CD154 Plays a Central Role in Regulating Dendritic Cell Activation During Infections That Induce Th1 or Th2 Responses[1]

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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, 4-1BBL, 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 of IFN-γ are required for host survival during both the acute and chronic stages of *Toxoplasma* infection (11).

Dendritic cells (DC)[4] are APC 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 *S. mansoni* infection, and compared

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[4] Abbreviations used in this paper; DC, dendritic cell; SEA, schistosome egg Ag; STAg, soluble tachyzoite Ag.

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it to that of DC from uninfected control animals as well as to animals infected with a Th1 response-inducing parasite, *T. gondii*. Additionally, we examined the effect of the absence of the CD40/CD154 intercellular communication pathway on DC activation in infected mice. Our data show that in comparison with splenic DC in mice infected with *T. gondii*, which become highly activated, splenic DC in schistosome-infected mice exhibit only a modest degree of increased MHC class II and costimulatory molecule expression. DC did not produce IL-12 during infection with *S. mansoni*. In contrast, infection with the Th1 response-inducing pathogen *T. gondii* resulted in dramatically increased expression of activation markers and promoted elevated IL-12 production by DC. The activation of DC observed during infection with either parasite was not apparent in DC from infected $CD154^{-/-}$ mice, revealing a major role for CD40 ligation in maintaining DC activation during infection regardless of whether the outcome is a Th1 or Th2 response. However, a significant difference between Th1 and Th2 responses is that in *S. mansoni*-infected mice, but not *T. gondii*-infected animals, IL-12 production by DC in response to CD40 ligation is restrained by Ag-responsive $CD11c^{-}$ cells.

Materials and Methods

Mice and experimental infections

Age-matched male or female C57BL/6 (Taconic Farms, Germantown, NY) and $CD154^{-/-}$ mice (The Jackson Laboratory, Bar Harbor, ME) were purchased and used at 6–12 wk of age. For experiments using $CD154^{-/-}$ mice, all animals were housed in filter-covered isolator cages and maintained on Bactrim (HiTech Pharmaceutical, Amityville, NY)-supplemented water. Mice were each infected by percutaneous exposure to 100 *S. mansoni* cercariae (Puerto Rican strain NMRI; Biomedical Research Institute, Rockville, MD) or by i.p. injection of 100 brain cysts of ME49 strain *T. gondii*, or 10^3 culture-derived tachyzoites of P strain *T. gondii*. ME49 and P are type 2 strains of *T. gondii*, and in our experiments results obtained using either strain were indistinguishable.

Reagents and Abs

FITC-conjugated Abs specific for CD8α, CD11c, CD80, CD86, and CD40; an APC-conjugated Ab to CD11c; a biotinylated Ab to OX40L; PE-conjugated Abs specific for CD11c, MHC class II (IA^b), and IL-12p40; a CyChrome-conjugated Ab against CD8α; an unconjugated Ab against CD40; and PerCP-conjugated streptavidin were purchased from BD PharMingen (San Diego, CA). PE-conjugated Abs specific for ICOSL and B7-DC, and a biotinylated Ab against 4-1BBL (CD137L) were obtained from eBioscience (San Diego, CA). Anti-CD11c microbeads and 25 MS MACS separation columns were purchased from Miltenyi Biotec (Auburn, CA). SEA was prepared from isolated schistosome eggs as previously described (26), and soluble tachyzoite Ag (STAg) was prepared from RH tachyzoites (30).

DC purification

Mouse spleens were harvested aseptically into DMEM (Mediatech, Herndon, VA) supplemented with 10 mM HEPES (Life Technologies, Gaithersburg, MD), 100 U/ml of penicillin plus 100 μg/ml of streptomycin (Life Technologies), and 2 mM EDTA (Fisher Scientific, Pittsburgh PA). Spleens were processed into single-cell suspensions by passage through 70-μm nylon cell strainers. Erythrocytes in the suspension were lysed using Red Cell Lysis Buffer (Sigma-Aldrich, St. Louis, MO), and viable nucleated cells that excluded trypan blue were enumerated. To purify DC, splenocytes were washed and resuspended at 2.5×10^8 cells/ml in MACS buffer (Dulbecco's PBS (Mediatech) containing 0.5% BSA, Cohn fraction V (Intergen, Purchase, NY), and 2 mM EDTA), incubated with CD11c microbeads for 15 min at 4°C, washed, and transferred to magnetic columns for positive selection of DC. In experiments requiring >95% purity, DC were passed consecutively through two columns.

