occur during infection with S. mansoni may be important for the generation of the Th2 response in this setting.

Despite the fact that ICOSL and OX40L have been implicated in Th2 response development (45–47, 67), while 4-1BBL and ICAM-1 have been shown or suggested to play a role in Th1 responses (48, 49), we have found that, in general, all these molecules were more highly expressed on DC from mice infected with T. gondii than on DC from mice with schistosomiasis (except ICAM-1, the expression of which on DC was not modulated by either infection). Overall, these data suggest that there are differences in the degree of DC activation in Th1 and Th2 responses rather than unique Th1- or Th2-associated patterns of accessory molecule expression.

This study has revealed distinct impacts of S. mansoni and T. gondii infection on splenic DC. In addition to the marked differences in DC expression of MHC and costimulatory molecules (Figs. 1 and 3 and Table I), ongoing production of IL-12 by DC was found only in T. gondii-infected animals and was not apparent in schistosome-infected or uninfected mice (Fig. 4). In previous studies splenic DC have been shown to produce IL-12 as early as 1 h following i.v. injection of STAg in a CD154-independent manner (40), but it is possible that later in infection and/or in infected vs Ag-injected mice, there is an important role for CD40/CD154 in the activation of DC to make IL-12 (68). Our data showing a lack of any increased expression of activation-associated surface proteins in DC from T. gondii-infected CD154−/− mice would support this view. The observation that active infection with T. gondii results in ongoing IL-12 production in the spleen as late as day 6 is consistent with the previously recognized requirement for continued production of IL-12 for maintenance of the Th1 response during this infection (69, 70).

Our study is the first to investigate changes in the activation status of splenic DC during the course of infection with a Th2 response-inducing pathogen. The data show that despite the many complicating factors of schistosome infection (including egg-induced tissue injury, inflammation, and intestinal mucosal epithelial layer perforation), DC in the spleen and draining lymph nodes retain a minimally activated phenotype, as assessed by the expression of MHC and costimulatory molecules, surface levels of which were increased maximally 2- to 3-fold over those on DC isolated from uninfected mice. This degree of activation is unimpressive compared with that evident in DC from mice infected with T. gondii. Nevertheless, it is notable that both infections have in common the fact that DC activation is fully dependent upon CD154, which presumably is functioning to ligate CD40 on DC. Although the major stimulus for DC activation in active schistosomiasis, and toxoplasmosis is immune system-intrinsic, there are clearly significant differences in the regulation of this signal during the two infections. The enhanced DC activation status in T. gondii-infected mice could be due to priming of DC by the pathogen itself or by TNF-α (40, 56), which is made in quantity during this infection (71). Alternatively, Th1 cells may express more CD154 than Th2 cells (72), resulting in a stronger T cell-derived activation signal for DC in the T. gondii- compared with S. mansoni-infected animals. Another possibility is that in the S. mansoni-infected, but not the T. gondii-infected mice, there are processes that regulate CD154-mediated activation. This view is supported by the findings that while DC isolated from schistosome-infected mice are capable of making IL-12 in response to CD40 ligation, this responsiveness is lost if the DC are cocultured with Ag-stimulated CD11c+ splenocytes from infected animals (Fig. 6A). Surprisingly, purified DC from T. gondii-infected mice made no more IL-12 in response to CD40 ligation than did purified DC from S. mansoni-infected animals (Fig. 6), indicating that both infections result in similar degrees of DC priming. However, the addition of Ag-responsive CD11c+ splenocytes from T. gondii-infected mice promoted DC IL-12 production in response to mAb antiCD40 (Fig. 6B) and also resulted in the production of IL-12p70, which we were unable to measure in the schistosome system. While our previous studies showed that bone marrow-derived DC are not phenotypically activated following in vitro exposure to SEA and yet are able to induce Th2 responses (26), the data presented here show that activation events do occur in vivo during Th2 response development.
initiated by the same Ag. It is becoming apparent that control of DC function during ongoing infection occurs via a series of signals from the pathogen itself, from CD154+ cells through the ligation of CD40, and finally from other Ag-responsive cells that are able to provide additional inhibitory or activating signals. It is the net effect of these signals that dictates DC activation status and which, by controlling the strength of signal- and T-cell-polarizing cytokines potentially deployable by DC to T cells, may play an important role in stabilizing the Th1 or Th2 phenotype of the immune response. The identities of the factors being made by CD11c+ splenic cells that are capable of inhibiting or promoting the responsiveness of DC to CD40 ligation are unknown at present. Future work will focus on understanding these interactions during Th2 response development and determining whether DC express genes unique from those associated with classical activation that may promote their ability to induce a Th2 response in vivo.

Acknowledgments

We thank Beverly Bauman for technical assistance.

References

10. Rutitzky, L. I., H. J. Hernandez, and M. J. Stadecker. 2001. Th1-polarizing cytokines potentially deployable by DC to T cells, may play an important role in stabilizing the Th1 or Th2 phenotype of the immune response. The identities of the factors being made by CD11c+ splenic cells that are capable of inhibiting or promoting the responsiveness of DC to CD40 ligation are unknown at present. Future work will focus on understanding these interactions during Th2 response development and determining whether DC express genes unique from those associated with classical activation that may promote their ability to induce a Th2 response in vivo.


