CD8$^-$ Dendritic Cell Activation Status Plays an Integral Role in Influencing Th2 Response Development[1]

Andrew S. MacDonald, Amy D. Straw, Beverley Bauman, and Edward J. Pearce[2]

Whether dendritic cells (DC) play a passive or active role in Th2 response induction is poorly understood. In this study, we show that CD8$^-$ DC pulsed with Th2-polarizing Ag (soluble egg Ag (SEA)) from *Schistosoma mansoni* potently stimulate Th2 responses in vivo and in vitro while failing to undergo a conventional maturation process. Thus, in contrast to DC pulsed with the Th1 response inducing Ag *Propionebacterium acnes*, SEA-exposed DC exhibit a phenotype that is most similar to that of immature DC, failing to up-regulate expression of CD40, CD54, CD80, CD86, or OX40L; producing no detectable IL-4, IL-10, or IL-12; and displaying only a minor increase in MHC class II expression. Importantly, in vitro derived DC exposed to SEA were phenotypically similar to CD8$^-$ DC isolated from active *S. mansoni* infection. By discriminating between different types of pathogen and responding appropriately, CD8$^-$ DC play a major role in the decision process to mount either a Th1 or Th2 response. ***The Journal of Immunology*****, 2001, 167: 1982–1988.**

Immune responses to pathogens are initiated when dendritic cells (DC)[3] present MHC class II/Ag peptide complexes to CD4$^+$ Th cells. In combination with costimulation, this signal drives T cells to produce IL-2 and to enter the cell cycle (1, 2). Proliferating Th cells initially express a spectrum of cytokine genes, but within a short period a population of cells, each producing a broadly similar array of cytokines, emerges as the dominant type (3–6). These Th cells have a stable, inheritable phenotype characterized by the production of signature effector cytokines that define the immune response (7). The most common effector cell types are Th1 lymphocytes, which produce IFN-γ and IL-2, and Th2 cells, which secrete IL-4, IL-5, and IL-13 (4, 8). A role for DC-derived IL-12 in Th1 response development has recently become clear (9). IL-12 strongly selects for Th cells making IFN-γ (10), and pathogens that induce Th1 responses are capable of activating DC to secrete IL-12 (11). Moreover, these interactions arm DC to produce additional IL-12 following stimulation through CD40 upon encounter with T cells expressing CD154 (12–16).

Given their importance for activating naive Th cells and their role in promoting Th1 responses (1, 17), a key question is whether DC also play a central role in promoting Th2 response development. The crucial role played by IL-4 in stabilizing Th2 cytokine expression (3, 4) makes it an ideal candidate for a DC-derived factor that could promote Th2 responses. Consistent with this idea, it was recently reported that life stages of *Candida* that induce Th2 responses are able to stimulate DC to make IL-4 (18). However, whether this represents the standard response of DC to Th2 Ag is currently unclear. Other possibilities are that exposure of DC to Th1 or Th2 Ag leads to differential costimulatory molecule expression, activation status, final Ag load, and/or differences in the duration of interaction with the Th cell, any or all of which could influence the outcome of the Th response (19–23). A final possibility, favored by a growing number of reports, is that different subsets of DC are specialized for priming Th1 or Th2 responses (2, 24, 25).

In this study, we focused on the issue of Th2 response induction by comparing the responses of a single type of DC, murine bone marrow-derived CD8$^-$ DC, with Th2- vs Th1-polarizing Ag. For a Th2 Ag, we used soluble egg Ag (SEA), a soluble extract of the eggs of the parasitic helminth, *Schistosoma mansoni*. Mice infected with this parasite mount strong Th2 responses that are essential for host survival (26), and detailed analysis of the immune response has revealed that the major Th2 Ag are soluble and released from the egg stage (27, 28). For a Th1 stimulus, we utilized the Gram-positive bacterium *Propionebacterium acnes* (previously known as *Corynebacterium parvum*) (29). The interaction of both of these Ag with DC has not previously been examined. Our results show that exposure of DC to SEA or *P. acnes* in vitro was sufficient to respectively induce in them the ability to promote Th2 or Th1 responses when subsequently injected into mice or cocultured with naive CD4 cells in vitro. Analysis of the effects on DC of exposure to SEA or *P. acnes* revealed striking differences in activation status that are most likely key to the ability of these cells to promote Th2 or Th1 responses. Cells exposed to *P. acnes* responded similarly to those activated by the prototypic DC maturation signal, LPS (30–32), up-regulating expression of a panel of costimulatory molecules and cytokine genes, including IL-12 and IL-18. In striking contrast, SEA-pulsed DC retained a phenotype most similar to that of immature DC, producing no measurable Th2 response skewing cytokines (e.g., IL-4, IL-10) and maintaining immature DC levels of CD40, CD80, CD86, and OX40L surface expression. Indeed, the only response measured in SEA-pulsed DC was a slight up-regulation in MHC class II expression, which coincided with the phenotype of CD8$^-$ DC isolated from

Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Received for publication April 3, 2001. Accepted for publication May 31, 2001.

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 a Wellcome Trust International Prize Traveling Fellowship to A.S.M. and National Institutes of Health Grant A132573 to E.J.P., who is a Burroughs Wellcome Fund Scholar in Molecular Parasitology. Schistosome life cycle stages for this work were supplied through National Institutes of Health-National Institute of Allergy and Infectious Diseases Contract NO1-AI-55270.

[2] Address correspondence and reprint requests to Dr. Edward J. Pearce, C5165 VMC, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14850. E-mail address: ejp2@cornell.edu

[3] Abbreviations used in this paper: DC, dendritic cell; iNOS, inducible NO synthase; PEC, peritoneal exudate cell; RPA, RNase protection assay; SEA, soluble egg Ag; wt, wild type.

0022-1767/01/$02.00

schistosome-infected mice. These findings demonstrate that pathogens inducing contrasting immune responses differentially activate $CD8^-$ DC, which in turn directly influences the phenotype of the developing immune response.