Cell culture

Purified DC were cultured at 10^5/round-bottom sterile polypropylene tube in RPMI containing 100 U/ml of penicillin, 100 μg/ml of streptomycin, 5×10^{-5} M 2-ME, 10% FCS, and L-glutamine (Mediatech). Some tubes received 10 μg/ml of mAb anti-CD40. After allowing 10 min on ice for the anti-CD40 to efficiently bind to CD40 on DC, 2×10^6 $CD11c^{-}$ cells not retained by the magnetic columns during the DC purification procedure (see above) were added to the DC with or without SEA (50 μg/ml) or soluble tachyzoites Ag (STAg) at 50 μg/ml. The final culture volume was adjusted to 400 μl/tube. Cells were cultured at 37°C for 18 h, following which DC were analyzed for intracellular IL-12 (see below), and supernatants were collected for ELISA measurement of IL-12 as described previously (26).

Flow cytometry

To analyze DC percentages in the spleens of normal and 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^b, and CyChrome-conjugated anti-CD8α at predetermined concentrations in flow 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 I, MHC class II, CD80, CD86, CD40, ICOSL, B7-DC, OX40L, ICAM-1, 4-1BBL, and CD8α. For detection of intracellular IL-12, DC were fixed in DPBS containing 3% paraformaldehyde (Sigma-Aldrich), 0.1 mM $CaCl_2$, and 0.1 mM $MgCl_2$ 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. $CD11c^{high}$ DC 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

Analysis of DC activation status during infection

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. 1*A*). 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. 1*A*). 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 is 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. 1*B*). Based on previous reports, these time points fall within the initial (day 3), peak (days 6–11), and subdued (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. 1*B*). 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

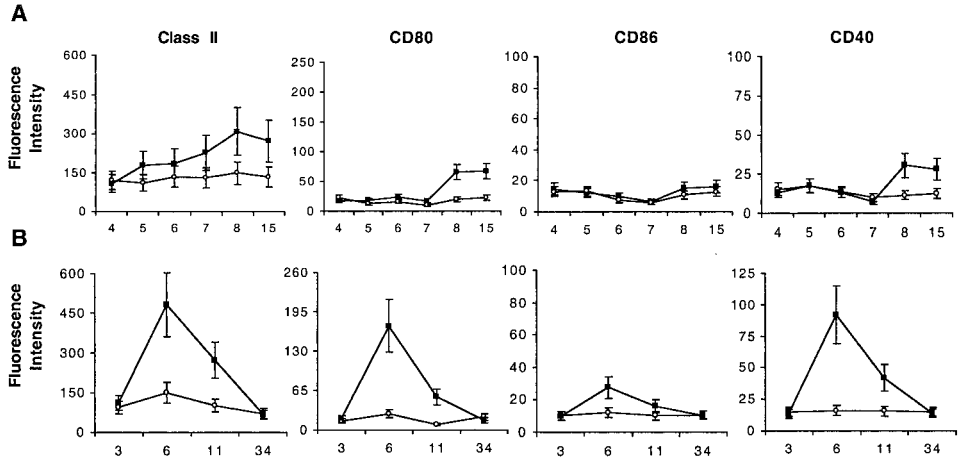

FIGURE 1. Activation phenotype of DC during infection with *S. mansoni* and *T. gondii*. DC were purified using MACS and stained with Abs against CD11c as well as MHC class II, CD80, CD86, or CD40. Graphs show the average geometric mean fluorescence intensity of each activation marker on gated DC from three individual mice; error bars indicate the SD. *A*, DC were obtained at wk 4, 5, 6, 7, 8, and 15 after infection with 100 *S. mansoni* cercariae (■) or from uninfected control animals (○). *B*, DC were purified and analyzed as described above from mice infected with 100 cysts of ME49 strain of *T. gondii* i.p. 3, 6, 11, and 34 days following infection (■) or from normal control animals (○). Representative data from one of two experiments are shown.

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 $CD11c^{high}$/MHC class II^+ DC was determined by flow cytometry. The calculated total number of splenic DC dramatically increased during *S. mansoni* infection (Fig. 2*A*). 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. 2*A*). In addition, analysis of $CD8\alpha^-$ and $CD8\alpha^+$ DC demonstrated that there was a significant increase in both subsets of DC during infection (Fig. 2*C*). 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 activation (Fig. 2*B*), but, interestingly, the increase in $CD8\alpha^+$ DC was less pronounced during infection with this parasite at this time point (Fig. 2*D*). These results suggest that there is increased recruitment or retention of viable DC in the spleen during infection with both pathogens.

