

Parasite-Induced Th2 Polarization Is Associated with Down-Regulated Dendritic Cell Responsiveness to Th1 Stimuli and a Transient Delay in T Lymphocyte Cycling

Dragana Jankovic,¹ Marika C. Kullberg, Patricia Caspar, and Alan Sher

The nature of the signals that bias Th effector choice is still not completely understood. Using parasite extracts from pathogens known to induce polarized Th1 or Th2 responses and an *in vitro* experimental model for priming murine CD4⁺ cells, we demonstrated that splenic dendritic cells (DC), but not B cells, promote Th1/Th2 differentiation of naive CD4⁺ lymphocytes. Th polarization in this system was found not to depend on DC secretion of the polarizing cytokines IL-12/IL-4, but instead correlated with distinct states of DC activation induced by the different parasite preparations. As expected, conditioning of DC for Th1 development was associated with up-regulation of costimulatory molecules and enhanced chemokine production and required intact MyD88 signaling. In contrast, conditioning of DC for Th2 differentiation correlated with down-regulation of many of the same functions and was MyD88 independent. This dampened DC activation was accompanied in the cocultures by a reduction in the frequency of CD4⁺ lymphocytes exiting the first division of the cell cycle. When the latter was mimicked by drug-induced arrest of peptide-primed CD4⁺ cells after the S phase of the first cycle, a marked Th2 polarization was also observed. Together, these findings suggest that the emergence of IL-4-producing CD4⁺ lymphocytes results from a suppression in DC function leading to a temporary delay in initial T cell cycling. *The Journal of Immunology*, 2004, 173: 2419–2427.

The polarization of CD4⁺ T cell responses into distinct Th1 and Th2 phenotypes is a well-studied, but incompletely understood aspect of the immune response. Although Th cell differentiation is clearly influenced by a diverse set of immunological factors, it is currently thought that signals provided by appropriately stimulated APCs, and in particular dendritic cells (DC),² are a major determinant underlying this form of effector choice. Although TCR cross-linking and engagement of costimulatory molecules are necessary for activation of CD4⁺ lymphocytes, these two events do not appear in themselves to explain Th1/Th2 commitment. Evidence for a third signal responsible for Th differentiation came initially from studies demonstrating that certain infectious agents trigger highly polarized Th1- or Th2-type responses (1–3). In this work, pathogen-stimulated APC were shown to promote Th1 development by means of a mechanism requiring IL-12, which by interacting with IL-12R activates STAT4 signaling in the responding CD4 cells (4–8). DC were shown to be a major source of the IL-12 driving Th1 polarization (4, 9). Nevertheless, recent studies have argued for the existence of additional, IL-12-independent pathways for Th1 effector choice (10–14).

Although the three-signal hypothesis predicts the existence of an alternative stimulus analogous to IL-12 for Th2 differentiation,

the nature of the APC-derived signal involved has been difficult to define. While IL-4 clearly promotes Th2 development by an IL-4R/STAT6-dependent mechanism (15–17), there is considerable debate as to whether pathogen-primed APC produce the cytokine. Moreover, evidence from multiple studies indicates that limited, but highly significant Th2 polarization can occur in the absence of IL-4R/STAT6 signaling (18–21). Based on the evidence that IL-12-induced IFN- γ prevents the development of Th2 cells and that IL-12 is minimally produced in response to Th2 stimuli, a default model was proposed to explain Th2 development (22). According to this hypothesis, Th1 or Th2 effector choice is determined by the presence or the absence of only a third signal, that required for Th1 priming, with IL-12 being the major candidate.

Although the default model successfully explains several key aspects of Th2 differentiation, it fails to account for a number of additional features of this process. First, there is now strong evidence that DC primed with Th2 stimuli (e.g., schistosome egg Ag (SEA)) can promote Th2 development both *in vitro* and *in vivo* (23–26). Additionally, recent studies conclusively demonstrate that in the absence of the Th1-polarizing signal IL-12, no default to a Th2 response occurs following stimulation with Th1-inducing pathogens (12–14, 27, 28). Interestingly, however, mice deficient in the TLR adapter molecule MyD88 do revert to a Th2 response after repeated exposure to Th1 microbial stimuli (14), as do T cells stimulated *in vitro* with LPS-primed MyD88-deficient DC (29), suggesting a different default pathway distinct from that postulated to involve IL-12.

As already introduced above, a likely source of the putative third signal involved in Th effector choice is the Ag-primed DC. One hypothesis, which has been considered heavily in the past, is that activation of different subsets of DC leads to differential Th1/Th2 commitment (30–32). Nevertheless, more recent data argue that a requirement for different subsets is not absolute and that their influence can be overridden by appropriate external signals that are

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Received for publication January 26, 2004. Accepted for publication June 4, 2004.

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 Address correspondence and reprint requests to Dr. Dragana Jankovic, Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 50, Room 6142, 50 South Drive, MSC 8003, Bethesda, MD 20892-8003. E-mail address: djankovic@niaid.nih.gov

² Abbreviations used in this paper: DC, dendritic cell; KO, knockout; SEA, soluble *S. mansoni* egg Ag; STAg, soluble *T. gondii* tachyzoite extract; Tg, transgenic; WT, wild type.

not subset specific (33–35). This process whereby microbial priming leads to DC with different Th1/Th2 polarizing potential has been termed conditioning.

To more directly define the mechanisms by which pathogen-conditioned DC prime Th1 vs Th2 responses and in particular to distinguish between the three signal (36) and default models of Th polarization (22), we used an in vitro system using highly purified CD11c⁺ DC as APC and naive transgenic (Tg) CD4⁺ lymphocytes specific for an unrelated Ag (OVA) as the responding cell population. Although similar to the in vitro model using human cells described by de Jong et al. (23), this protocol differs in the use of in vivo derived splenic DC and a monoclonal T cell readout. Using this system, we demonstrate that Th2 conditioning by a helminth extract manifests as an actual suppression of DC function, an effect revealed in cultures simultaneously exposed to a Th1 parasite stimuli. Moreover, we demonstrate that this down-regulation is associated with a subsequent delay in CD4⁺ T cell cycling and the emergence of IL-4-producing cells. Together, these results suggest a model in which Th1/Th2 induction by pathogens depends on stimulatory vs inhibitory signals delivered to DC, which in turn regulate the cytokine secretion capacity of newly primed T lymphocytes through cell cycle regulation.

Materials and Methods

Animals

C57BL/6 and BALB/c wild-type (WT) mice were obtained from the Division of Cancer Treatment, National Cancer Institute (Frederick, MD). BALB/c IL-4^{-/-} (37), BALB/c DO.11.10 TCR Tg animals on the RAG-2^{-/-} background (38), and MyD88^{-/-} mice (39) backcrossed for four to five generations onto the C57BL/6 background were maintained at an American Association of Laboratory Animal Care accredited facility at the National Institute of Allergy and Infectious Diseases (Bethesda, MD). OT-II TCR Tg C57BL/6 animals (40) and IL-12^{-/-} mice (41) backcrossed for five generations onto the BALB/c background were provided by the National Institute of Allergy and Infectious Diseases Animal Supply Contract at Taconic Farms (Germantown, NY).

Female mice were used for the purification of splenic DC and B cells, while either males or females were used as Tg CD4⁺ cell donors. Eight- to 12-wk-old animals were used in all experiments.

Parasite extract preparations

Soluble tachyzoite Ag (STAg) was prepared from sonicated *Toxoplasma gondii* tachyzoites, as described (42). SEA was obtained from eggs of *Schistosoma mansoni*, according to a standard procedure (43).

Cell preparation and flow cytometric sorting

Spleen cell suspensions were prepared after Liberase (Roche, Indianapolis, IN) digestion and lysis of RBC by osmotic treatment. DC and B cells were purified as single-stained populations from total splenocytes stained with PE anti-CD11c and FITC anti-B220 mAb (BD Pharmingen, San Diego, CA) using a FACStar sorter (BD Biosystems, San Jose, CA). The purity of CD11c⁺ DC and B220⁺ B cells was 97.9 ± 1.8% and >99%, respectively.