Materials and Methods

Animals and reagents

Female wild-type (wt) C57BL/6 and MHC class II-deficient (Abb) mice were purchased from Taconic Farms (Germantown, NY). Six- to 12-wk-old females were used as bone marrow donors for DC culture, for in vivo immunizations, or as splenocyte donors for CD4 T cell purification for use in some in vitro experiments. SEA was prepared from isolated schistosome eggs, as previously described (33, 34). Care was used to prevent endotoxin contamination of the Ag preparation, and using a timed gel endotoxin detection kit (Sigma, St. Louis, MO), we found the SEA to be contamination free. Moreover, the nonresponsiveness of DC to SEA (see *Results*) is a strong indicator that the SEA was endotoxin free. A stock of heat-killed *P. acnes* was kindly provided by the Trudeau Institute (Saranac Lake, NY). LPS (*Escherichia coli* 0111:B4) was purchased from Difco Laboratories (Detroit, MI).

We chose these Ag preparations (soluble SEA and whole *P. acnes*) for their physiological relevance. Schistosome eggs are metabolic, and during infection release proteins into the tissues surrounding their site of deposition (35). SEA contains these released Ag, and is widely used in the schistosomiasis field to assess Th responses during infection (36–38). Similarly, infection with *P. acnes* involves exposure of the host to whole bacteria, which are small enough to be phagocytosed, and thus we felt it relevant to stimulate the DC with the whole organism rather than a soluble extract. To control for the particulate vs soluble difference of *P. acnes* and SEA, we additionally used a soluble bacterial product (LPS) in many of the experiments.

DC generation

DC were generated as previously described (39). Briefly, bone marrow was collected from femurs of mice, and clusters within the bone marrow suspension were dispersed by vigorous pipetting. Cells were seeded into bacteriological petri dishes at 2×10^5/ml in 10 ml medium (RPMI 1640; Sigma) supplemented with 10% heat-inactivated and filtered FCS (HyClone), 2 mM L-glutamine (Life Technologies, Gaithersburg, MD), 100 U/ml penicillin plus 100 μg/ml streptomycin (Life Technologies), and 50 μM 2-ME (Sigma), with the addition of 20 ng/ml GM-CSF (Peprotech, Rocky Hill, NJ). At day 3, a further 10 ml of medium containing 20 ng/ml GM-CSF was added. At days 6 and 8, 10 ml culture supernatant was removed and replaced with 10 ml fresh culture medium containing 20 ng/ml GM-CSF. For generation of immature DC, at day 10 plates were fed as at days 6 and 8, but only 5 ng/ml GM-CSF was added in fresh media, and cells were harvested 18 h later (day 11). After this time, harvested cells comprised 95% DC (class II^+, $CD11c^+$), with the remainder of the cells being predominantly granulocytes. As expected, no contaminating B cells, macrophages, CD4 or CD8 T cells, or $CD8^+$ DC were generated under these conditions, as determined by FACS using mAbs specific for B220, F4/80, CD4, and CD8-α (not shown). For activation of DC, cells were treated in the same way, but with the addition of the appropriate Ag (1 μg/ml LPS, 50 μg/ml SEA, or 25 μg/ml *P. acnes*) for the final 18-h incubation. In our study, we based our choice of concentrations of SEA and *P. acnes* on analysis of DC dose-response curves (not shown).

Determination of DC activation state

Expression of surface molecules was quantified by flow cytometry using FITC- or PE-conjugated Ab ($I\text{-}A^b$, B220, CD4, CD8-α, CD11c, CD80, CD86) or biotin-conjugated Ab, followed by FITC streptavidin (OX40L), all purchased from PharMingen (San Diego, CA). Samples were analyzed using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson, Franklin Lakes, NJ). Total RNA was extracted from differentially activated day 11 DC using RNA-STAT 50 (Tel-Test, Friendswood, TX). DNA probe templates for genes of interest were purchased from PharMingen. Target RNA (2 μg) was used for hybridization with [α-^{32}P]UTP (Amersham, Arlington Heights, IL)-labeled probe. Probe synthesis and RNase treatment were conducted using standard reagents and protocols (PharMingen). The QuickPoint Rapid Nucleic Acid Separation System (Novex, San Diego, CA) was used for gel resolution of protected probes, which was detected and quantified using a phosphor screen with a Storm 860 PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). Cytokine ELISAs were performed on culture supernatants using paired mAb purchased from PharMingen or purified from hybridoma supernatants in our laboratory. The TNF-α Duoset (R&D Systems, Minneapolis, MN) was used for measurement of TNF-α. For cytocentrifuge preparations (cytospins), DC were centrifuged onto a glass slide using a Cytospin-2 (Shandon, Pittsburgh, PA). Cytospins were air dried and fixed in methanol before staining using Hema-3 (Fisher Scientific, Pittsburgh, PA). Photomicrographs were taken using a Zeiss Axioskop microscope at $\times$400 magnification. Splenic DC were isolated from C57BL/6 mice that had been infected with approximately 70 *S. mansoni* cercariae percutaneously 8 wk previously (26) by magnetic sorting using directly conjugated CD11c-specific microbeads and MS^+ or VS^+ columns in conjunction with MiniMACS or VarioMacs magnets (Miltenyi Biotec, Auburn, CA).