Analysis of $CD8\alpha^+$ and $CD8\alpha^-$ DC phenotype during infection

We and others have shown that $CD8\alpha^-$ 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 $CD8\alpha^+$ DC in Th1 response induction and for $CD8\alpha^-$ 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 $CD8\alpha^+$ and $CD8\alpha^-$ gated cells (Fig. 3*A*). In keeping with our observations for

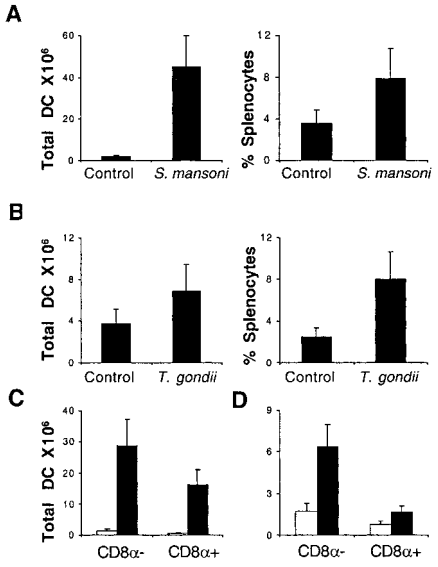

FIGURE 2. Splenic DC from mice infected with *S. mansoni* or *T. gondii*. Individual spleens were harvested after 8 wk of infection with *S. mansoni* (*A*) or 6 days of infection with *T. gondii* (*B*). Total splenocytes were counted and stained with FITC-conjugated CD11c and PE-conjugated IA^b Abs. The percentage of DC for each spleen was determined by flow cytometric analysis, and total DC were calculated. *C* and *D*, DC were purified by MACS and the percentages of $CD8\alpha^+$ and $CD8\alpha^-$ DC were determined by flow cytometry. □, Total DC from each subset in normal spleens from uninfected animals; ■, total DC from mice infected with either *S. mansoni* (*C*) or *T. gondii* (*D*). Values for all graphs indicate mean values from three individual spleens; error bars indicate the SD. Representative data from one of two experiments are shown.

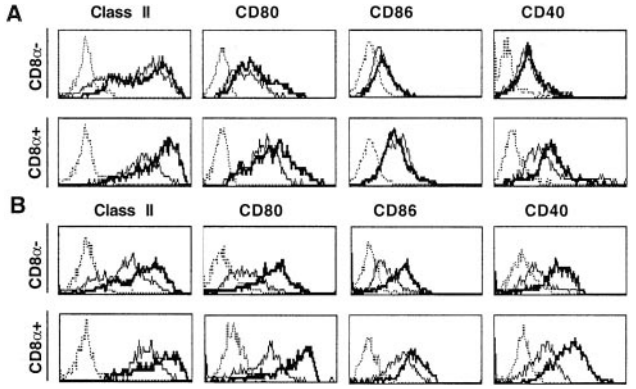

FIGURE 3. DC activation phenotype during infection with *T. gondii* or *S. mansoni*. DC were purified from mouse spleens after 8 wk of infection with *S. mansoni* (*A*) or 6 days of infection with *T. gondii* (*B*) and were stained with Abs for CD11c and CD8α as well as MHC class II, CD80, CD86, and CD40. Histograms show the fluorescence intensity of staining for each indicated marker on gated CD11c^{high} DC from uninfected control animals (fine line), infected animals (bold line), or cells pooled from all mice stained with isotype-matched control Abs (dotted line). Representative data from one of at least three experiments are shown.

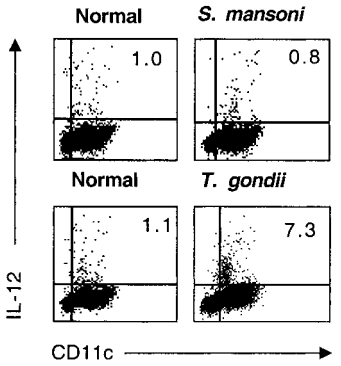

FIGURE 4. DC IL-12 production during infection with *S. mansoni* or *T. gondii*. DC were purified from splenocytes at peak activation time points and were stained with FITC-conjugated CD11c Ab and PE-conjugated IL-12 Ab. Numbers indicate the percentage of DC positive for IL-12, as calculated by subtracting isotype control staining from each sample. Representative data from one of three experiments are shown.

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 of MHC class I on DC from schistosome-infected mice was either unaffected (ICOSL, ICAM-1) or moderately 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. 3*B* and data not shown). In addition there was a higher proportion of activated DC within each subset (Fig. 3*B* 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.

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 schistosome infections 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. 5*A*). 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. 5*A*). Having found

Table I. *Expression of MHC class I and other DC surface molecules*[a]

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

[a] 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.