DO.11.10 TCR Tg CD4⁺ lymphocytes were positively sorted (>99% purity) from spleen cell suspensions after staining with Cy anti-CD4, FITC anti-CD11c, and FITC anti-DX5 mAb (BD Pharmingen). Similar results were obtained when DO.11.10 T cells were sorted using clonotypic KJ1.26 mAb (data not shown). OT-II TCR Tg CD4⁺ lymphocytes were purified (>99%) by cell sorting as a CD4⁺CD62L^{high} cell population from total splenocytes stained with Cy anti-CD4 and FITC anti-CD62L (BD Pharmingen).

Cell culture conditions

CD4⁺ lymphocytes (5 × 10⁵/ml) were incubated with DC or B cells (2.5 × 10⁵/ml) in RPMI 1640 complete medium (19) in the presence or absence of 1 μM OVA_{323–339} in 0.2 ml vol in round-bottom 96-well plates. When indicated, rIL-12 (10 ng/ml), rIL-4 (10 ng/ml), STAg (5 μg/ml), SEA (20 μg/ml), or STAg plus SEA (at the same concentrations as when tested individually) was added to the wells. After 72-h culture, supernatants were replaced with fresh medium containing 10 U/ml rIL-2, and after an additional 2–3 days, intracellular cytokine staining was performed as described below. In some experiments, DC were preincubated in medium alone or

with SEA (50 μg/ml) before addition to wells containing CD4⁺ cells and peptide with or without STAg or SEA. Progression of the cell cycle of DO.11.10 Tg cells was inhibited by addition of 200 nM paclitaxel (Valeant Pharmaceuticals, Costa Mesa, CA) (44).

In some experiments, DO.11.10 Tg CD4⁺ cells (1 × 10⁶/ml) were stimulated with plate-bound anti-CD3 mAb (10 μg/ml) (clone 145-2C11; BD Pharmingen) in the presence of soluble anti-CD28 mAb (1 μg/ml) (clone 37.51; BD Pharmingen). On day 3 culture supernatants were collected and CD4⁺ lymphocytes were transferred to new wells with fresh medium containing IL-2 (10 U/ml) and cultured for an additional 2–3 days before intracellular cytokine staining.

Cell labeling

FACS-purified DO.11.10 Tg CD4⁺ lymphocytes (1 × 10⁷ cells/ml) were incubated in serum-free RPMI 1640 medium containing CFSE (2.5 μM; Molecular Probes, Eugene, OR) for 8 min in the dark at room temperature, followed by incubation for 1 min with 2 ml of FCS. Cells were then washed three times with complete medium, counted, and plated (10⁵ cells/well) together with DC or B cells.

Measurement of cell surface marker expression

For the expression of surface markers and chemokine secretion, DC (10⁵ cells/well) were cultured under the same conditions as described above in the presence or absence of STAg and/or SEA, but without CD4⁺ lymphocytes and OVA peptide. After 18-h culture, supernatants were collected, and DC were incubated with anti-CD16/32 mAb and then stained with PE anti-CD11c and FITC anti-CD40, FITC anti-CD80, or FITC anti-CD86 (BD Pharmingen) mAb for 15 min at 4°C. Flow cytometric analysis was performed using a FACScan (BD Biosystems).

Intracellular cytokine staining

Analysis of intracellular cytokine expression was performed, as described (19). Briefly, on day 3 of culture (before adding IL-2) or on day 5–6 (after expansion in IL-2), cells were stimulated with PMA (10 ng/ml; Sigma-Aldrich, St. Louis, MO) and ionomycin (1 μg/ml; Sigma-Aldrich) for 4.5 h with addition of brefeldin A (10 μg/ml; Sigma-Aldrich) during the last 2 h. Intracellular staining was then conducted, as described previously (19), and cell fluorescence was measured using a FACScan (BD Biosystems). Data were analyzed using CellQuest software.

Cytokine and chemokine ELISA

Production of IL-4 was assayed in 72-h supernatants from cultures containing CD4⁺ cells, peptide, and DC with or without parasite extracts using an ELISA kit from Pierce (Woburn, MA). MIP-1α and MIP-1β secreted by STAg- and/or SEA-conditioned DC were measured in 18-h supernatants by ELISA (R&D Systems, Minneapolis, MN). The limits of detection were: IL-4 = 62 pg/ml, MIP-1α = 4.7 pg/ml, and MIP-1β = 7.8 pg/ml. The statistical significance of differences in amounts of cytokines secreted was evaluated using Student's two-tailed *t* test.

Results

Exposure of DC to parasite extracts results in induction of polarized Th responses to an unrelated Ag

During *T. gondii* or *S. mansoni* infection (45, 46) or after priming with corresponding soluble extract (STAg or SEA) in the absence of any adjuvant (14), parasite-specific CD4⁺ T cells display highly polarized Th1 or Th2 cytokine profiles, respectively. To determine whether STAg and SEA are able to promote the development of Th1 and Th2 cells independently of direct TCR triggering, we used an in vitro model system in which FACS-sorted splenic CD11c⁺ B220⁺ DC are cocultured with naive CD4⁺ T cells from TCR Tg DO.11.10 × RAG2 knockout (KO) mice together with an optimal concentration of the nominal OVA_{323–339} peptide. In the absence of conditioning by parasite extracts, DO.11.10 T cells proliferate extensively, but only a fraction (10–20%) of them acquires the capacity to produce lymphokines as measured by intracellular cytokine staining (Fig. 1). Addition of STAg or SEA to these cultures triggers the development of either IFN-γ- or IL-4-producing DO.11.10 CD4⁺ cells, respectively, while at the same time inhibiting the generation of the opposing subset. In the case of SEA, the observed parasite extract-induced polarization was equivalent to

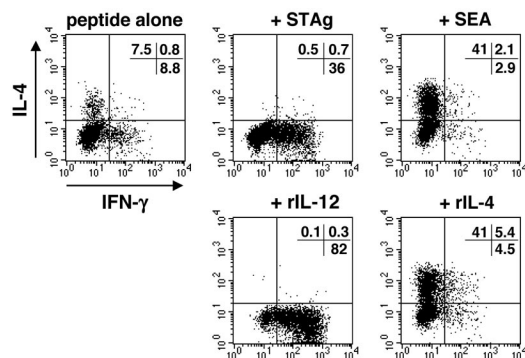


FIGURE 1. Parasite extracts promote Th polarization of DO.11.10 Tg CD4⁺ T cells. Naive DO.11.10 Tg CD4⁺ lymphocytes were cultured with FACS-purified splenic CD11c⁺ B220[−] DC and OVA_{323–329} peptide in the presence or absence of STAg, SEA, rIL-12, or rIL-4 for 3 days. Culture supernatants were next removed, and the cells were expanded in IL-2-containing medium for an additional 3-day period. Each culture was then stimulated with PMA/ionomycin in the presence of brefeldin A, followed by three-color staining for CD4, IL-4, and IFN-γ. The FACS dot plots shown are gated on CD4⁺ cells and are representative of >15 experiments performed.

that resulting from addition of the Th2-differentiative cytokine IL-4. In the presence of STAg, the degree of polarization was somewhat lower than that induced by the corresponding Th1-differentiative cytokine IL-12. STAg and SEA maintained their Th1- vs Th2-polarizing effects over a wide (100-fold) range of doses tested (data not shown).

Th polarization by parasite extracts depends on the presence of conditioned DC

Although we used OVA-specific DO.11.10 cells as the readout in our assay, it was still possible that the polarizing influence of STAg and SEA results from an Ag-nonspecific effect on CD4⁺ T cells rather than on DC. To test this hypothesis, we analyzed the phenotype of DO.11.10 cells stimulated with anti-CD3 plus anti-CD28 mAb in the presence of STAg or SEA, but in the absence of APC. As shown in Fig. 2A, addition of either parasite extract failed to alter the Th1 phenotype observed when CD4⁺ cells are activated by this APC-independent mechanism.