Determination of DC-priming ability

For in vivo experiments, C57BL/6 mice were injected i.p. with 5×10^5 DC, or DC that had been pulsed overnight with SEA or *P. acnes* (as above). After 7 days, spleens were removed aseptically, and splenocytes were incubated with media, SEA (50 μg/ml), *P. acnes* (25 μg/ml), or mAb anti-CD3 (0.5 μg/well, plate bound; PharMingen). For these assays, the media used was DMEM (Sigma) supplemented with 3% normal mouse serum (Cedarlane Laboratories, Ontario, Canada), 2 mM L-glutamine (Life Technologies), 100 U/ml penicillin plus 100 μg/ml streptomycin (Life Technologies), and 50 μM 2-ME (Sigma). After 72 h, cytokine levels in supernatants harvested from these cultures were measured by ELISA. For in vitro experiments, 5×10^5 control DC, or DC pulsed with SEA or *P. acnes* were cultured with 2.5×10^6 $CD4^+$ T cells (purified from naive C57BL/6 splenocytes using CD4 T cell subset purification columns; R&D Systems). After 48-h culture, rIL-2 was added at 20 U/ml. Cells were then rested for 72–96 h, before being restimulated with plate-bound anti-CD3 (0.5 μg/well) for 72 h. Cytokine in supernatants harvested from these cultures was then measured by ELISA. To determine DC responsiveness to stimulation via CD40 ligation, differentially activated DC were incubated with 10 μg/ml agonistic anti-CD40 Ab (3/23; PharMingen). After 72 h, culture supernatants were measured for IL-12p40 production by ELISA.

Results

Generation and characterization of murine $CD8^-$ DC

To generate DC for these studies, we utilized a recently published technique in which murine bone marrow cells are cultured in GM-CSF for 10 days (39). The cells that emerge from this culture were >95% $CD8^-$ myeloid DC, expressing CD11c, and low levels of MHC class II (Fig. 1*A*). $CD11c^-$ cells were MHC class II^- and were predominantly granulocytic. Following overnight exposure to the prototypic DC activation signal LPS, the $CD11c^+$ population uniformly expressed high levels of MHC class II. Morphologically, unstimulated DC were round and rather nondendritic in appearance, whereas activated DC were highly dendritic (Fig. 1*B*).

SEA-pulsed DC induce Th2 polarization in vivo and in vitro

To test the ability of Ag-pulsed DC to induce Th1 or Th2 responses in vivo, cells were collected on day 11 after overnight

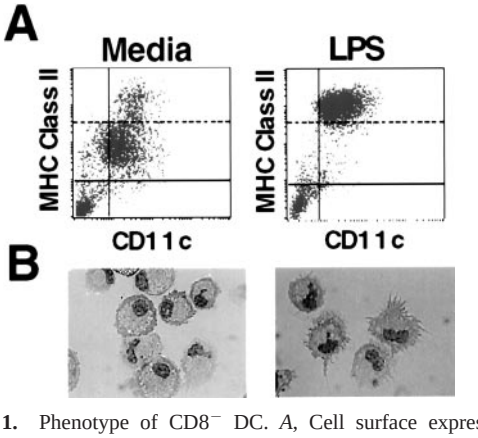

FIGURE 1. Phenotype of $CD8^-$ DC. *A*, Cell surface expression of MHC class II and CD11c; *B*, morphology of either unstimulated DC (media) or DC stimulated overnight with 1 μg/ml LPS.

culture with SEA or *P. acnes*, resuspended in PBS, and injected i.p. into naive mice. One week later, spleen cells were recovered and stimulated in vitro with SEA, *P. acnes*, anti-CD3, or media alone. Cells from mice injected with SEA-pulsed DC made IL-4, IL-5, and IL-13, but no IFN-γ in response to stimulation with SEA, whereas cells from mice injected with an identical number of DC that had instead been pulsed with *P. acnes* made IFN-γ, but no IL-4, IL-5, or IL-13 in response to stimulation with *P. acnes* (Fig. 2*A*). Remarkably, a similar result was also apparent when spleen cells were stimulated with anti-CD3 (Fig. 2*B*). Under these conditions, spleen cells from mice injected with DC alone made low amounts of IL-4, IL-5, and IL-13, and of IFN-γ. In contrast, spleen cells from mice injected with *P. acnes*-pulsed DC made high levels of IFN-γ (greater than 20 ng/ml) and lower amounts of the Th2 cytokines than were made by the spleen cells from the mice injected with immature DC. Spleen cells from mice injected with SEA-pulsed DC made IL-4, IL-5, IL-13, and intermediate levels of IFN-γ (15 ng/ml) in response to anti-CD3. A further indication of Th2 response induction by SEA-pulsed DC was provided by cytospin examination of the peritoneal exudate cells (PEC) lavaged from injected mice. Of the PEC from the SEA-DC-injected mice, 3.6% were eosinophils, whereas this cell type comprised $<0.5\%$ of the PEC lavaged from *P. acnes*-DC-injected mice. PEC produced the same pattern of cytokines as was seen in splenocyte cultures (not shown). SEA-pulsed DC therefore induced a marked Ag-specific Th2 response, whereas *P. acnes*-pulsed DC induced a strong Th1 response.

To determine whether the induction of Ag-specific Th responses required presentation of Ag by the injected DC, we compared the ability of differentially activated wt or MHC class $II^{-/-}$ DC to induce Th2 or Th1 responses in vivo. One week after injection of DC, spleen cells were recovered and stimulated in vitro with SEA or *P. acnes*. Ag-pulsed MHC class $II^{-/-}$ DC failed to induce Ag-specific Th responses after in vivo injection (Fig. 3). Spleen cells from mice that had been injected with SEA-pulsed wt DC produced SEA-specific IL-5 (Fig. 3), IL-4, and IL-13 (not shown), which were not seen with SEA-pulsed MHC class $II^{-/-}$ DC (Fig. 3). Similarly, spleen cells from mice that had been injected with *P. acnes*-pulsed wt DC produced *P. acnes*-specific IFN-γ, but this was not observed with *P. acnes*-pulsed MHC class $II^{-/-}$ DC. These data provide strong evidence that Ag is being presented in a physiological context by the injected DC, and not by resident cells that acquire Ag from these DC.

To examine the ability of DC to prime Th responses in vitro, DC cultured overnight in media, or with SEA, *P. acnes*, or LPS were cocultured with CD4 T cells isolated from naive C57BL/6 mice, at a ratio of 1 DC:5 CD4 T cells. One week later, cells were recovered from the cultures and stimulated with plate-bound anti-CD3, and secreted cytokines were measured. CD4 T cells cultured with immature DC made low levels of IL-4 (Fig. 4*A*). In contrast, SEA-pulsed DC primed CD4 cells to make more than twice the amount of IL-4 made by cells primed with immature DC, whereas CD4 T cells cultured with *P. acnes*- or LPS-pulsed DC made little or no IL-4. CD4 cells cocultured with all four populations of DC were able to make IFN-γ following stimulation (Fig. 4*B*).