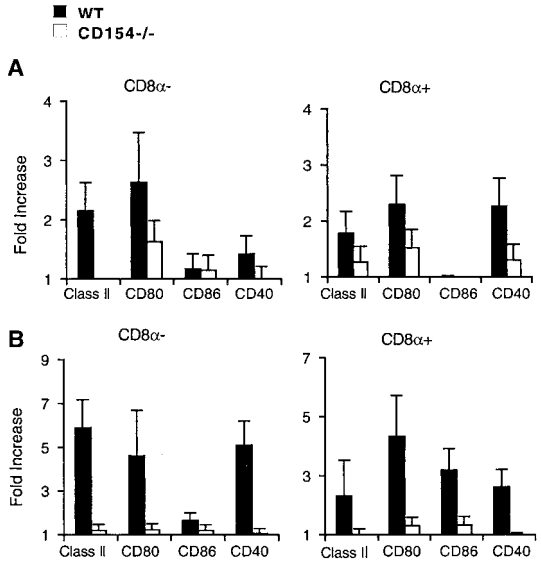

FIGURE 5. Fold change in the expression of DC activation markers during schistosome infection in WT and CD154$^{-/-}$ mice. DC were purified from splenocytes after 8 wk of infection and stained with Abs to CD11c, CD8α, and DC activation markers. Graphs show the mean fold increase in fluorescence intensity for each marker on DC from three individual animals infected with *S. mansoni* (*A*) or *T. gondii* (*B*) compared with normal controls from wild-type (■) and CD154$^{-/-}$ (□) mice. Error bars indicate the SE. Representative data from one of at least two experiments are shown.

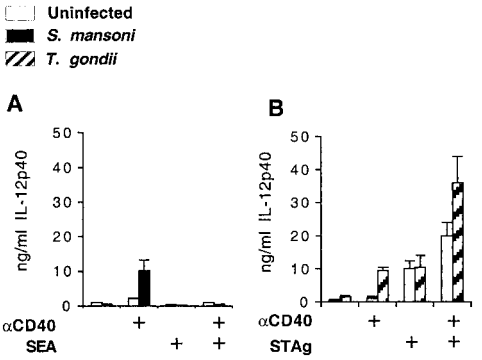

FIGURE 6. Response of DC from infected mice to CD40 ligation. DC were highly purified from splenocytes of normal control animals (□), mice infected with *S. mansoni* (■; *A*), or mice infected with *T. gondii* (▨; *B*). mAb anti-CD40 was incubated with highly purified CD11c$^+$ DC on ice. CD11c$^-$ cells were added to anti-CD40-stimulated DC in the presence or the absence of SEA (*A*) or STAg (*B*). Cultures were transferred to 37°C for 18 h, and IL-12 in supernatants was measured by ELISA. Bars indicate the mean concentration, and error bars indicate the SD.

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 on day 6 of *T. gondii* infection was completely lost in the absence of CD154 (Fig. 5*B*). These results imply a crucial role for CD154, and therefore of T cells, in the continued activation of DC during infections that induce either Th1 or Th2 responses.

Regulation of IL-12 production by DC in response to CD40 ligation

Because DC in schistosome-infected mice are clearly being activated via CD40 (Fig. 5), and CD40 ligation is a known stimulus for IL-12 production (54), it was somewhat surprising that DC recovered from the spleens of schistosome-infected animals were not making IL-12 (Fig. 4). To begin to examine this issue, we isolated DC from the spleens of normal or schistosome-infected mice to assess IL-12 production in response to agonistic mAb anti-CD40. ELISA measurements revealed that DC from infected mice produced ~10-fold more IL-12 in response to this stimulus than did DC from normal mice (Fig. 6*A*). Flow cytometric analysis of intracellular IL-12 in DC fully corroborated this finding (data not shown). Thus, schistosome infection appears to prime DC for IL-12 production in response to CD40 ligation. This finding is inconsistent with the observed Th2 bias of the immune response during schistosomiasis, however, and with the lack of evidence for ongoing IL-12 production by DC recovered from infected mice (Fig. 4). A possible explanation for these findings is that other cells within the spleen negatively regulate IL-12 production by DC in vivo. To address this possibility we cocultured CD11c$^-$ cells from the spleens of control or infected mice with the mAb anti-CD40-treated DC in either the absence or the presence of SEA, which we reasoned would activate Ag-responsive cells and so mimic the situation in vivo. Analysis of IL-12 in the supernatants by ELISA (Fig. 6*A*) and in DC by intracellular staining (data not shown) revealed that stimulation of SEA-responsive cells markedly inhibited IL-12 production by DC in response to CD40 ligation.