To address whether DC are specifically required for both Th1 and Th2 parasite-induced polarization, we compared the ability of highly purified DC and B cells to mediate these effects. In this experiment, we used CFSE-labeled DO.11.10 cells to simultaneously assess induction of T cell proliferation when peptide is presented by either APC population. Both DC and B cells stimulated comparable proliferative responses visualized by CFSE dilution on day 3 of culture, and the response observed was not altered by the addition of either parasite extract (Fig. 2B). In contrast, there was a major difference in the capacity of DC and B cells to promote Th1 vs Th2 differentiation, with DC, but not B cells mediating polarization (Fig. 2B). Interestingly, in this experiment, IFN-γ- or IL-4-producing cells were evident as early as the third division following stimulation.

The contribution of DC to Th2 polarization was further dissected in experiments in which DC preincubated in medium alone or with SEA were tested for their ability to induce Th2 polarization in subsequent DO.11.10 CD4 cell cultures containing peptide only. As shown in Fig. 3A, DC exposed to SEA for 10, but not 5, h successfully triggered Th2 commitment. DC preincubated with STAg were likewise able to promote Th1 polarization, although in

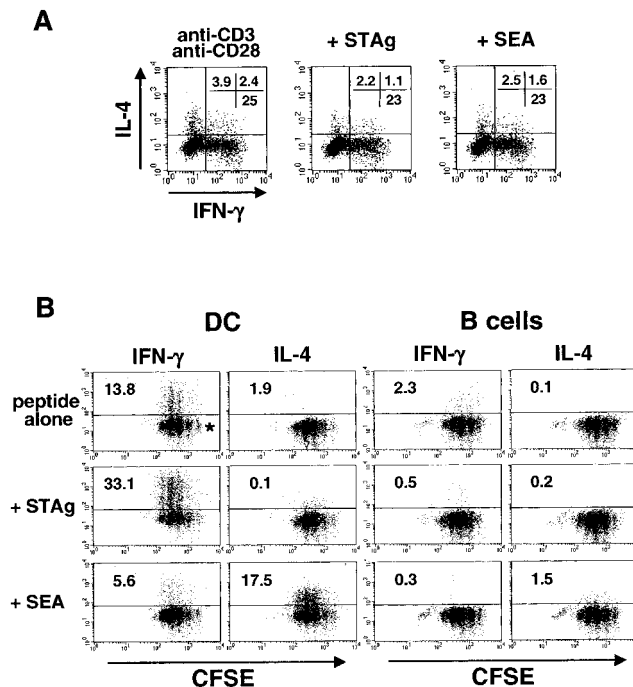


FIGURE 2. The Th-polarizing effects of STAg and SEA depend on the presence of DC, but not B cells. *A*, Naive DO.11.10 Tg CD4⁺ cells were stimulated by plate-bound anti-CD3 and soluble anti-CD28 mAb with or without STAg or SEA. On day 3, culture supernatants were removed and the lymphocytes were transferred to uncoated wells and cultured in IL-2-containing medium for an additional 3 days, followed by PMA/ionomycin stimulation and staining for CD4, IL-4, and IFN-γ. The FACS dot plots shown are gated on CD4⁺ cells. Results are representative of three separate experiments. *B*, CFSE-labeled DO.11.10 cells were cultured with FACS-purified splenic CD11c⁺ B220[−] DC or CD11c[−] B220⁺ B cells and OVA_{323–329} peptide in the presence or absence of STAg or SEA. On day 3, culture supernatants were removed and cells were immediately stimulated with PMA/ionomycin in the presence of brefeldin A, followed by staining for CD4 and IFN-γ or IL-4. The FACS dot plots shown are gated on CD4⁺ cells and show the extent of proliferation of DO.11.10 cells as well as the frequency of IFN-γ⁺ or IL-4⁺ cells for each culture condition. The CFSE intensity of cells cultured in medium alone, corresponding to undivided cells, is indicated by the asterisk in the top left dot plot. The findings shown were confirmed in a second repeat experiment.

that case 5 h were sufficient to achieve maximal conditioning (data not shown).

Th2 polarization by SEA-conditioned DC is not due to DC exhaustion

To exclude the possibility that the Th2-inducing capacity of SEA-pretreated DC is the result of the development of a nonresponsive state (DC exhaustion (47)), we tested the capacity of such DC to induce Th1 polarization following subsequent exposure to STAg. Importantly, in the presence of STAg, SEA-preconditioned DC induced only a slightly lower frequency of IFN-γ⁺ CD4⁺ T cells compared with control DC preincubated in medium alone (Fig. 3B). The above results indicate that the DC that polarize toward Th2 in this system remain functional in terms of their ability to respond to Th1-inducing stimuli, and therefore are not exhausted. In the same type of experiments, addition of SEA was able to reverse the Th1-polarizing effect of STAg-preconditioned DC (data not shown). The competence of DC to respond adequately to opposing stimuli in a sequential order might be an important feature of DC that provides the basis for their plasticity.

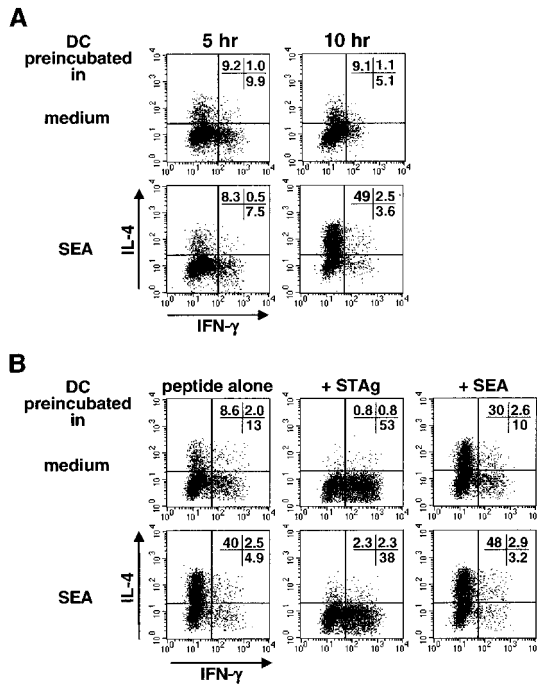


FIGURE 3. DC preincubated with SEA promote Th2 polarization. DO.11.10 Tg CD4⁺ cells were stimulated with OVA₃₂₃₋₃₂₉ peptide presented on DC preincubated in medium alone or with SEA for 5 or 10 h (A) or for 12 h (B) added alone (A) or in the presence or absence of STAg or SEA (B). On day 3, culture supernatants were removed and the cells were expanded in IL-2-containing medium for an additional 3 days, followed by staining for CD4, IL-4, and IFN- γ . The FACS dot plots shown are gated on CD4⁺ cells. Similar results were obtained in a repeat experiment.

Polarization does not depend on DC production of either IL-4 or IL-12

To determine whether the opposing effects of SEA/STAg are the result of their ability to selectively induce secretion of the polarizing cytokines IL-12/IL-4 by DC, we compared the Th phenotype of DO.11.10 CD4⁺ cells activated by WT DC and DC from IL-4 KO or IL-12 KO animals conditioned by each parasite extract. As shown in Fig. 4, IL-4-deficient DC exposed to SEA were unimpaired in their ability to drive Th2 polarization measured by either intracellular cytokine staining (Fig. 4A) or the level of IL-4 found in the culture supernatants (Fig. 4B). Similarly, STAg-primed DC from IL-12 KO mice effectively induced Th1 differentiation, although the frequency of IFN- γ ⁺ cells was decreased relative to cultures stimulated with WT DC (Fig. 4C). In each case, the absence of IL-4/IL-12 failed to result in a compensatory increase in CD4⁺ cells with the opposing phenotype (i.e., IFN- γ or IL-4, respectively).

