Concurrent Bacterial Stimulation Alters the Function of Helminth-Activated Dendritic Cells, Resulting in IL-17 Induction

Georgia Perona-Wright, Rachel J. Lundie, Stephen J. Jenkins, Lauren M. Webb, Richard K. Grencis and Andrew S. MacDonald

*J Immunol* 2012; 188:2350-2358; Prepublished online 27 January 2012; doi: 10.4049/jimmunol.1101642 http://www.jimmunol.org/content/188/5/2350

---

**References**  This article cites 86 articles, 35 of which you can access for free at: http://www.jimmunol.org/content/188/5/2350.full#ref-list-1

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

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

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Concurrent Bacterial Stimulation Alters the Function of Helminth-Activated Dendritic Cells, Resulting in IL-17 Induction

Georgia Perona-Wright,*1 Rachel J. Lundie,* Stephen J. Jenkins,* Lauren M. Webb,* Richard K. Grencis,† and Andrew S. MacDonald*

Infection with schistosome helminths is associated with granulomatous inflammation that forms around parasite eggs trapped in host tissues. In severe cases, the resulting fibrosis can lead to organ failure, portal hypertension, and fatal bleeding. Murine studies identified IL-17 as a critical mediator of this immunopathology, and mouse strains that produce high levels of IL-17 in response to schistosome infection show increased mortality. In this article, we demonstrate that schistosome-specific IL-17 induction by dendritic cells from low-pathology C57BL/6 mice is normally regulated by their concomitant induction of IL-10. Simultaneous stimulation of schistosome-exposed C57BL/6 dendritic cells with a heat-killed bacterium enabled these cells to overcome IL-10 regulation and induce IL-17, even in wild-type C57BL/6 recipients. This schistosome-specific IL-17 was dependent on IL-6 production by the copulsed dendritic cells. Coimmunization of C57BL/6 animals with bacterial and schistosome Ags also resulted in schistosome-specific IL-17, and this response was enhanced in the absence of IL-10–mediated immune regulation. Together, our data suggest that the balance of pro- and anti-inflammatory cytokines that determines the severity of pathology during schistosome infection can be influenced not only by host and parasite, but also by concurrent bacterial stimulation. The Journal of Immunology, 2012, 188: 2350–2358.

Schistosomiasis is a cause of significant morbidity and mortality, affecting >200 million people worldwide and resulting in ≥300,000 deaths annually (1, 2). Adult Schistosoma mansoni live in the vasculature of the human intestine, and each female releases up to 350 eggs/d into the bloodstream (3). To complete the parasite life cycle, these eggs are required to move out of the blood vessel, across the gut wall, and into the intestinal lumen, from where they can exit the host. However, blood flow causes many eggs to be swept into the liver sinusoids, where they become trapped and form the foci of immune-mediated granulomas (4). The fibrosis associated with these granulomas, as well as the tissue destruction and portal hypertension that result, is responsible for much of the pathology of infection (5).

*Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JT, United Kingdom; and Manchester Immunology Group, Faculty of Life Sciences, University of Manchester, Manchester M13 9PT, United Kingdom

1Current address: Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada.

Received for publication June 7, 2011. Accepted for publication December 23, 2011.

This work was supported by the Wellcome Trust and the Medical Research Council U.K. The schistosome life-cycle stages used in this research were supplied by the National Institute of Allergy and Infectious Diseases Schistosomiasis Resource Center at the Biomedical Research Institute (Rockville, MD) through Contract N01-AI-30026.

Address correspondence and reprint requests to Dr. Andrew S. MacDonald and Dr. Georgia Perona-Wright, Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh EH9 3JT, U.K. (A.S.M.) or Department of Microbiology and Immunology, University of British Columbia, Vancouver V6T 1Z3, BC, Canada (G.P.W.). E-mail addresses: andrew.macdonald@ed.ac.uk (A.S.M.) and gperona@mail. ubc.ca (G.P.W.)

Abbreviations used in this article: BM, bone marrow; DC, dendritic cell; MHCII, MHC class II; SEA, soluble egg Ag from Schistosoma mansoni; WT, wild-type.