Thus, DC exposed to SEA potently drive Th2 development both in vitro and in vivo. Since the only cells exposed directly to Ag in these experiments were DC, these data suggest that Ag need not interact with cells other than DC to be able to induce polarized Th responses, and that a major part of the decision to mount a Th1 or Th2 response rests with the DC.

Comparison of DC activation by Th2 or Th1 Ag

The majority of our understanding of DC activation processes has been gained from studies involving microbes or microbial products that typically induce Th1 responses (11, 17), but comparatively little is known about the effects of Th2-inducing pathogens on DC. To determine whether the response of DC to SEA, a potent Th2 inducer, differs from that to microbial products that induce Th1 responses, DC were examined after exposure to SEA, *P. acnes*,

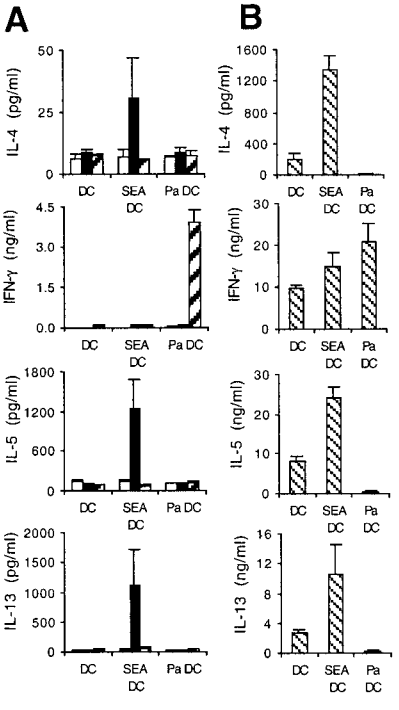

FIGURE 2. T cell-polarizing ability of differentially primed DC in vivo. Cytokine production as measured by ELISA of culture supernatants from spleen cells taken from C57BL/6 mice that had been injected i.p. with unstimulated DC, SEA-pulsed DC, or *P. acnes* (Pa)-pulsed DC 7 days previously. Splenocytes were then stimulated: *A*, with media (□), SEA (■), or *P. acnes* (▨), or *B*, with plate-bound anti-CD3 (▧) for 72 h before supernatant harvest. Data are means ± SD of triplicate wells from three mice per group and are representative from one of at least six experiments.

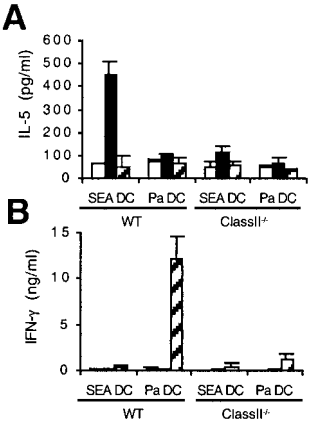

FIGURE 3. T cell-polarizing ability of differentially primed DC in vivo requires DC MHC class II. IL-5 or IFN-γ levels as measured by ELISA in culture supernatants from spleen cells taken from C57BL/6 mice that had been injected i.p. with wt or MHC class $II^{-/-}$, SEA-pulsed DC, or *P. acnes*-pulsed DC 7 days previously. Splenocytes were then stimulated with media (□), SEA (■), or *P. acnes* (Pa; ▨) for 72 h before supernatant harvest. Data are means ± SD of triplicate wells from four mice per group analyzed individually.

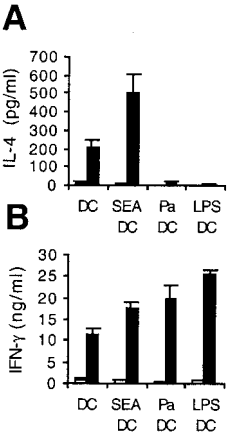

FIGURE 4. T cell-polarizing ability of differentially primed DC in vitro. IL-4 (*A*) and IFN-γ (*B*) production, as measured by ELISA in supernatants from CD4 T cells that had been cultured for 7 days with unstimulated DC, or SEA-, *P. acnes* (Pa)-, or LPS-pulsed DC, then stimulated for 72 h with media (□) or plate-bound anti-CD3 (■). Data are means ± SD of triplicate wells and are representative results from one of three experiments.

LPS, or media alone. Following overnight culture in Ag or media, cells were collected and analyzed by FACS for expression of surface proteins involved in Th cell activation (MHC class II, CD54, CD80, CD86), Th1 response polarization (CD40), and Th2 response polarization (OX40L, CD80, and CD86) (12, 13, 19, 40–42). Phenotypic analysis of differentially activated DC showed a marked elevation in expression of MHC class II, CD80, CD86, and CD40 (Fig. 5) (Table I), as well as CD54 (not shown), on DC stimulated with either *P. acnes* or LPS, but not on those exposed to SEA, which induced only a minor up-regulation of class II. Analysis of the proportion of DC from each culture that expressed OX40L showed a similar pattern, with *P. acnes* and LPS, but not SEA, inducing increased expression (Fig. 5).