Essential role for MyD88 in Th1, but not Th2 priming by DC

We have recently shown that MyD88 expression is required for the *in vivo* development of STAg-specific Th1 responses following multiple priming with this parasite extract, while generation of SEA-specific Th2 responses occurs independently of MyD88 signaling (14). To analyze the influence of MyD88 on parasite extract-induced conditioning of DC for Th1/Th2 responses, we compared the ability of sort-purified DC from WT and MyD88-deficient mice to prime OVA-specific CD4⁺ lymphocytes for *in vitro* IFN- γ and IL-4 production in the presence of either STAg or SEA.

Because the MyD88 KO mice are on an H-2^b background, we used OT-II Tg CD4⁺ T cells that recognize the same OVA peptide

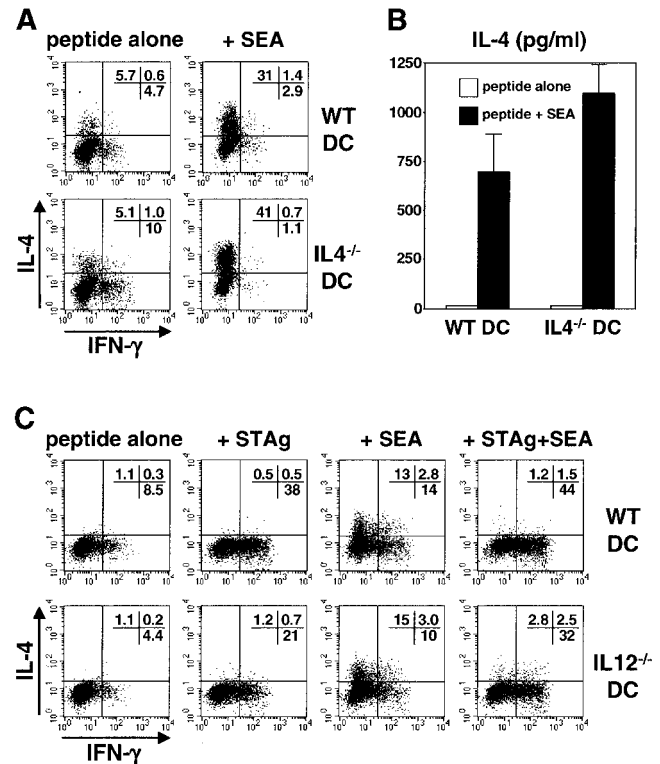


FIGURE 4. Th polarization by SEA and STAg does not require DC production of IL-4 and IL-12, respectively. Parallel cultures of DO.11.10 CD4⁺ lymphocytes were performed with peptide and DC isolated either from WT and IL-4 KO (A) or WT and IL-12 KO (C) animals. The parasite extracts added as polarization stimuli are indicated. Intracellular cytokine staining was performed as in Fig. 1. The FACS dot plots shown are gated on CD4⁺ cells and are representative of three experiments performed for each set of DC. B, IL-4 was measured in supernatants collected at 72 h from cultures containing CD4⁺ cells, peptide, and WT or IL-4 KO DC shown in A. The bars represent the mean value $\pm$ SD.

as DO.11.10 Tg T cells, but in the context of I-A^b (40). In the presence of WT DC, addition of STAg or SEA promoted the development of IFN- γ - or IL-4-producing OT-II CD4⁺ lymphocyte, respectively (Fig. 5A), confirming that polarization by these extracts is not qualitatively dependent on MHC. Nevertheless, the extent of Th2 polarization by SEA was lower in the B6 vs BALB/c background setting (compare the frequency of IL-4⁺ cells in Figs. 1 and 5A).

When MyD88 KO DC were substituted for WT DC, the Th2-polarizing activity of SEA was unaltered over a wide range of SEA concentrations (Fig. 5, A and B), while the ability of STAg to promote the differentiation of IFN- γ ⁺ OT-II T cells was completely lost (Fig. 5A). Unexpectedly, in the latter cultures, despite the presence of a lymphoproliferative response, there was no increase in the frequency of IL-4-producing cells, as previously observed in *in vivo* experiments involving Th1 stimulation (14). Together, these findings indicate that MyD88 is critical for STAg-induced Th1 polarization by DC, but fails to regulate Th2 priming stimulated by SEA-exposed DC.

Recent studies have suggested a role for TLR4 in the induction of Th2 differentiation by lacto-N-fucopentaose III carbohydrates present in SEA (48). Because this TLR can signal through alternative pathways not involving MyD88, it remained possible that the MyD88-independent Th2 induction by SEA-primed DC results from TLR4 triggering. Nevertheless, when tested in the *in vitro* assay used in the analysis of MyD88 function, SEA-treated TLR4

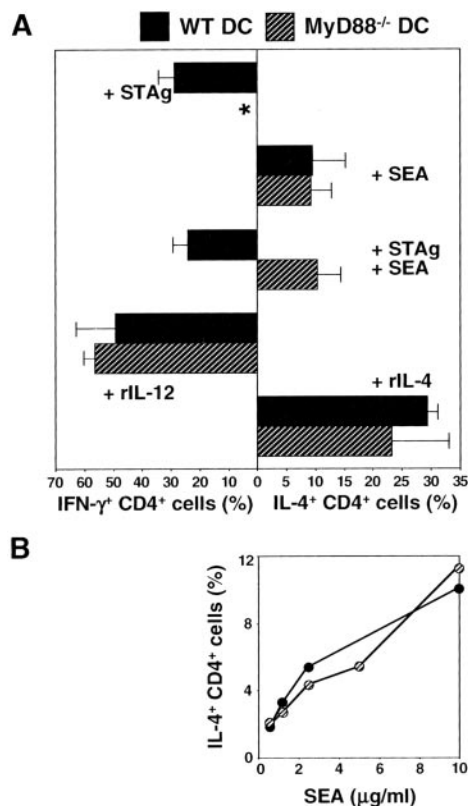


FIGURE 5. Differential requirement for MyD88 in STAg- vs SEA-induced Th polarization. **A**, OT-II Tg CD4⁺ lymphocytes were cultured with FACS-purified splenic CD11c⁺ B220⁻ DC from WT or MyD88 KO mice and OVA_{323–329} peptide in the presence or absence of STAg (5 μg/ml), SEA (20 μg/ml), rIL-12, or rIL-4 for 3 days, followed by 3-day expansion in IL-2-containing medium. Cells were then stimulated with PMA/ionomycin in the presence of brefeldin A, and three-color staining for CD4, IL-4, and IFN- γ was performed. The graph shows the percentage of IL-4⁺ and IFN- γ ⁺ CD4⁺ cells above the background levels observed in cultures with peptide alone. The bars represent the mean value $\pm$ SD of data obtained from two experiments performed. **B**, OT-II Tg CD4⁺ lymphocytes were cultured with splenic DC from WT or MyD88 KO mice, as described in **A**, but with varying concentrations of SEA. The graph shows the percentage of IL-4⁺ CD4⁺ cells above the background levels observed in cultures with peptide alone.

KO DC induced significant priming of IL-4⁺ OT-II cells (8 vs 1% with peptide alone) comparable to that observed with WT DC. Moreover, in parallel experiments, TLR4-deficient and WT mice developed equivalent splenic Th2 cytokine responses (IL-4, IL-5, IL-10, IL-13) following *in vivo* priming with SEA (data not shown).

Effect of simultaneous exposure to STAg and SEA on Th development vs DC function

To better understand the opposing effects of STAg- and SEA-conditioned DC on Th development, we analyzed the phenotype of DO.11.10 Tg CD4⁺ cells in cultures in which both parasite extracts were added simultaneously. Under these conditions, only IFN- γ -producing cells were generated (Fig. 4C). A dominant effect of STAg vs SEA was observed when either WT or IL-12-deficient DC were used to present OVA peptide, consistent with the finding that polarization by STAg alone does not require IL-12. As expected, when WT DC were replaced with MyD88 KO DC, the Th1 skewing induced by STAg plus SEA was completely abolished, while the frequency of IL-4⁺ cells was similar to that induced by MyD88 KO DC conditioned with SEA alone (Fig. 5A).