Infection-induced priming of DC to make IL-12 in response to ligation of CD40 was found to occur to a similar degree in *T. gondii*-infected mice as in *S. mansoni*-infected animals (Fig. 6, *B* vs *A*). However, control of IL-12 production by DC in response to CD40 ligation was markedly different in the *T. gondii*-infected animals, in that Ag-activated CD11c$^-$ cells promoted, rather than inhibited, IL-12 production (Fig. 6*B*). Therefore, the inhibition of DC 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. 6*B*). Specific analysis for IL-12p70 rather than p40 revealed this bioactive form of the cytokine only in the culture supernatants of DC from uninfected or *Toxoplasma*-infected mice following stimulation with mAb anti-CD40 in the presence of STAg and CD11c$^-$ cells (uninfected, 30 ± 13 pg/ml; *Toxoplasma*-infected, 130 ± 18.7 pg/ml). Specific ELISAs failed to detect any IL-4, IL-10, or IL-13 in the supernatants of DC cultured under any of the conditions described (data not shown).

Discussion

Previous work revealed that DC exhibit different activation responses to different classes of pathogens in vitro and that there appears to be a correlation between the activation status of DC and the type of Th cell response that they are able to induce (26, 43). Accordingly, CD8α^- DC grown in vitro from bone marrow and exposed to helminth Ag exhibit little evidence of conventional activation and preferentially initiate Th2 responses, whereas the same type of DC primed with bacterial Ag become highly activated and induce Th1 responses (26, 43). In the present study we examined the activation status of DC in spleens of schistosome-infected mice both before and during development of the Th2 response that characterizes this infection. Our analysis included both CD8α^- and CD8α^+ DC and indicates that in vivo both types of cells undergo a degree of activation at times coincident with the establishment of the Th2 response (Figs. 1 and 3 and Table I). Activation in the CD8α^- subset was marked by 2- to 3-fold increases in the expression of MHC classes I and II, CD80, 4-1BBL,

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-x_L 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 schistosome 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. 6*A*). 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$^-$ spleen cells from *T. gondii*-infected mice promoted DC IL-12 production in response to mAb antiCD40 (Fig. 6*B*) 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.