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12/$16.00

In both human infection and mouse models, parasite burden is a poor correlate of disease severity (6, 7). Instead, the level of pathology closely reflects the cytokine balance achieved by the infected host. In low-pathology C57BL/6 mice, S. mansoni elicits a CD4+ T cell response that initially displays a mixed Th1/Th2 profile but becomes highly Th2 dominated once egg-laying begins (4, 8). Th2 cells release IL-4 and IL-13 and although IL-13 is profibrotic (9, 10), IL-4 is protective and essential for host survival (11, 12). In contrast, the severe or fatal fibrotic pathology that characterizes schistosomiasis in high-pathology CBA mice is associated with a persistent Th1 response (13) and production of schistosome-specific IL-17 (14, 15). IL-17 contributes to florid granuloma formation through its recruitment of neutrophils and its inhibition of alternative activation of macrophages, both of which exacerbate inflammation (15). The immunosuppressive cytokine IL-10 was shown to limit inflammatory pathology during schistosomiasis (16, 17), but the relationship between IL-10 and IL-17 in this disease remains unexplored.

IL-17 has been implicated in the pathogenesis of chronic inflammation in several disease models, including eczema (18, 19), arthritis (20), and fungal (21, 22) and bacterial infections (23, 24). Its release from CD4+ T cells is dependent on IL-6, TGF-β, and IL-23 (25–27), with IL-23 likely acting during murine schistosomiasis, we used a technique of adoptive transfer of DCs that allowed us to restrict cytokine deficiency either
to the priming APCs or to the network of responding cells in the recipient mice (36, 37). We show that, in low-pathology C57BL/6 mice, DCs exposed to soluble egg Ags of *S. mansoni* (SEA) possess the ability to drive SEA-specific IL-17, but that this response is normally suppressed by IL-10 released by nonlymphoid cells of the host. Strikingly, this IL-10–mediated regulation is overcome if the schistosome-conditioned DCs encounter simultaneous bacterial stimulation. Together, these data highlight a three-way interaction between host, helminth, and coincident bacteria that can influence the pathogenic capacity of the ensuing cytokine response.

**Materials and Methods**

**Mice**

C57BL/6, *il10<sup>-/-</sup>*, *rag1<sup>-/-</sup>*, and *p10<sup>-/-</sup>* mice were bred and maintained under specific pathogen-free conditions in the animal facilities of the School of Biological Sciences at the University of Edinburgh (Edinburgh, U.K.), Dr. Maurice Gallagher (University of Edinburgh). Both were heat killed by incubation at 80°C for 30 min. The anti-CD25 Ab (clone PC61) was produced from a hybridoma in-house.

**DC culture**

DCs were generated in the presence of RGM-CSF (PeproTech, Rocky Hill, NJ) for 11 d, as described (36, 38). Cells were lethally gamma irradiated with 1250 rad and rescued 24 h later by i.v. administration of 5 × 10<sup>6</sup> bone marrow (BM) cells from *rag1<sup>-/-</sup>* and *il10<sup>-/-</sup>* donors mixed at a ratio of 80:20. Control chimeras received 20% WT BM in place of the *il10<sup>-/-</sup>* cells. Mature T cells were removed from all BM preparations prior to transfer, using anti-Th1.2 MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Chimeras were allowed to reconstitute for 6–8 wk before use.

**Ags and Abs**

Endotoxin-free SEA was prepared from *S. mansoni* eggs taken from the livers of infected C57BL/6 mice, as previously described (36). *Propionibacterium acnes* was obtained from American Type Culture Collection (#6919; Manassas, VA), and *Salmonella typhimurium* was provided by Dr. Maurice Gallagher (University of Edinburgh). Both were heat killed by incubation at 80°C for 30 min. The anti-CD25 Ab (clone PC61) was produced from a hybridoma in-house.

**Assessment of DC activation**

The expression of surface molecules on DCs was assessed by flow cytometry using Abs against MHC class II (MHCII), CD11c, and CD80, or purchased from BD Europe (Oxford, U.K.). Ab expression was measured by direct or indirect labeling. Cells were analyzed with FACSCalibur flow cytometer and analyzed using FlowJo software (Tree Star), gating on live, CD11c<sup>+</sup> cells. Cytokine ELISAs were performed on culture supernatants using paired mAbs purified from hybridomas in-house.

**RNA extraction, RT-PCR, and real time RT-PCR**

Total RNA was isolated from 1 × 10<sup>6</sup> DCs using TRIzol reagent (Invitrogen, Paisley, U.K.) and standard phenol-chloroform extraction. cDNA was generated using the Reverse Transcriptase System with random hexamers (Promega, Southamp ton, U.K.). PCR was performed using Taq Supreme polymerase and buffers from Helena Biosciences (Sunderland, U.K.), as described (41). For quantitative analysis, PCR was performed using SYBR green (Invitrogen), a Chromo4 detector, and Opticon Monitor software (MJ Research). Expression values for the gene of interest relative to that of 18S rRNA were calculated using the equation 2<sup>-ΔΔCt</sup>.