To understand why in the case of WT DC the Th2-promoting effect of SEA is lost in the presence of STAg, we next examined the influence of the two extracts on DC function. FACS-sorted WT CD11c⁺ splenic DC were cultured in medium, STAg, SEA, or STAg plus SEA, and the level of costimulatory molecule expression was analyzed. DC cultured in medium alone displayed a significant level of CD40, CD80, and CD86 (Fig. 6A). In the presence of STAg, this basal expression was increased, as assessed by mean fluorescence intensity for each marker. In contrast, addition of SEA clearly failed to activate DC, as none of the markers were up-regulated.

Unexpectedly, DC cultured in the presence of STAg plus SEA had an intermediate phenotype. Expression of all three surface markers was elevated when compared with cells cultured in the presence of SEA alone, while the induction of CD40 and CD86 was decreased with respect to cultures exposed to STAg alone. Interestingly, a decrease in the expression of the same two markers was also evident when DC cultured in the presence of SEA alone were compared with parallel cultures containing medium only (Fig. 6A). A marked down-regulatory effect of SEA addition was also evident when MIP-1 β , a chemokine previously shown to be

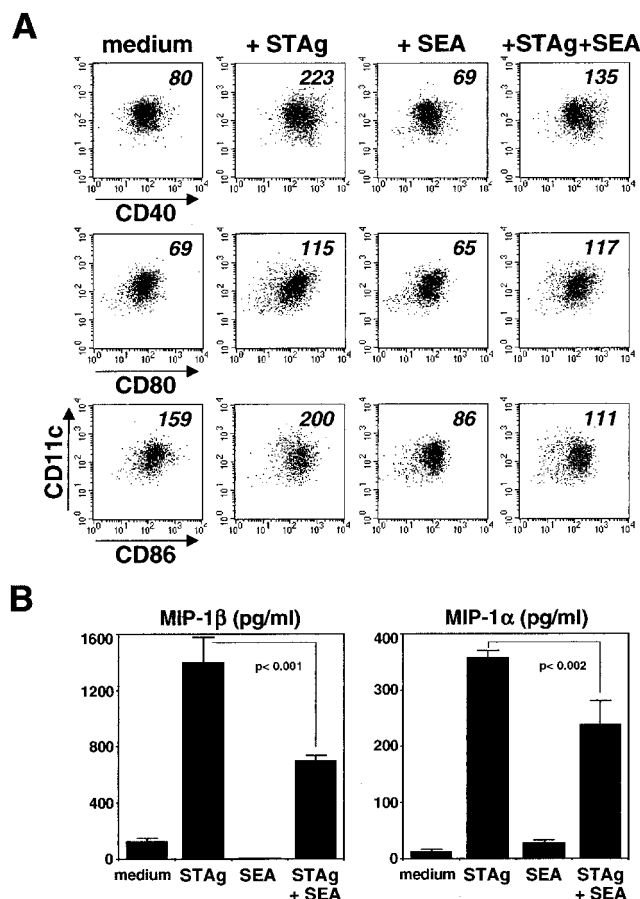


FIGURE 6. Suppression of STAg-induced DC activation by SEA. The expression of costimulatory molecules was analyzed on CD11c⁺ B220⁻ DC exposed for 18 h to medium, STAg, SEA, or STAg plus SEA. **A**, The FACS dot plots shown are gated on live cells and represent DC stained for CD11c and CD40 (top row), CD80 (middle row), or CD86 (bottom row). The numbers in the top right corner indicate the geometric mean fluorescence intensity for the costimulatory molecule analyzed. **B**, Supernatants from the DC cultures with or without STAg, SEA, or STAg plus SEA collected after 18 h were analyzed for the presence of MIP- α and MIP- β . The bars represent the mean value $\pm$ SD. The data shown are representative of three independent experiments performed.

secreted by STAg-stimulated DC (49), and MIP-1 α were measured in supernatants from the same cultures (Fig. 6B). A similar degree of suppression of MIP-1 β expression was observed from CD11c⁺ DC preincubated with anti-CD40 mAb and when isolated CD8 α ⁺ or CD8 α ⁻ DC subpopulations were tested in this assay (data not shown). SEA addition reduced rather than increased the expression of the apoptotic marker CD95 on STAg-exposed DC, and it had no effect on STAg-induced IL-12 (data not shown), arguing that the observed suppression by the helminth extract is not the result of increased cell death or selective loss of CD8 α ⁺ DC, respectively. Of note, despite their down-regulated state of activation, DC isolated from WT or IL-12 KO mice and conditioned with STAg in the presence of SEA display the ability to polarize CD4⁺ cells toward Th1 phenotype (Fig. 4C).

SEA-conditioned DC induce a transient block in the first cell cycle of Ag-driven T cell proliferation

The observation that SEA down-regulates certain DC functions was unexpected because the degree of CD4⁺ T cell stimulation, measured by proliferation on day 3, was similar in cultures containing peptide with or without SEA or STAg (Fig. 2). We therefore decided to assess the effects of SEA-conditioned DC on cell division at earlier time points. As shown in Fig. 7A, when assayed at 36 h following the stimulation with peptide alone, ~17% of DO.11.10 Tg T cells were found within the gate corresponding to the population that has undergone one cell division compared with only ~2% in cultures without peptide. Although STAg addition resulted in a marginal increase in this percentage, addition of SEA caused a 73% inhibition in the number of cells exiting the first cycle compared with cultures stimulated with peptide alone. Interestingly, initial cell activation as measured by size increase (Fig. 7A) and [³H]thymidine incorporation reflecting the number of cells in S phase (data not shown) was comparable in cultures stimulated with peptide in the presence or absence of SEA.

To explore the possibility that the observed delay in initial cell cycling may directly promote Th2 development, we tested the effect of paclitaxel, a known inhibitor of metaphase/anaphase transition, on the phenotype of CD4⁺ T cells emerging in cultures containing DC and peptide alone. When added simultaneously with the peptide, paclitaxel dramatically augmented the frequency of IL-4-producing Tg CD4⁺ cells while concomitantly reducing the number of IFN- γ ⁺ cells when compared with parallel untreated cultures (Fig. 7, B and C). Importantly, in cultures stimulated with STAg, no significant influence of the drug on the frequency of Th1 cells was observed (Fig. 7C), arguing against the possibility that paclitaxel has nonspecific effects on cell survival. Thus, consistent with the dominant effect of STAg vs SEA on CD4⁺ polarization (in the absence of the drug treatment; Fig. 4C), STAg-conditioned DC are able to override the Th2-priming effect provided by paclitaxel.

When T cells cultured with DC and peptide alone were arrested in the G₂/M phase of the first cell cycle by addition of nocodazole, an inhibitor of metaphase initiation, a similar increase in the frequency of Th2 cells was observed (data not shown). In contrast, addition of L-mimosine, which arrests cells in the late G₁ phase, failed to promote Th2 development even when the drug was removed after 48 h, allowing the DO.11.10 T cells to resume proliferation (data not shown). Together, our results argue that IL-4-producing CD4⁺ cells selectively emerge from precursors whose progression through the first cell cycle is impeded after the S phase in the presence of DC that have not been Th1 conditioned.