Activation of DC in response to *P. acnes* and LPS was accompanied by increased levels of IL-12 (p40 and p35), TNF-α, IL-6, inducible NO synthase (iNOS), and IL-10, as measured by RNase protection assay (RPA) (Fig. 6). Reinforcing these data, elevated levels of IL-12 (p40 and p70), TNF-α, IL-6, NO, and IL-10 were measured by ELISA or Greiss reaction in culture supernatants

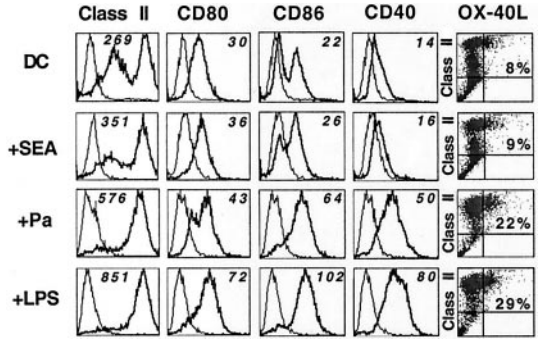

FIGURE 5. Phenotype of DC activated by Th1- or Th2-polarizing Ag. Cell surface expression of MHC class II, CD80, CD86, CD40, or OX40L on unstimulated DC, or DC that had been stimulated overnight with SEA, *P. acnes* (Pa), or LPS. Expression of the indicated markers is shown by the bold lined histograms, whereas cells stained with control Ab are indicated by the fine lined histograms. Values represent the mean fluorescence intensity of a given marker. For OX40L, control Ab staining is indicated by the quadrant boundaries, and values represent the percentage of cells staining positive for that marker. Representative data from one of at least eight experiments.

Table I. *DC activation by Th2 or Th1 Ag*[a]

	Class II^{low}	Class II^{high}	CD80	CD86	CD40
DC	51	45	69	51	27
+ SEA	31	57	65	49	17
+ Pa	20	74	75	86	82
+ LPS	15	82	89	92	93

[a] Values represent the percentage of unstimulated DC, or DC that had been stimulated overnight with SEA, *P. acnes*, (Pa), or LPS identified as staining positive for a given marker (CD80, CD86, and CD40) or the percentage of cells bearing Class II^{high} or Class II^{low}, as determined by FACS. Representative data from one of at least eight experiments are shown.

taken from DC that had been stimulated overnight with *P. acnes* or LPS (Fig. 7). Exposure to SEA did not measurably affect expression or secretion of any of these mediators (Figs. 6 and 7). For the RPA experiments, we also used whole schistosome eggs to stimulate DC, reasoning that these are most likely the relevant Th2 stimulus during infection. However, eggs had no more measurable effect on DC than did SEA. The RPA also revealed that DC were activated by *P. acnes* and LPS to make IL-15 and IL-18; again, neither SEA nor eggs had any effect on expression of these cytokines. We were unable to detect IFN-γ (43) or IL-13 (44) in any of the cultures by ELISA or RPA (not shown). Importantly, given a recent report suggesting that DC may produce IL-4 under certain stimuli (18), IL-4 was not detected under any conditions either by ELISA or RPA (not shown).

DC isolated from S. mansoni*-infected mice exhibit a phenotype analogous to that of DC exposed to SEA in vitro*

During infection with *S. mansoni*, DC in the lymphoid organs draining affected sites are presumably constantly exposed to parasite Ag. To determine whether DC isolated from an active Th2-dominated infection were phenotypically similar to in vitro generated DC exposed to Th2 Ag, we used magnetic microbeads conjugated to CD11c to purify DC from spleens of mice infected with *S. mansoni*. CD8$^-$ DC were then assessed by flow cytometry

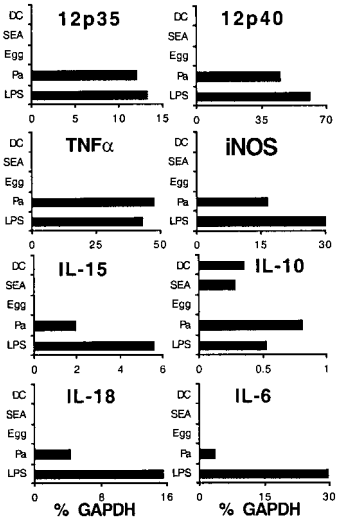

FIGURE 6. Cytokine and iNOS gene expression by DC in response to Th1- or Th2-polarizing Ag. Expression of IL-12p35, IL-12p40, IL-18, TNF-α, iNOS, IL-6, IL-15, and IL-10, by unstimulated DC, or DC that had been stimulated overnight with SEA, *S. mansoni* eggs, *P. acnes* (Pa), or LPS, as measured by RPA. Bars show quantification of message band intensity relative to expression of the housekeeping gene GAPDH for each sample. Representative data from one of four experiments.

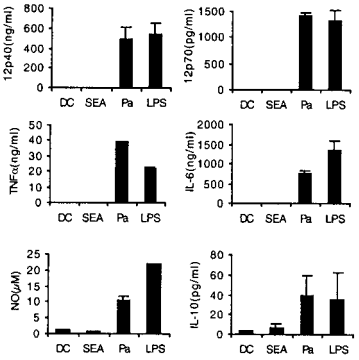

FIGURE 7. Cytokine and NO protein production by DC in response to Th1- or Th2-polarizing Ag. Cytokine and NO production, as measured by ELISA or Greiss reaction, in culture supernatants from DC that had been stimulated overnight with media, SEA, *P. acnes* (Pa), or LPS before supernatant harvest. Data are means ± SD of triplicate wells and are representative results from one of at least eight experiments.

for expression of MHC class II, CD86, and CD40 (Fig. 8). Consistent with the data obtained from SEA-primed, in vitro culture-derived DC, $CD8^-$ DC isolated from *S. mansoni* infection displayed minor up-regulation of MHC class II, but not CD86 or CD40, when compared with $CD8^-$ DC isolated from uninfected control mice.

DC exposed to SEA are not primed for IL-12 production in response to stimulation via CD40

DC exposed to microbial pathogens are armed for a second round of IL-12 production in response to stimulation through CD40 (12–14). The latter allows these cells to influence IFN-γ production in the lymphatics as well as at the site of infection. To assess whether SEA-pulsed DC were capable of producing IL-12 in response to CD40 ligation, DC were cultured overnight in media alone or with SEA, *P. acnes*, or LPS, then stimulated with an agonistic anti-CD40 mAb. Cells that had been exposed to *P. acnes* or LPS responded to CD40 ligation by making large amounts of IL-12 (Fig. 9), whereas immature DC and DC exposed to SEA did not make detectable levels of this cytokine after CD40 ligation (Fig. 9). Thus, the failure to up-regulate CD40 expression in response to SEA correlated with an inability of SEA-pulsed DC to make IL-12 via activation through this receptor.