**Results**

**Exposure to SEA stimulates IL-23 p19 expression by C57BL/6 DCs**

Previous work showed that DCs from the low-pathology C57BL/6 mouse strain display minimal signs of activation in response to schistosome egg Ags (35, 36, 43). Consistent with this, SEA caused only a minor increase in C57BL/6 DC expression of MHCII and little change in surface levels of CD80, CD86, or CD40 compared with DCs incubated in medium alone (Fig. 1A), and it did not alter DC expression of either the *p40* or *p35* subunit of IL-12 (Fig. 1B). Surprisingly, however, SEA did elicit a detectable increase in DC expression of mRNA for *p19*, the protein product of which pairs with that of *p40* to form IL-23 (44). The upregulation of *p19* expression by SEA was confirmed using quantitative real-time PCR (Fig. 1C). Secretion of IL-23 protein in response to SEA remained undetectable (Fig. 1D), in keeping with previous reports (35). In contrast, stimulation with a heat-killed preparation of the bacterium *P. acnes* triggered conventional DC maturation, involving marked upregulation of MHCII and little change in surface levels of CD80, CD86, or CD40 compared with DCs incubated in medium alone (Fig. 1A), and it did not alter DC expression of either of the *p40* or *p35* subunit of IL-12 (Fig. 1B). The induction of *p19* gene expression in DCs exposed to SEA prompted us to examine the ability of these cells to promote IL-17 production in vivo. We adoptively transferred DCs into recipient mice and analyzed the cytokine composition of the resulting immune response. Injection of SEA-pulsed DCs i.p. into WT animals induced a strong, SEA-specific Th2 response (Fig. 2) but no detectable IL-17 or IFN-γ (Fig. 2). Transfer of the same DCs into *il10<sup>-/-</sup>* recipients had little effect on the Th2 response but instead resulted in clear production of SEA-specific IL-17 (Fig. 2). The absence of recipient IL-10 also unveiled a SEA-specific IFN-γ response, as reported previously (39). *P. acnes*-pulsed DCs elicited *P. acnes*-specific IL-17 and IFN-γ in both WT and *il10<sup>-/-</sup>* mice, with the IFN-γ response markedly enhanced in *il10<sup>-/-</sup>* animals (Fig. 2) (39). Together, these data suggested that C57BL/6 DCs exposed to SEA are able to induce Ag-specific IL-17 in vivo but that this process is ordinarily inhibited by the action of IL-10.
Lymphocyte-derived IL-10 is dispensable in the control of DC-induced, SEA-specific IL-17

We investigated the source of the IL-10 that regulates DC-induced, SEA-specific IL-17 in vivo, first anticipating that CD25+ regulatory T cells would be likely candidates (45, 46). To test this hypothesis, we depleted CD25+ cells in WT C57BL/6 mice prior to DC transfer. Administration of PC61 mAb effectively removed CD25+ expressing CD4+ cells from the blood of naive animals (42), and this depletion was maintained in the spleen for the duration of the experiment (Fig. 3A). There was no concurrent increase in the FoxP3+ CD25− population (Fig. 3A). However, the absence of CD25+ cells did not result in the emergence of SEA-specific IL-17 following immunization with SEA-pulsed DCs (Fig. 3B). The IL-17 response to P. acnes-activated DCs was similarly unaffected by the depletion of CD25+ cells in recipient animals. The induction of IFN-γ by SEA-pulsed DCs was also unchanged in the presence or absence of CD25+ cells, whereas the quantity of IFN-γ released after transfer of P. acnes-pulsed DCs was significantly, but modestly, increased (p = 0.04, Fig. 3B). The Th2 response to SEA-pulsed DCs was not affected by the depletion of CD25+ cells, as shown previously (Fig. 3C) (42). These data suggest that CD4+ CD25+ regulatory T cells are not required to prevent the induction of SEA-specific IL-17 by C57BL/6 DCs.