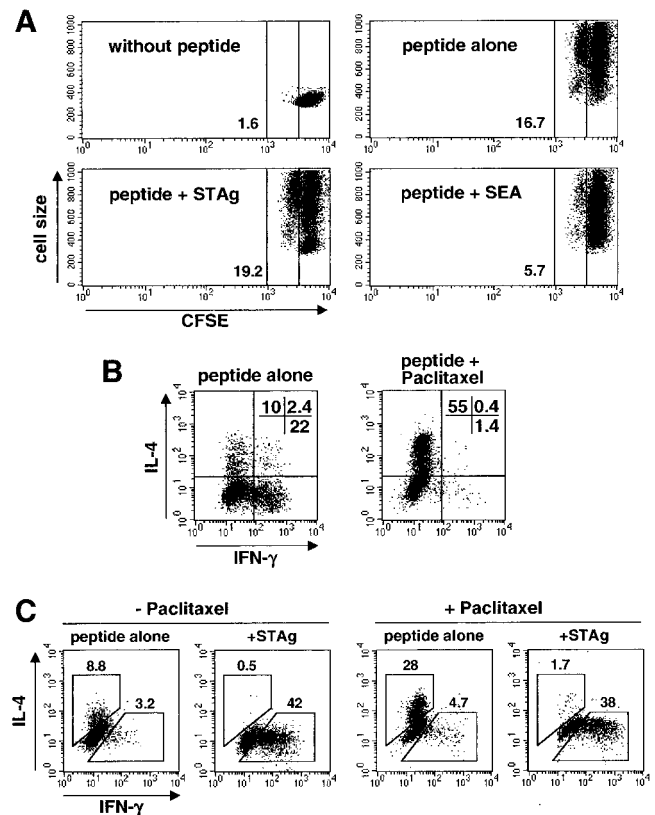


FIGURE 7. Th2 priming by SEA correlates with a delay in exit from the first T cell division and can be mimicked by drug-induced mitotic arrest. **A**, CFSE-labeled DO.11.10 Tg CD4⁺ cells were cultured with DC in medium alone or in the presence of peptide with or without STAg or SEA for 36 h. The cells were then subjected to flow cytometry for measurement of CFSE, as described in Fig. 2B. The FACS dot plots shown are gated on CD4⁺ cells. The percentage of cells that have completed the first cell cycle, defined by decreased CFSE staining, is indicated on each plot. Forward scatter is shown on the y-axis as a parameter of cell size. The findings shown were confirmed in a repeat experiment. **B**, DO.11.10 Tg CD4⁺ lymphocytes were cultured with DC and peptide alone or in the presence of paclitaxel for 3 days. Following an additional incubation for 2 days in IL-2-containing medium, cells were stimulated with PMA/ionomycin in the presence of brefeldin A, and three-color staining for CD4, IFN- γ , and IL-4 was performed. The FACS dot plots shown are gated on CD4⁺ cells. Similar results were obtained in two additional experiments. **C**, DO.11.10 Tg CD4⁺ lymphocytes were cultured with DC and peptide with or without STAg in the presence or absence of paclitaxel, as described in **B**. The FACS dot plots shown are gated on CD4⁺ cells. The data shown are representative of two independent experiments performed.

Discussion

As shown in this work, DC conditioned with *T. gondii* or *S. mansoni* extracts promote the development of Th1 or Th2 DO.11.10 Tg CD4⁺ cells in vitro following priming with nominal peptide. They do so by augmenting the frequency of cytokine-positive cells and function as a third signal for T cell activation. Nevertheless, as documented in the present study, the nature of the third signals leading to Th1 vs Th2 differentiation involves distinct, opposing effects on both the APC and responding T cells. Importantly, while DC must receive a strong positive signal to prime for Th1 development, Th2 differentiation is associated with an actual suppression in DC responses normally induced by Th1 stimuli.

Although the polarizing parasite extracts we studied induce markedly different responses in both DC and T cells, they unexpectedly share a number of important functional properties. Neither STAg nor SEA exert their polarizing effect by acting directly

on CD4⁺ T cells, but instead require APC and in particular DC to promote Th differentiation. Moreover, in each case, the biasing of Th1 or Th2 development by the extracts is not dependent on the induction of the polarizing cytokines IL-12 or IL-4 from DC. Finally, in Tg T cells stimulated with DC conditioned with either parasite stimulus, the signature lymphokines IFN- γ and IL-4 appear in differentiating cells after the third cell division. Previous studies have suggested that Th polarization is associated with a difference in the number of CD4 cell divisions undergone before phenotypic determination (50, 51). Our observation that differentiated Th1 and Th2 effectors arise after the same number of cell divisions argues against this as an obligatory mechanism.

Despite the above similarities, conditioning of DC by STAg vs SEA differs in several important aspects. First, while Th1 polarization by STAg requires MyD88⁺ DC, Th2 polarization by SEA is MyD88 independent. This finding is in agreement with our previous studies indicating that MyD88-deficient mice develop normal Th2 responses following multiple immunization with SEA (14). Taken together, both observations suggest that Th2 polarization by SEA does not involve triggering of the classical MyD88-dependent pathway in DC. Inasmuch as SEA-induced Th2 differentiation is maintained in the TLR4^{-/-} setting, it appears that Th2 development in this model does not require TLR4-dependent MyD88-independent signaling either. Nevertheless, the development of Th2 responses to inhaled OVA plus low dose LPS (52) and Th2 polarization by helminth glycan (48) have both been recently shown to be TLR4 dependent, suggesting that under certain circumstances TLR signaling may promote Th2 development.

A second distinction between STAg and SEA concerns the nature of the DC populations required for polarization. Thus, as previously demonstrated by MacDonald et al. (24) and independently confirmed by us (data not shown), SEA-conditioned bone marrow-derived DC are fully capable of inducing Th2 polarization. In contrast, the same cells fail to polarize toward Th1 when exposed to STAg (J. Aliberti and D.J., unpublished data). The latter observations argue that the receptors involved in Th2 polarization by SEA are more widely distributed among DC populations than those mediating STAg-induced Th1 polarization.

The key distinction between Th1 and Th2 polarization highlighted in this study relates to the state of DC activation and subsequent T cell proliferative responses triggered by different parasite extracts. The existence of this dichotomy was previously noted by investigators examining Th differentiation in both human and murine in vitro systems (23, 24, 53). MacDonald and colleagues (24) have shown that bone marrow-derived DC exposed to SEA or *Propionibacterium acnes* extract, a Th1-polarizing agent, potentially stimulate Ag-specific Th2 or Th1 responses in vivo as well as polyclonal Th2 or Th1 responses in vitro. In that report, bone marrow DC exposed to SEA were shown to be minimally activated in comparison with the same APC exposed to *P. acnes* Ag. The phenotype of DC simultaneously exposed to both SEA and *P. acnes* Ag was not analyzed in the latter study.

Our observations are consistent with the above results as well as data from a recent study by Zacone and colleagues (54) in which SEA was shown to inhibit LPS-induced IL-12 production by bone marrow-derived DC. In the experiments reported here, the inhibitory effect of SEA was revealed in cultures in which DC were simultaneously exposed to STAg and SEA and was shown to be selective in terms of the cellular functions targeted. Thus, while addition of SEA resulted in decreased chemokine secretion and surface expression of CD40 and CD86, responses such as IL-12 production and CD80 expression were not affected in the STAg-stimulated splenic DC cultures used. This differential influence of SEA may be explained by SEA targeting DC populations other

than the CD8 α ⁺ subset previously implicated in the response to STAg (49). Alternatively, its effect may be the result of a specific interference with a pathway(s) that is more widely distributed and not involved in Th1 conditioning of DC. Although both of these interpretations are consistent with the finding that SEA fails to influence the outcome of STAg stimulation as assessed by the degree of Th1 polarization of Tg CD4⁺ cells (Fig. 4), we favor the latter hypothesis because: 1) the decrease in the intensity of CD40 and CD86 staining on DC cultured in the presence of STAg plus SEA vs STAg alone appears to affect all CD11c⁺ cells (Fig. 6), and 2) SEA is able to exert a Th2-polarizing effect on both isolated CD8 α ⁻ DC and CD8 α ⁺ DC (data not shown). A comparative analysis of gene expression in STAg vs STAg plus SEA-stimulated CD11c⁺ DC has been initiated to attempt to identify the precise signaling pathways involved.