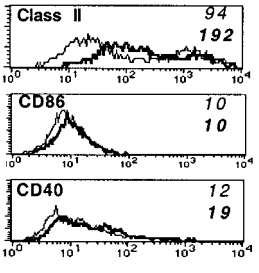

FIGURE 8. Phenotype of $CD8^-$ DC isolated from *S. mansoni* infection. Cell surface expression of MHC class II, CD86, and CD40 on $CD8^-$ DC isolated using CD11c-conjugated magnetic microbeads from the spleens of uninfected control C57BL/6 mice (fine lined histograms) or mice that had been infected for 8 wk with *S. mansoni* (bold lined histograms). Values represent the mean fluorescence intensity of a given marker for DC isolated from uninfected (fine) or infected (bold) mice. Representative data from one of three experiments.

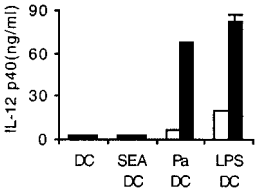

FIGURE 9. Production of IL-12 by differentially activated DC in response to CD40 ligation. IL-12p40 as measured by ELISA in supernatants from cultures of unstimulated, SEA-, *P. acnes* (Pa)-, or LPS-pulsed DC that had been incubated for 72 h with media (□) or agonistic anti-CD40 Ab (■). Data are means ± SD of triplicate wells and are representative results from one of at least three experiments.

Discussion

Our data demonstrate that following exposure to *S. mansoni* egg Ag in vitro, immature $CD8^-$ DC increase expression of MHC class II, but not of CD40, CD54, CD80, CD86, OX40L, or of TNF-α, IL-6, IL-10, IL-12, IL-15, IL-18, or NO. This contrasts markedly with DC exposed to *P. acnes* or LPS, which up-regulate expression of all of these molecules. These data would suggest that DC are largely nonresponsive to SEA. Nevertheless, this was clearly not the case, since DC pulsed with this Ag were capable stimulators of strong Th2 responses both in vivo and in vitro. Our finding that $CD8^-$ DC isolated from the spleens of infected mice display a phenotype that is similar to that of the in vitro SEA-pulsed $CD8^-$ DC supports the view that the observed phenotype is physiologically relevant.

Recently, it was shown that DC produce IL-4 in response to *Candida* hyphae (18). This finding raised the attractive possibility that, in general, Ag that induce Th2 responses do so because they promote DC to make IL-4. However, we were unable to detect IL-4 production by SEA-pulsed DC either at the protein or mRNA level, arguing strongly that this is not a general feature of DC activation by Th2 Ag. The available data suggest two possibilities to explain the ability of SEA-pulsed DC to prime Th2 responses. First, DC fail to undergo any major activation events in response to SEA, and in the absence of increased expression of IL-12, IL-18, CD40, CD80, or CD86, but in the presence of Ag, this is sufficient to drive the development of a Th2 response. In this scenario, Th2 response induction would be the default pathway when Ag presentation occurs in the absence of overt DC activation. A more attractive model is that DC actively promote Th2 responses as a result of undergoing an alternative activation process. Alternatively activated macrophages have been described recently (45, 46), and it is conceivable that analogous activation states exist in DC. We intend to further address these issues by more fully defining changes in gene expression in DC as a result of exposure to SEA.

In a recent report of the effect of another helminth Ag, *Acanthocheilonema viteae* ES-62, on DC function in vitro, it was shown that ES-62 plus OVA-pulsed DC primed naive DO.11.10 CD4 T cells to become Th2-like (47). This occurred in the absence of significantly increased MHC class II expression or costimulatory molecule up-regulation. While this study did not assess whether DC pulsed with ES-62 could induce an ES-62-specific Th2 response in vivo, we would predict on the basis of our data that this would be the case. Overall, the data support the view that SEA and possibly other Th2-inducing Ag alternatively activate DC in a way that allows sufficient MHC class II/peptide formation, in the absence of other changes indicative of maturation, to allow Th cell activation. The minor increase in expression of MHC class II that we have observed on DC following exposure to SEA is suggestive

of such a process, and indeed, low levels of MHC class II/peptide complexes may actually favor induction of Th2 responses (48). Access to a mAb that recognizes MHC class II/SEA peptide complexes would greatly facilitate a detailed analysis of this process, but unfortunately, such reagents are not currently available.

Naive CD4 T cells require costimulation to respond fully following activation through their TCR. Costimulation is provided primarily via molecules on the surface of APC, the most well characterized being CD80 and CD86, which bind CD28 on T cells (19, 40). Without costimulation, T cells enter an anergic stage in which they are minimally responsive to subsequent signals (49). Many reports have stressed the importance of appropriate costimulation for the development of Th2 responses, with CD80, CD86, and OX40L being implicated as playing major roles in Th2 response induction (41, 42). For example, expression of the OX40-binding partner for OX40L on Th cells is dependent on CD28 ligation by CD80/CD86, and activation of naive CD4 cells in vitro by targeted ligation of TCR, CD28, and OX40L promotes increased IL-4 production compared with that observed when OX40L is not involved (42). Thus, OX40L would appear to be an attractive candidate for involvement in SEA-driven Th2 responses. However, our analyses of DC phenotype following exposure to SEA have failed to reveal any obvious pattern of costimulatory molecule expression that could account for the ability of these cells to drive Th2 responses. We found no overt effect of SEA on DC CD54, CD80, CD86, or OX40L expression, although enhanced expression of all four costimulatory molecules was triggered by exposure to *P. acnes* or LPS. Since immature and SEA-pulsed DC express CD54, CD80, CD86, and OX40L at low but detectable levels, it is feasible that these cells deliver sufficient signal to drive T cell activation in the absence of CD40 signaling, a scenario that could support Th2 response development.