To investigate whether any lymphocyte population provides the IL-10 necessary to enforce this suppression, we generated mixed BM chimeric mice in which IL-10 deficiency was limited to B and T cells (Fig. 4A). Compared with control chimeras, immunization of the lymphocyte-restricted il10−/− chimeras with SEA-pulsed DCs resulted in a slight increase in SEA-specific IFN-γ (p = 0.039, Fig. 4B; compare with Fig. 2), but there was no detectable induction of SEA-specific IL-17 (Fig. 4B). SEA-pulsed DCs elicited equivalent Th2 responses in both WT and il10−/− chimeras (Fig. 4C) (39). The absence of lymphocyte-derived IL-10 in recipient mice markedly increased the IFN-γ response to P. acnes-conditioned DCs, as reported previously (Fig. 4B) (39), but P. acnes-specific IL-17 was not significantly affected (Fig. 4B). Together with Fig. 2, these data indicate that, in C57BL/6 animals, the induction of IL-10 by SEA-activated DCs ordinarily inhibits the release of SEA-specific IL-17 and IFN-γ and that the critical source of this IL-10 is nonlymphocyte cells in the recipient mice.

Coincident bacterial stimulation of DCs elicits SEA-specific IL-17

Schistosome-specific IL-17 is a critical component of the extensive immunopathology that characterizes S. mansoni infection in high-pathology CBA mice (14). Neither IL-17 nor severe pathology is normally observed during infection of WT C57BL/6 animals, but both can be induced by immunization with SEA emulsified in...
CFA prior to infection (14, 15). The importance of the CFA adjuvant in this model suggested that coincident inflammatory signals delivered at the time of T cell priming might result in the expression of SEA-specific IL-17. To test this, we compared C57BL/6 DCs that had been exposed to SEA alone or to both SEA and a heat-killed preparation of the bacterium *P. acnes*. Consistent with previous data (47, 48), the copulsed DCs appeared phenotypically activated, as evidenced by their upregulation of surface MHCII and strong expression of the costimulatory molecules CD80, CD86, and CD40 (Fig. 5A). The presence of SEA during DC activation potently reduced the *P. acnes*-stimulated release of IL-12p70 (Fig. 5B) (47, 48). However, the inhibition of DC production of IL-12/23 p40 by coincident SEA exposure was less extensive, and its impact on IL-23 was minor: the concentration of IL-23 released by copulsed DCs was similar to that produced by DCs activated by *P. acnes* alone (Fig. 5B). IL-6 and IL-1b were also secreted in similar quantities by copulsed and by *P. acnes*-stimulated DCs, whereas IL-10 production by copulsed DCs was greater than that of DCs activated by *P. acnes* alone (Fig. 5B).

When transferred in vivo, DCs concurrently activated with SEA and *P. acnes* induce simultaneous, yet distinct, Th responses: SEA-specific T cell differentiation is appropriately skewed toward a Th2, and not a Th1, phenotype, and *P. acnes*-specific CD4+ cells retain Th1 polarization (48). Consistent with this data, we observed similarly low levels of SEA-specific IFN-γ induction following transfer of copulsed WT DCs or WT DCs pulsed with SEA alone, as well as an equivalently strong *P. acnes*-specific IFN-γ response in mice that received either copulsed or *P. acnes* only-pulsed DCs (Fig. 5C). Copulsed DCs were also capable of eliciting an SEA-specific Th2 response, although it was less potent than that generated by DCs pulsed with SEA alone (Fig. 5D) (48). In contrast, adoptive transfer of WT C57BL/6 DCs that had been activated with both SEA and *P. acnes* resulted in the clear induction of SEA-specific IL-17, a response completely absent in mice immunized with DCs exposed to SEA alone (Fig. 5C). This was not a peculiarity of *P. acnes*, because it was also true of DCs pulsed with SEA and heat-killed *S. typhimurium* and transferred into WT recipients (Fig. 5E). The ability of WT DCs to elicit *P. acnes*-specific IL-17 was modestly, but consistently, reduced in

**FIGURE 3.** CD25+ cells do not affect IL-17 induction by SEA-pulsed DCs. WT mice were injected i.p. with an anti-CD25–depleting Ab (PC61; 1 mg/mouse) or polyclonal rat IgG as an irrelevant Ab control (1 mg/mouse). Three days later, WT DCs that had been cultured for 18 h with medium alone (M), SEA (S), or *P. acnes* (P) were injected s.c. Mice were analyzed 7 d later. (A) Splenocytes were assessed for their expression of CD25 (using mAb 7D2) and Foxp3. (B and C) Splenocytes were restimulated for 72 h in medium (white bars), with SEA (black bars), or with *P. acnes* (gray bars), and their cytokine production was measured by ELISA. No IL-4 or IL-13 was detected following *P. acnes* restimulation. Data shown are representative of three experiments, and error bars indicate SEM of four to six mice/group.