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References

1. Pearce, E. J., and A. S. MacDonald. 2002. The immunobiology of schistosomiasis. *Nat. Rev. Immunol. 2:499.*
2. Grzych, J. M., E. Pearce, A. Cheever, Z. A. Caulada, P. Caspar, S. Heiny, F. Lewis, and A. Sher. 1991. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J. Immunol. 146: 1322.*
3. Vella, A. T., and E. J. Pearce. 1992. $CD4^+$ Th2 response induced by *Schistosoma mansoni* eggs develops rapidly, through an early, transient, Th0-like stage. *J. Immunol. 148:2283.*
4. Okano, M., A. R. Satoskar, K. Nishizaki, M. Abe, and D. A. Harn, Jr. 1999. Induction of Th2 responses and IgE is largely due to carbohydrates functioning as adjuvants on *Schistosoma mansoni* egg antigens. *J. Immunol. 163:6712.*
5. Brunet, L. R., F. D. Finkelman, A. W. Cheever, M. A. Kopf, and E. J. Pearce. 1997. IL-4 protects against TNF-α-mediated cachexia and death during acute schistosomiasis. *J. Immunol. 159:777.*
6. Hoffmann, K. F., A. W. Cheever, T. A. Wynn. 2000. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J. Immunol. 164:6404.*
7. Chiaramonte, M. G., D. D. Donaldson, A. W. Cheever, and T. A. Wynn. 1999. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J. Clin. Invest. 104:777.*
8. Dessein, A. J., D. Hillaire, N. E. Elwali, S. Marquet, Q. Mohamed-Ali, A. Mirghani, S. Henri, A. A. Abdelhameed, O. K. Saeed, M. M. Magzoub, et al. 1999. Severe hepatic fibrosis in *Schistosoma mansoni* infection is controlled by a major locus that is closely linked to the interferon-γ receptor gene. *Am. J. Hum. Genet. 65:709.*
9. Fallon, P. G., E. J. Richardson, G. J. McKenzie, and A. N. McKenzie. 2000. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J. Immunol. 164:2585.*
10. Rutitzky, L. I., H. J. Hernandez, and M. J. Stadecker. 2001. Th1-polarizing immunization with egg antigens correlates with severe exacerbation of immunopathology and death in schistosome infection. *Proc. Natl. Acad. Sci. USA 98:13243.*
11. Denkers, E. Y. 1999. T lymphocyte-dependent effector mechanisms of immunity to *Toxoplasma gondii*. *Microbes Infect. 1:699.*
12. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
13. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. *Annu. Rev. Immunol. 18:767.*
14. Mellman, I., and R. M. Steinman. 2001. Dendritic cells: specialized and regulated antigen processing machines. *Cell 106:255.*
15. Kalinski, P., C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today 20:561.*
16. Moser, M., and K. M. Murphy. 2000. Dendritic cell regulation of TH1-TH2 development. *Nat. Immunol. 1:199.*
17. Reid, S. D., G. Penna, and L. Adorini. 2000. The control of T cell responses by dendritic cell subsets. *Curr. Opin. Immunol. 12:114.*
18. Maldonado-Lopez, R., and M. Moser. 2001. Dendritic cell subsets and the regulation of Th1/Th2 responses. *Semin. Immunol. 13:275.*
19. Pulendran, B., K. Palucka, and J. Banchereau. 2001. Sensing pathogens and tuning immune responses. *Science 293:253.*
20. Vremec, D., M. Zorbas, R. Scollay, D. J. Saunders, C. F. Ardavin, L. Wu, and K. Shortman. 1992. The surface phenotype of dendritic cells purified from mouse thymus and spleen: investigation of the CD8 expression by a subpopulation of dendritic cells. *J. Exp. Med. 176:47.*
21. Pulendran, B., J. Lingappa, M. K. Kennedy, J. Smith, M. Teepe, A. Rudensky, C. R. Maliszewski, and E. Maraskovsky. 1997. Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. *J. Immunol. 159:2222.*
22. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, and M. Moser. 1999. $CD8\alpha^+$ and $CD8\alpha^-$ subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med. 189:587.*
23. Pulendran, B., J. L. Smith, G. Caspary, K. Brasel, D. Pettit, E. Maraskovsky, and C. R. Maliszewski. 1999. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc. Natl. Acad. Sci. USA 96:1036.*
24. Vieira, P. L., E. C. de Jong, E. A. Wierenga, M. L. Kapsenberg, and P. Kalinski. 2000. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. *J. Immunol. 164:4507.*
25. De Smedt, T., E. Butz, J. Smith, R. Maldonado-Lopez, B. Pajak, M. Moser, and C. Maliszewski. 2001. $CD8\alpha^-$ and $CD8\alpha^+$ subclasses of dendritic cells undergo phenotypic and functional maturation in vitro and in vivo. *J. Leukocyte Biol. 69:951.*