Our data suggest that up-regulation of CD40 on DC following exposure to *P. acnes* or LPS makes them receptive to a secondary round of stimulation and IL-12 production. This second round of stimulation would presumably occur in vivo via interaction with CD154 on T cells, in which the resulting IL-12 production might help to amplify the developing Th1 response (16). The inability of SEA-pulsed DC to make IL-12 following exposure to agonistic anti-CD40 mAb argues that these cells are unlikely to make this cytokine following interactions with Th cells; an absence of IL-12 at this stage would be expected to favor Th2 response development. Our finding that Th1 Ag induce DC IL-15 production is also interesting in this context, as IL-15 has been shown to act in synergy with IL-2 to up-regulate CD154 expression on T cells (50). Furthermore, ligation of DC CD40 has been shown to promote increased surface expression of CD80 and CD86, which subsequently are capable of ligating CD28 on T cells and providing the crucial costimulatory signal that can up-regulate CD154 expression on the T cell (17, 51).

Consistent with our data illustrating the ability of Th1-, but not Th2-polarizing Ag-pulsed DC to make IL-12 following ligation of CD40, interaction between CD40 on DC and CD154 on T cells has in certain circumstances been shown to be essential for Th1 response induction. For example, mouse strains that normally mount Th1 responses and are resistant to *Leishmania* parasites fail to mount Th1 responses and succumb to infection when CD40 signaling is blocked (52, 53), presumably due to a failure to make IL-12. In light of this, it is possible that Th2 response induction is favored in the absence of CD40 ligation (51). The finding that $CD8^-$ DC isolated from *S. mansoni*-infected mice do not show elevated expression of CD40, while the infection is characterized by a strong Th2 response, promotes the view that DC CD40 may be more important in Th1-dominated infections.

There are several mechanisms that may contribute to DC activation at sites of infection. Most prominently, many microbial pathogens appear capable of activating DC directly (11), as illustrated by the long-recognized response of DC to LPS (30–32). This is evident as increased expression of MHC class II, CD40, CD54, CD80, and CD86, reduced phagocytic ability and production of high levels of the inflammatory mediators TNF-α, IL-6, IL-12, and IL-18. Our data indicate that the Gram-positive bacterium *P. acnes* is also highly effective in this capacity. The ability of DC to respond to pathogens by secreting inflammatory mediators implies that this cell type plays an important role in initiating the local inflammatory response to invading Th1-inducing pathogens as well as in launching the appropriate adaptive immune response to these organisms.

We have shown that the nature of the activation stimulus influences the ability of DC to induce polarized T cell responses. The ability of microbial pathogens to induce DC activation, while simultaneously promoting IL-12 synthesis by these cells and arming them for a second round of IL-12 production in response to CD154, explains in part how Th1 responses are induced. It remains unclear how DC orchestrate Th2 responses, but the underlying mechanisms appear distinct from those induced by Th1 pathogens. Our data suggest that DC take up and present Th2 Ag without undergoing concurrent activation, or that these Ag promote an alternative activation process that is essential for the inductive phase of the Th2 response.

Acknowledgments

We thank Drs. Erik Denkers, Phillip Scott, David Artis, Anne La Flamme, and Elisabeth Patton and the students of the 2000 Biology of Parasitism course for helpful discussion, and Eric Denkers for critical review of this manuscript.

References

1. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
2. Reid, S. D., G. Penna, and L. Adorini. 2000. The control of T cell responses by dendritic cell subsets. *Curr. Opin. Immunol. 12:114.*
3. Abbas, A. K., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper T lymphocytes. *Nature 383:787.*
4. O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity 8:275.*
5. Bix, M., and R. M. Locksley. 1998. Independent and epigenetic regulation of the interleukin-4 alleles in $CD4^+$ T cells. *Science 281:1352.*
6. Bird, J. J., D. R. Brown, A. C. Mullen, N. H. Moskowitz, M. A. Mahowald, J. R. Sider, T. F. Gajewski, C. R. Wang, and S. L. Reiner. 1998. Helper T cell differentiation is controlled by the cell cycle. *Immunity 9:229.*
7. Reiner, S. L., and R. A. Seder. 1999. Dealing from the evolutionary pawnshop: how lymphocytes make decisions. *Immunity 11:1.*
8. Mosmann, T. R., and R. L. Coffman. 1989. Heterogeneity of cytokine secretion patterns and functions of helper T cells. *Adv. Immunol. 46:111.*
9. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive $CD4^+$ T cells. *J. Immunol. 154:5071.*
10. Gately, M. K., L. M. Renzetti, J. Magram, A. S. Stern, L. Adorini, U. Gubler, and D. H. Presky. 1998. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu. Rev. Immunol. 16:495.*
11. Reis e Sousa, C., A. Sher, and P. Kaye. 1999. The role of dendritic cells in the induction and regulation of immunity to microbial infection. *Curr. Opin. Immunol. 11:392.*
12. Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, C. Van Kooten, I. Durand, and J. Banchereau. 1994. Activation of human dendritic cells through CD40 cross-linking. *J. Exp. Med. 180:1263.*
13. 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.*
14. Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kampgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: up-regulation via MHC class II and CD40 molecules and down-regulation by IL-4 and IL-10. *J. Exp. Med. 184:741.*
15. Trinchieri, G. 1998. Interleukin-12: a cytokine at the interface of inflammation and immunity. *Adv. Immunol. 70:83.*