**FIGURE 4.** Lymphocyte-derived IL-10 is dispensable in the control of SEA-specific IL-17. WT mice were lethally irradiated and rescued with a mixture of *rag2*−/− and either WT or *il10*−/− BM. (A) Resulting genotype of different cell populations within the chimeric mice. ❖ denotes majority (80% versus 20%) of cells. (B and C) Chimeras were injected i.p. with WT DCs that had been cultured for 18 h with medium alone (M), SEA (S), or *P. acnes* (P). Seven days later, splenocytes were restimulated for 72 h in medium (white bars), with SEA (black bars), or with *P. acnes* (gray bars), and cytokine production was measured by ELISA. No IL-5 or IL-13 was detected following *P. acnes* restimulation. Data shown are representative of two independent experiments, and error bars indicate SEM of four mice/group.
FIGURE 5. Bacterial stimulation of schistosome-activated DCs elicits SEA-specific IL-17. WT or il6−/− DCs were cultured for 18 h in medium alone (M) or in the presence of SEA (S), P. acnes (P), or both SEA and P. acnes (S/P). (A) Surface phenotype, assessed by flow cytometry. Open graphs indicate staining with isotype controls. Data shown are from WT DCs. (B) Cytokine levels in culture supernatants were measured by ELISA. Error bars indicate SEM of five mice/group. (C) Surface phenotype, assessed by flow cytometry. Open graphs indicate staining with isotype controls. Data shown are from WT DCs. (D) Cytokine levels in culture supernatants were measured by ELISA. Error bars indicate SEM of five mice/group. (E) WT DCs were pulsed with SEA, S. typhimurium (St), or SEA and S. typhimurium and injected i.p. into WT recipients. Seven days later, splenocytes were restimulated for 72 h in medium (white bars) or with SEA (black bars), and cytokine production was measured by ELISA. Error bars indicate SEM of five mice/group. (F) WT or il6−/− DCs were pulsed with SEA, P. acnes, or SEA and P. acnes and injected i.p. into DCs exposed simultaneously to SEA and P. acnes compared with DCs activated by P. acnes alone (Fig. 5C).

The induction of SEA-specific IL-17 by copulsed DCs is dependent on their production of IL-6

To investigate the mechanism by which coincident stimulation with P. acnes enables SEA-pulsed, C57BL/6 DCs to generate SEA-specific IL-17 in recipient mice, we compared the immune responses elicited in vivo by WT and cytokine-deficient DCs. The induction of p19 gene expression in SEA-conditioned DCs (Fig. 1B, 1C) prompted us to consider the role of IL-23 (35). However, equivalent levels of IL-17 were induced by both WT and il6−/− copulsed DCs (Fig. 6A), suggesting that IL-23 production by the priming DCs was dispensable in this process. WT and il6−/− DCs also elicited equivalent levels of IFN-γ (Fig. 6B).

Copulsed DCs released high levels of IL-6 in addition to IL-23 (Fig. 5B), and our previous work identified IL-6 as essential for the promotion of Th17 cells by bacterially activated DCs (33); therefore, we next tested the importance of IL-6 in the promotion of SEA-specific IL-17 by copulsed DCs. Strikingly, the ability of copulsed DCs to elicit SEA-specific IL-17 was completely abrogated by the absence of DC-derived IL-6 (Fig. 5C). No SEA-specific IL-17 was detected in response to il6−/− DCs, despite equivalent levels of IL-23 and IL-1β production from WT and il6−/− C57BL/6 DCs (Fig. 5B). IL-6–deficient DCs were also severely impaired in their ability to induce a P. acnes-specific IL-17 response after transfer into WT recipients (Fig. 5C) (33), irrespective of whether the DCs were activated with P. acnes alone or with both P. acnes and SEA. Neither the P. acnes-nor the SEA-specific IFN-γ response was impaired following transfer of il6−/− DCs (Fig. 5C). These findings demonstrated that the IL-6 triggered by concurrent bacterial stimulation of SEA-activated DCs is essential for the ability of these cells to initiate schistosome-specific IL-17 production in recipient C57BL/6 mice.