26. MacDonald, A. S., A. D. Straw, B. Bauman, and E. J. Pearce. 2001. $CD8^-$ dendritic cell activation status plays an integral role in influencing Th2 response development. *J. Immunol. 167:1982.*
27. Maldonado-Lopez, R., C. Maliszewski, J. Urbain, and M. Moser. 2001. Cytokines regulate the capacity of $CD8\alpha^+$ and $CD8\alpha^-$ dendritic cells to prime Th1/Th2 cells in vivo. *J. Immunol. 167:4345.*
28. MacDonald, A. S., A. D. Straw, N. M. Dalton, and E. J. Pearce. 2002. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J. Immunol. 168:537.*
29. MacDonald, A. S., and E. J. Pearce. 2002. Cutting edge: polarized Th cell response induction by transferred antigen-pulsed dendritic cells is dependent on IL-4 or IL-12 production by recipient cells. *J. Immunol. 168:3127.*
30. Grunvald, E., M. Chiaramonte, S. Hieny, M. Wysocka, G. Trinchieri, S. N. Vogel, R. T. Gazzinelli, and A. Sher. 1996. Biochemical characterization and protein kinase C dependency of monokine-inducing activities of *Toxoplasma gondii*. *Infect. Immun. 64:2010.*
31. Pearce, E. J., P. Caspar, J. M. Grzych, F. A. Lewis, and A. Sher. 1991. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med. 173:159.*
32. Kuchroo, V. K., M. P. Das, J. A. Brown, A. M. Ranger, S. S. Zamvil, R. A. Sobel, H. L. Weiner, N. Nabavi, and L. H. Glimcher. 1995. B7-1 and B7-2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. *Cell 80:707.*
33. Subramanian, G., J. W. Kazura, E. Pearlman, X. Jia, I. Malhotra, and C. L. King. 1997. B7-2 requirement for helminth-induced granuloma formation and CD4 type 2 T helper cell cytokine expression. *J. Immunol. 158:5914.*
34. Cheever, A. W., D. Jankovic, G. S. Yap, M. C. Kullberg, A. Sher, and T. A. Wynn. 1998. Role of cytokines in the formation and downregulation of hepatic circumoval granulomas and hepatic fibrosis in *Schistosoma mansoni*-infected mice. *Mem. Inst. Oswaldo Cruz 93:25.*
35. Sher, A., E. Y. Denkers, and R. T. Gazzinelli. 1995. Induction and regulation of host cell-mediated immunity by Toxoplasma gondii. *Ciba Found. Symp. 195:95.*
36. Denkers, E. Y., and R. T. Gazzinelli. 1998. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clin. Microbiol. Rev. 11:569.*
37. Caux, C., S. Ait-Yahia, K. Chemin, O. de Bouteiller, M. C. Dieu-Nosjean, B. Homey, C. Massacrier, B. Vanbervliet, A. Zlotnik, and A. Vicari. 2000. Dendritic cell biology and regulation of dendritic cell trafficking by chemokines. *Springer Semin. Immunopathol. 22:345.*
38. McDermott, D. H., and P. M. Murphy. 2000. Chemokines and their receptors in infectious disease. *Springer Semin. Immunopathol. 22:393.*
39. Kamath, A. T., J. Pooley, M. A. O'Keeffe, D. Vremec, Y. Zhan, A. M. Lew, A. D'Amico, L. Wu, D. F. Tough, and K. Shortman. 2000. The development, maturation, and turnover rate of mouse spleen dendritic cell populations. *J. Immunol. 165:6762.*
40. Sousa, C. R., S. Hieny, T. Scharton-Kersten, D. Jankovic, H. Charest, R. N. Germain, and A. Sher. 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med. 186:1819.*
41. Bourguin, I., M. Moser, D. Buzoni-Gatel, F. Tielemans, D. Bout, J. Urbain, and O. Leo. 1998. Murine dendritic cells pulsed in vitro with *Toxoplasma gondii* antigens induce protective immunity in vivo. *Infect. Immun. 66:4867.*
42. Marovich, M. A., M. A. McDowell, E. K. Thomas, and T. B. Nutman. 2000. IL-12p70 production by *Leishmania major*-harboring human dendritic cells is a CD40/CD40 ligand-dependent process. *J. Immunol. 164:5858.*
43. Whelan, M., M. M. Harnett, K. M. Houston, V. Patel, W. Harnett, and K. P. Rigley. 2000. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of Th2 cells. *J. Immunol. 164:6453.*
44. Kronin, V., C. J. Fitzmaurice, I. Caminschi, K. Shortman, D. C. Jackson, and L. E. Brown. 2001. Differential effect of $CD8^+$ and $CD8^-$ dendritic cells in the stimulation of secondary $CD4^+$ T cells. *Int. Immunol. 13:465.*
45. Akiba, H., Y. Miyahira, M. Atsuta, K. Takeda, C. Nohara, T. Futagawa, H. Matsuda, T. Aoki, H. Yagita, and K. Okumura. 2000. Critical contribution of OX40 ligand to T helper cell type 2 differentiation in experimental leishmaniasis. *J. Exp. Med. 191:375.*
46. Kopf, M., A. J. Coyle, N. Schmitz, M. Barner, A. Oxenius, A. Gallimore, J. C. Gutierrez-Ramos, and M. F. Bachmann. 2000. Inducible costimulator protein (ICOS) controls T helper cell subset polarization after virus and parasite infection. *J. Exp. Med. 192:53.*