16. 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.*
17. 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.*
18. d'Ostiani, C. F., G. Del Sero, A. Bacci, C. Montagnoli, A. Spreca, A. Mencacci, P. Ricciardi-Castagnoli, and L. Romani. 2000. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*: implications for initiation of T helper cell immunity in vitro and in vivo. *J. Exp. Med. 191:1661.*
19. 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.*
20. Hosken, N. A., K. Shibuya, A. W. Heath, K. M. Murphy, and A. O'Garra. 1995. The effect of antigen dose on $CD4^+$ T helper cell phenotype development in a T cell receptor-$\alpha\beta$-transgenic model. *J. Exp. Med. 182:1579.*
21. Constant, S. L., and K. Bottomly. 1997. Induction of Th1 and Th2 $CD4^+$ T cell responses: the alternative approaches. *Annu. Rev. Immunol. 15:297.*
22. Iezzi, G., E. Scotet, D. Scheidegger, and A. Lanzavecchia. 1999. The interplay between the duration of TCR and cytokine signaling determines T cell polarization. *Eur. J. Immunol. 29:4092.*
23. 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.*
24. 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.*
25. Rissoan, M. C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. de Waal Malefyt, and Y. J. Liu. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. *Science 283:1183.*
26. 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.*
27. Pearce, E. J., P. Casper, 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.*
28. 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.*
29. Matsui, K., T. Yoshimoto, H. Tsutsui, Y. Hyodo, N. Hayashi, K. Hiroishi, N. Kawada, H. Okamura, K. Nakanishi, and K. Higashino. 1997. *Propionibacterium acnes* treatment diminishes $CD4^+$ $NK1.1^+$ T cells but induces type I T cells in the liver by induction of IL-12 and IL-18 production from Kupffer cells. *J. Immunol. 159:97.*
30. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: down-regulation by cytokines and bacterial products. *J. Exp. Med. 182:389.*
31. Winzler, C., P. Rovere, M. Rescigno, F. Granucci, G. Penna, L. Adorini, V. S. Zimmermann, J. Davoust, and P. Ricciardi-Castagnoli. 1997. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J. Exp. Med. 185:317.*
32. Verhasselt, V., C. Buelens, F. Willems, D. De Groote, N. Haeffner-Cavaillon, and M. Goldman. 1997. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells: evidence for a soluble CD14-dependent pathway. *J. Immunol. 158:2919.*
33. Boros, D. L., R. Tomford, and K. S. Warren. 1977. Induction of granulomatous and elicitation of cutaneous sensitivity by partially purified SEA of *Schistosoma mansoni*. *J. Immunol. 118:373.*
34. Dalton, J. P., S. R. Day, A. C. Drew, and P. J. Brindley. 1997. A method for the isolation of schistosome eggs and miracidia free of contaminating host tissues. *Parasitology 115:29.*
35. Dunne, D. W., and M. J. Doenhoff. 1983. *Schistosoma mansoni* egg antigens and hepatocyte damage in infected T cell-deprived mice. *Contrib. Microbiol. Immunol. 7:22–9.*
36. Boros, D. L., and K. S. Warren. 1970. Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. *J. Exp. Med. 132:488.*
37. Boros, D. L., and K. S. Warren. 1971. Specific granulomatous hypersensitivity elicited by bentonite particles coated with soluble antigens from schistosome eggs and tubercle bacilli. *Nature 229:200.*
38. Colley, D. G. 1972. *Schistosoma mansoni*: eosinophilia and the development of lymphocyte blastogenesis in response to soluble egg antigen in inbred mice. *Exp. Parasitol. 32:520.*
39. Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods 223: 77.*
40. Bluestone, J. A. 1995. New perspectives of CD28–B7-mediated T cell costimulation. *Immunity 2:555.*
41. Gause, W. C., M. J. Halvorson, P. Lu, R. Greenwald, P. Linsley, J. F. Urban, and F. D. Finkelman. 1997. The function of costimulatory molecules and the development of IL-4-producing T cells. *Immunol. Today 18:115.*
42. Lane, P. 2000. Role of OX40 signals in coordinating CD4 T cell selection, migration, and cytokine differentiation in T helper (Th)1 and Th2 cells. *J. Exp. Med. 191:201.*
43. Ohteki, T., T. Fukao, K. Suzue, C. Maki, M. Ito, M. Nakamura, and S. Koyasu. 1999. Interleukin 12-dependent interferon γ production by $CD8\alpha^+$ lymphoid dendritic cells. *J. Exp. Med. 189:1981.*
44. De Saint-Vis, B., I. Fugier-Vivier, C. Massacrier, C. Gaillard, B. Vanbervliet, S. Ait-Yahia, J. Banchereau, Y. J. Liu, S. Lebecque, and C. Caux. 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. *J. Immunol. 160:1666.*
45. Goerdt, S., and C. E. Orfanos. 1999. Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity 10:137.*
46. Mills, C. D., K. Kincaid, J. M. Alt, M. J. Heilman, and A. M. Hill. 2000. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J. Immunol. 164:6166.*
47. 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.*
48. Ruedl, C., M. F. Bachmann, and M. Kopf. 2000. The antigen dose determines T helper subset development by regulation of CD40 ligand. *Eur. J. Immunol. 30: 2056.*
49. Slavik, J. M., J. E. Hutchcroft, and B. E. Bierer. 1999. CD28/CTLA-4 and CD80/CD86 families: signaling and function. *Immunol. Res. 19:1.*
50. Skov, S., M. Bonyhadi, N. Odum, and J. A. Ledbetter. 2000. IL-2 and IL-15 regulate CD154 expression on activated CD4 T cells. *J. Immunol. 164:3500.*
51. Grewal, I. S., and R. A. Flavell. 1998. CD40 and CD154 in cell-mediated immunity. *Annu. Rev. Immunol. 16:111.*
52. Kamanaka, M., P. Yu, T. Yasui, K. Yoshida, T. Kawabe, T. Horii, T. Kishimoto, and H. Kikutani. 1996. Protective role of CD40 in *Leishmania major* infection at two distinct phases of cell-mediated immunity. *Immunity 4:275.*
53. Campbell, K. A., P. J. Ovendale, M. K. Kennedy, W. C. Fanslow, S. G. Reed, and C. R. Maliszewski. 1996. CD40 ligand is required for protective cell-mediated immunity to *Leishmania major*. *Immunity 4:283.*