The concept that Th2 polarization by SEA involves a blunting of early activation signals is also supported by our observation of retarded CD4 T cell cycling in cultures containing DC exposed to this extract. The mechanism underlying this transitory delay, which persists for one cell division, is unclear. Although we cannot rule out the involvement of an inhibitory factor produced by SEA-stimulated DC, it is likely that the observed impairment in initial T cell responsiveness results from the down-regulation in DC function documented in this study. Indeed, SEA was shown to decrease the expression of two important costimulatory molecules (CD40 and CD86) that play a role in T cell priming, and it is probable that additional signals involved in stimulation of T cell function are also affected by exposure to the extract. Because CD40 has been shown to be essential for SEA-induced Th2 responses in vivo (55), its reduced expression on DC is unlikely on its own to be responsible for Th2 polarization.

Regardless of the precise inhibitory signal delivered by SEA-conditioned DC, our findings with cell cycle inhibitors support the concept that the induction of a delay in exiting the first division can directly promote Th2 differentiation. One possibility is that this retardation in early cell cycling leads to a more rapid induction of autocrine IL-4, which is known to be sufficient for promoting Th2 development (21, 56). Indeed, at 72 h, we observed significant levels of IL-4 in cultures exposed to SEA, but not peptide alone or peptide plus STAg, and this cytokine production occurred even when IL-4-deficient DC were used as APC. Our observation that Th2 differentiation involves a delay in the first cell cycle is consistent with previous studies demonstrating Th2 polarization by low doses of nominal Ag (57–59) in that subthreshold TCR signaling might be expected to result in suboptimal early T cell cycling. The mechanism by which delayed cell cycling could lead to early IL-4 expression upon activation of Th cells is unclear. At present, both positive activators such as GATA-3 (60–62) and inhibitors such as ROG, FOG-1, or Runx 1 (63–65) have been implicated as molecular determinants of IL-4 gene activation within differentiating CD4 lymphocytes.

The major implication of the present study is that in contrast to Th1 polarization, Th2 differentiation is promoted by negative signals. This concept is consistent with observations in other Th2 induction systems involving allergic (66, 67) as well as helminth-triggered responses (53, 68), in which decreased rather than increased CD4 T cell function has been noted. The findings reported in this work now link this behavior to down-regulatory signals in the APC compartment itself, which in turn are associated with retarded lymphocyte activation.

Acknowledgments

We are grateful to C. Eigsti and R. Swofford, present and past member of the National Institute of Allergy and Infectious Diseases Flow Cytometry

Facility, for their expert assistance in cell sorting. We also thank Drs. R. Germain, P. Schwartzberg, M. Udey, and C. Reis e Sousa for their critical reading of this manuscript, and Dr. Reis e Sousa for invaluable advice and discussions during the initial phase of this study.

References

- Sher, A., and R. L. Coffman. 1992. Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annu. Rev. Immunol.* 10:385.
- Jankovic, D., and A. Sher. 1996. Initiation and regulation of CD4⁺ T-cell function in host-parasite models. *Chem. Immunol.* 63:51.
- Abbas, A. K., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper T lymphocytes. *Nature* 383:787.
- Trinchieri, G. 1995. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251.
- Kaplan, M. H., Y. L. Sun, T. Hoey, and M. J. Grusby. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. *Nature* 382:174.
- Mattner, F., J. Magram, J. Ferrante, P. Launois, K. Di Padova, R. Behin, M. K. Gately, J. A. Louis, and G. Alber. 1996. Genetically resistant mice lacking interleukin-12 are susceptible to infection with *Leishmania major* and mount a polarized Th2 cell response. *Eur. J. Immunol.* 26:1553.
- Mattner, F., K. Di Padova, and G. Alber. 1997. Interleukin-12 is indispensable for protective immunity against *Leishmania major*. *Infect. Immun.* 65:4378.
- Tarleton, R. L., M. J. Grusby, and L. Zhang. 2000. Increased susceptibility of Stat4-deficient and enhanced resistance in Stat6-deficient mice to infection with *Trypanosoma cruzi*. *J. Immunol.* 165:1520.
- 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.
- Kaplan, M. H., A. L. Wurster, and M. J. Grusby. 1998. A signal transducer and activator of transcription (Stat)4-independent pathway for the development of T helper type 1 cells. *J. Exp. Med.* 188:1191.
- Altare, F., A. Durandy, D. Lamm, J. F. Emile, S. Lamhamed, F. Le Deist, P. Drysdale, E. Jouanguy, R. Doffinger, F. Bernaudin, et al. 1998. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 280:1432.
- Brombacher, F., A. Dorfmueller, J. Magram, W. J. Dai, G. Kohler, A. Wunderlin, K. Palmer-Lehmann, M. K. Lately, and G. Alber. 1999. IL-12 is dispensable for innate and adaptive immunity against low doses of *Listeria monocytogenes*. *Int. Immunol.* 11:325.
- Cai, G., T. Radzanowski, E. N. Villegas, R. Kastelein, and C. A. Hunter. 2000. Identification of STAT4-dependent and independent mechanisms of resistance to *Toxoplasma gondii*. *J. Immunol.* 165:2619.
- Jankovic, D., M. C. Kullberg, S. Hieny, P. Caspar, C. M. Collazo, and A. Sher. 2002. In the absence of IL-12, CD4⁺ T cell responses to intracellular pathogens fail to default to a Th2 pattern and are host protective in an IL-10^{-/-} setting. *Immunity* 16:429.
- O'Garra, A. 1998. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 8:275.
- Kaplan, M. H., U. Schindler, S. T. Smiley, and M. J. Grusby. 1996. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. *Immunity* 4:313.
- Shimoda, K., J. van Deursen, M. Y. Sangster, S. R. Sarawar, R. T. Carson, R. A. Tripp, C. Chu, F. W. Quelle, T. Nosaka, D. A. Vignali, et al. 1996. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. *Nature* 380:630.
- Finkelman, F. D., S. C. Morris, T. Orekhova, M. Mori, M., D. Donaldson, S. L. Reiner, N. L. Reilly, L. Schopf, and J. F. Urban, Jr. 2000. Stat6 regulation of in vivo IL-4 responses. *J. Immunol.* 164:2303.
- Jankovic, D., M. C. Kullberg, N. Noben-Trauth, P. Caspar, W. E. Paul, and A. Sher. 2000. Single cell analysis reveals that IL-4 receptor/Stat6 signaling is not required for the in vivo or in vitro development of CD4⁺ lymphocytes with a Th2 cytokine profile. *J. Immunol.* 164:3047.
- Ouyang, W., M. Löhning, Z. Gao, M. Assenmacher, S. Ranganath, A. Radbruch, and K. M. Murphy. 2000. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 12:27.
- Noben-Trauth, N., J. Hu-Li, and W. E. Paul. 2000. Conventional, naive CD4⁺ T cells provide an initial source of IL-4 during Th2 differentiation. *J. Immunol.* 165:3620.
- Moser, M., and K. M. Murphy. 2000. Dendritic cell regulation of TH1-TH2 development. *Nat. Immunol.* 1:199.
- 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 cell-polarizing signals. *J. Immunol.* 168:1704.
- MacDonald, A. S., A. D. Straw, B. Bauman, and E. J. Pearce. 2001. CD8 α ⁻ dendritic cell activation status plays an integral role in influencing Th2 response development. *J. Immunol.* 167:1982.
- 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.
- Faveeuw, C., T. Mallevaey, K. Paschinger, I. B. Wilson, J. Fontaine, R. Mollicone, R. Oriol, F. Altmann, P. Lerouge, M. Capron, and F. Trottein. 2003. Schistosome N-glycans containing core α 3-fucose and core β 2-xylose epitopes are strong inducers of Th2 responses in mice. *Eur. J. Immunol.* 33:1271.
- Del Rio, L., A. J. Buendia, J. Sanchez, M. C. Gallego, R. M. Caro, N. Ortega, J. Seva, F. J. Pallares, F. Cuello, and J. Salinas. 2001. Endogenous interleukin-12 is not required for resolution of *Chlamydia abortus* (*Chlamydia psittaci* serotype 1) infection in mice. *Infect. Immun.* 69:4808.
- Makeham, J., J. Wang, J. Magram, K. Croitoru, R. Harkness, P. Dunn, A. Zganiacz, and Z. Xing. 1998. Lack of both types 1 and 2 cytokines, tissue inflammatory responses, and immune protection during pulmonary infection by *Mycobacterium bovis* bacille Calmette-Guerin in IL-12-deficient mice. *J. Immunol.* 160:6101.
- Kaisho, T., K. Hoshino, T. Iwabe, O. Takeuchi, T. Yasui, and S. Akira. 2002. Endotoxin can induce MyD88-deficient dendritic cells to support Th2 cell differentiation. *Int. Immunol.* 14:695.
- 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.
- 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 α ⁺ and CD8 α ⁻ subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med.* 189:587.
- Pulendran, B., J. L. Smith, G. Caspar, 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.
- Cella, M., F. Facchetti, A. Lanzavecchia, and M. Colonna. 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. *Nat. Immunol.* 1:305.
- Edwards, A. D., S. P. Manickasingham, R. Sporri, S. S. Diebold, O. Schulz, A. Sher, T. Kaisho, S. Akira, and C. Reis e Sousa. 2002. Microbial recognition via Toll-like receptor-dependent and -independent pathways determines the cytokine response of murine dendritic cell subsets to CD40 triggering. *J. Immunol.* 169:3652.
- Manickasingham, S. P., A. D. Edwards, O. Schulz, and C. Reis e Sousa. 2003. The ability of murine dendritic cell subsets to direct T helper cell differentiation is dependent on microbial signals. *Eur. J. Immunol.* 33:101.
- 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.
- Noben-Trauth, N., G. Kohler, K. Burki, and B. Ledermann. 1996. Efficient targeting of the IL-4 gene in a BALB/c embryonic stem cell line. *Transgenic Res.* 5:487.
- Noben-Trauth, N., J. Hu-Li, and W. E. Paul. 2002. IL-4 secreted from individual naive CD4⁺ T cells acts in an autocrine manner to induce Th2 differentiation. *Eur. J. Immunol.* 32:1428.
- Adachi, O., T. Kawai, K. Takeda, M. Matsumoto, H. Tsutsui, M. Sakagami, K. Nakanishi, and S. Akira. 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. *Immunity* 9:143.
- Barnden, M. J., J. Allison, W. R. Heath, and F. R. Carbone. 1998. Defective TCR expression in transgenic mice constructed using cDNA-based α - and β -chain genes under the control of heterologous regulatory elements. *Immunol. Cell Biol.* 76:34.
- Magram, J., S. E. Connaughton, R. R. Warrier, D. M. Carvajal, C. Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty, and M. K. Lately. 1996. IL-12-deficient mice are defective in IFN- γ production and type 1 cytokine responses. *Immunity* 4:471.
- 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.
- 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.
- Richter, A., M. Löhning, and A. Radbruch. 1999. Instruction for cytokine expression in T helper lymphocytes in relation to proliferation and cell cycle progression. *J. Exp. Med.* 190:1439.
- Yap, G. S., and A. Sher. 1999. Cell-mediated immunity to *Toxoplasma gondii*: initiation, regulation and effector function. *Immunobiology* 201:240.
- Pearce, E. J., and A. S. MacDonald. 2002. The immunobiology of schistosomiasis. *Nat. Rev. Immunol.* 2:499.
- Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallusto. 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat. Immunol.* 1:311.
- Thomas, P. G., M. R. Carter, O. Atochina, A. A. Da'Dara, D. Piskorska, E. McGuire, and D. A. Harn. 2003. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. *J. Immunol.* 171:5837.
- 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 α ⁺ dendritic cells. *Nat. Immunol.* 1:83.
- 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.
- Gett, A. V., and P. D. Hodgkin. 1998. Cell division regulates the T cell cytokine repertoire, revealing a mechanism underlying immune class regulation. *Proc. Natl. Acad. Sci. USA* 95:9488.
- Eisenbarth, S. C., D. A. Piggott, J. W. Huleatt, I. Visintin, C. A. Herrick, and K. Bottomly. 2002. Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* 16:1645.