47. Ohshima, Y., L. P. Yang, T. Uchiyama, Y. Tanaka, P. Baum, M. Sergerie, P. Hermann, and G. Delespesse. 1998. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human $CD4^+$ T cells into high IL-4-producing effectors. *Blood 92:3338.*
48. Isobe, M., J. Suzuki, S. Yamazaki, Y. Yazaki, S. Horie, Y. Okubo, K. Maemura, and M. Sekiguchi. 1997. Regulation by differential development of Th1 and Th2 cells in peripheral tolerance to cardiac allograft induced by blocking ICAM-1/LFA-1 adhesion. *Circulation 96:2247.*
49. Kim, Y. J., S. H. Kim, P. Mantel, and B. S. Kwon. 1998. Human 4–1BB regulates CD28 co-stimulation to promote Th1 cell responses. *Eur. J. Immunol. 28:881.*
50. Kelsall, B. L., E. Stuber, M. Neurath, and W. Strober. 1996. Interleukin-12 production by dendritic cells: the role of CD40-CD40L interactions in Th1 T-cell responses. *Ann. NY Acad. Sci. 795:116.*
51. O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity 8:275.*
52. Trinchieri, G. 1998. Interleukin-12: a cytokine at the interface of inflammation and immunity. *Adv. Immunol. 70:83.*
53. Banchereau, J., F. Bazan, D. Blanchard, F. Briere, J. P. Galizzi, C. van Kooten, Y. J. Liu, F. Rousset, and S. Saeland. 1994. The CD40 antigen and its ligand. *Annu. Rev. Immunol. 12:881.*
54. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med. 184:747.*
55. Screaton, G., and X. N. Xu. 2000. T cell life and death signalling via TNF-receptor family members. *Curr. Opin. Immunol. 12:316.*
56. Trevejo, J. M., M. W. Marino, N. Philpott, R. Josien, E. C. Richards, K. B. Elkon, and E. Falck-Pedersen. 2001. TNF-α-dependent maturation of local dendritic cells is critical for activating the adaptive immune response to virus infection. *Proc. Natl. Acad. Sci. USA 98:12162.*
57. Aliberti, J., C. Reis e Sousa, M. Schito, S. Hieny, T. Wells, G. B. Huffnagle, and A. Sher. 2000. CCR5 provides a signal for microbial induced production of IL-12 by $CD8\alpha^+$ dendritic cells. *Nat. Immunol. 1:83.*
58. De Smedt, T., B. Pajak, G. G. Klaus, R. J. Noelle, J. Urbain, O. Leo, and M. Moser. 1998. Antigen-specific T lymphocytes regulate lipopolysaccharide-induced apoptosis of dendritic cells in vivo. *J. Immunol. 161:4476.*
59. McLellan, A., M. Heldmann, G. Terbeck, F. Weih, C. Linden, E. B. Brocker, M. Leverkus, and E. Kampgen. 2000. MHC class II and CD40 play opposing roles in dendritic cell survival. *Eur. J. Immunol. 30:2612.*
60. Harris, N. L., and F. Ronchese. 1999. The role of B7 costimulation in T-cell immunity. *Immunol. Cell. Biol. 77:304.*
61. Salomon, B., and J. A. Bluestone. 2001. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. *Annu. Rev. Immunol. 19:225.*
62. King, C. L., J. Xianli, C. H. June, R. Abe, and K. P. Lee. 1996. CD28-deficient mice generate an impaired Th2 response to *Schistosoma mansoni* infection. *Eur. J. Immunol. 26:2448.*
63. Padrid, P. A., M. Mathur, X. Li, K. Herrmann, Y. Qin, A. Cattamanchi, J. Weinstock, D. Elliott, A. I. Sperling, and J. A. Bluestone. 1998. CTLA4Ig inhibits airway eosinophilia and hyperresponsiveness by regulating the development of Th1/Th2 subsets in a murine model of asthma. *Am. J. Respir. Cell Mol. Biol. 18:453.*
64. Okano, M., M. Azuma, T. Yoshino, H. Hattori, M. Nakada, A. R. Satoskar, D. A. Harn, Jr., E. Nakayama, T. Akagi, and K. Nishizaki. 2001. Differential role of CD80 and CD86 molecules in the induction and the effector phases of allergic rhinitis in mice. *Am. J. Respir. Crit. Care Med. 164:1501.*
65. Hernandez, H. J., A. H. Sharpe, and M. J. Stadecker. 1999. Experimental murine schistosomiasis in the absence of B7 costimulatory molecules: reversal of elicited T cell cytokine profile and partial inhibition of egg granuloma formation. *J. Immunol. 162:2884.*
66. MacDonald A. S., E. A. Patton, A. C. La Flamme, M. I. Araujo, Cl. R. Huxtable, B. Bauman, and E. J. Pearce. 2002. Impaired Th2 development and increased mortality during *Schistosoma mansoni* infection in the absence of CD40/CD154 interaction. *J. Immunol. 168:4643.*
67. de Jong, E. C., P. L. Vieira, P. Kalinski, J. H. Schuitemaker, Y. Tanaka, E. A. Wierenga, M. Yazdanbakhsh, and M. L. Kapsenberg. 2002. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells in vitro with diverse Th cell-polarizing signals. *J. Immunol. 168:1704.*
68. Schulz, O., A. D. Edwards, M. Schito, J. Aliberti, S. Manickasingham, A. Sher, and C. Reis e Sousa. 2000. CD40 triggering of heterodimeric IL-12 p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity 13:453.*
69. Yap, G, M. Pesin, A. Sher. Cutting edge: IL-12 is required for the maintenance of IFN-γ production in T cells mediating chronic resistance to the intracellular pathogen, *Toxoplasma gondii. J. Immunol. 165:628.*
70. Park, A. Y., and P. Scott. 2001. IL-12: keeping cell-mediated immunity alive. *Scand. J. Immunol. 53:529.*
71. Johnson, L. L. 1992. A protective role for endogenous tumor necrosis factor in *Toxoplasma gondii* infection. *Infect. Immun. 60:1979.*
72. Lee, B. O., L. Haynes, S. M. Eaton, S. L. Swain, and T. D. Randall. 2002. The biological outcome of CD40 signaling is dependent on the duration of CD40 ligand expression: reciprocal regulation by interleukin (IL)-4 and IL-12. *J. Exp. Med. 196:693.*