53. 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.
54. Zaccane, P., Z. Fehérvári, F. M. Jones, S. Sidobre, M. Kronenberg, D. W. Dunne, and A. Cooke. 2003. *Schistosoma mansoni* antigens modulate the activity of the innate immune response and prevent onset of type 1 diabetes. *Eur. J. Immunol.* 33:1439.
55. MacDonald, A. S., A. D. Straw, N. M. Dalton, and E. J. Pearce. 2002. Th2 response induction by dendritic cells: a role for CD40. *J. Immunol.* 168:537.
56. Schmitz, J., A. Thiel, R. Kuhn, K. Rajewsky, W. Muller, M. Assenmacher, and A. Radbruch. 1994. Induction of interleukin 4 (IL-4) expression in T helper (Th) cells is not dependent on IL-4 from non-Th cells. *J. Exp. Med.* 179:1349.
57. Constant, S., C. Pfeiffer, A. Woodard, T. Pasqualini, and K. Bottomly. 1995. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4⁺ T cells. *J. Exp. Med.* 182:1591.
58. Jorritsma, P. J., J. L. Brogdon, and K. Bottomly. 2003. Role of TCR-induced extracellular signal-regulated kinase activation in the regulation of early IL-4 expression in naive CD4⁺ T cells. *J. Immunol.* 170:2427.
59. Boonstra, A., C. Asselin-Paturel, M. Gilliet, C. Crain, G. Trinchieri, Y. J. Liu, and A. O'Garra. 2003. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential Toll-like receptor ligation. *J. Exp. Med.* 197:101.
60. Zheng, W., and R. A. Flavell. 1997. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell* 89:587.
61. Kurata, H., H. J. Lee, A. O'Garra, and N. Arai. 1999. Ectopic expression of activated Stat6 induces the expression of Th2-specific cytokines and transcription factors in developing Th1 cells. *Immunity* 11:677.
62. Farrar, J. D., W. Ouyang, M. Lohning, M. Assenmacher, A. Radbruch, O. Kanagawa, and K. M. Murphy. 2001. An instructive component in T helper cell type 2 (Th2) development mediated by GATA-3. *J. Exp. Med.* 193:3643.
63. Miaw, S. C., A. Choi, E. Yu, H. Kishikawa, and I. C. Ho. 2000. ROG, repressor of GATA, regulates the expression of cytokine genes. *Immunity* 12:323.
64. Zhou, M., W. Ouyang, O. Gong, S. G. Katz, J. M. White, S. H. Orkin, and K. M. Murphy. 2001. Friend of GATA-1 represses GATA-3-dependent activity in CD4⁺ T cells. *J. Exp. Med.* 194:1461.
65. Komine, O., K. Hayashi, W. Natsume, T. Watanabe, Y. Seki, N. Seki, R. Yagi, W. Sukzuki, H. Tamauchi, K. Hozumi, et al. 2003. The Runx1 transcription factor inhibits the differentiation of naive CD4⁺ T cells into the Th2 lineage by repressing GATA3 expression. *J. Exp. Med.* 198:51.
66. Schulz, O., H. F. Sewell, and F. Shakib. 1998. Proteolytic cleavage of CD25, the α subunit of the human T cell interleukin 2 receptor, by Der p 1, a major mite allergen with cysteine protease activity. *J. Exp. Med.* 187:271.
67. Saarelainen, S., T. Zeiler, J. Rautiainen, A. Narvanen, M. Rytönen-Nissinen, R. Mantyjarvi, P. Vilja, and T. Virtanen. 2002. Lipocalin allergen Bos d 2 is a weak immunogen. *Int. Immunol.* 14:401.
68. Boitelle, A., H. E. Scales, C. Di Lorenzo, E. Devaney, M. W. Kennedy, P. Garside, and C. E. Lawrence. 2003. Investigating the impact of helminth products on immune responsiveness using a TCR transgenic adoptive transfer system. *J. Immunol.* 171:447.