DC-derived IL-6 inhibits IL-10 production by recipient cells

Our data showed that DCs exposed to SEA elicit schistosome-specific IL-17 in IL-10–deficient animals (Fig. 2) and that copulsed DCs generated schistosome-specific IL-17 in WT recipients (Fig. 5C, 5E). Because the latter observation was dependent on IL-6, we examined the relationship between DC-derived IL-6 and the induction of SEA-specific IL-17 by copulsed DCs. Strikingly, the ability of copulsed DCs to elicit SEA-specific IL-17 was completely abrogated by the absence of DC-derived IL-6 (Fig. 5C). No SEA-specific IL-17 was detected in response to il6−/− DCs, despite equivalent levels of IL-23 and IL-1β production from WT and il6−/− C57BL/6 DCs (Fig. 5B). IL-6–deficient DCs were also severely impaired in their ability to induce a P. acnes-specific IL-17 response after transfer into WT recipients (Fig. 5C) (33), irrespective of whether the DCs were activated with P. acnes alone or with both P. acnes and SEA. Neither the P. acnes-nor the SEA-specific IFN-γ response was impaired following transfer of il6−/− DCs (Fig. 5C). These findings demonstrated that the IL-6 triggered by concurrent bacterial stimulation of SEA-activated DCs is essential for the ability of these cells to initiate schistosome-specific IL-17 production in recipient C57BL/6 mice.

WT recipients. Seven days later, splenocytes were restimulated for 72 h in medium (white bars) or with SEA (black bars), and IL-10 production was measured by ELISA. Error bars indicate SEM of five mice/group. All data are representative of at least two independent experiments.
Coinjection of *S. mansoni* eggs and *P. acnes* results in schistosome-specific IL-17 that is enhanced in the absence of recipient IL-10

To investigate whether coincident bacterial stimulation could also elicit schistosome-specific IL-17 upon direct immunization with pathogen preparations, we injected *S. mansoni* eggs s.c. into WT and *il10*<sup>−/−</sup> mice, either alone or in combination with *P. acnes* (Fig. 7). IFN-γ production following the solo injection of either *S. mansoni* eggs or *P. acnes* was markedly enhanced in the absence of recipient IL-10, as expected (39). However, the immunization of *il10*<sup>−/−</sup> animals also elicited significantly higher levels of *P. acnes*-specific IL-17 than that of WT recipients, both when *P. acnes* was administered alone and when the inoculum was a combination of *P. acnes* and *S. mansoni* eggs (*p* = 0.048 and *p* = 0.0018, respectively; Fig. 7). Importantly, and similarly to the cytokine induction triggered by the transfer of copulsed DCs (Fig. 2), only the coinjection of eggs and *P. acnes* elicited an SEA-specific IL-17 response in WT mice. The injection of *S. mansoni* eggs alone was insufficient to do this (Fig. 7). The SEA-specific IL-17 that emerged when mice were coinimmunized with *S. mansoni* eggs and *P. acnes* was significantly elevated in the absence of recipient IL-10 (*p* = 0.0058, Fig. 7). These data suggest that schistosome-specific IL-17 can be induced in C57BL/6 mice in response to *S. mansoni* egg immunization, but only when coincident microbial stimulation is provided, and that this helminth-specific IL-17 is further elevated in the context of diminished IL-10 regulation. Thus, the development of pathological cytokine responses to schistosome Ags can be influenced by the host, parasite, and coincident bacterial stimulation.

**Discussion**

Surviving infection involves a balance between the proinflammatory cytokines that neutralize a pathogen and anti-inflammatory signals that limit collateral damage to the host. In schistosomiasis, severe disease is associated with a persistent, granulomatous inflammatory response that can cause extensive fibrosis (5), and recent work identified IL-17 as a critical mediator of this pathology (15, 35). In this article, we describe a regulatory circuit in which IL-10 restricts the induction of IL-17 by schistosome Ags, and we show that this IL-10–mediated suppression is abrogated by coincident microbial stimulation of the Ag-presenting DC. These data indicate that concurrent bacterial presence can alter DC function and modify the consequent schistosome-specific cytokine balance.

The morbidity and mortality in schistosome-infected, C57BL/6 mice are characteristically mild in comparison with some other inbred mouse strains, and this correlates with the ability of C57BL/6 mice to mount a protective Th2 response during the chronic stages of disease (5, 7). C57BL/6 DCs exposed to schistosome eggs or egg Ags also generate Th2 responses both in vitro and in vivo (35, 36, 40). Such Th2 induction was proposed to result from the absence of Th1-driving cytokines, a model known as “the default hypothesis” (49). Our data instead provide additional evidence to support an active, not passive, process of Th2 polarization by SEA-exposed DCs, in which WT DCs trigger IL-10 induction and consequently suppress the production of both IFN-γ and IL-17 (Fig. 2) (39). Other examples of dominant Th2 responses have been reported (48, 50), and a consensus is now emerging that Th2 induction against helminths requires a positive, multistep dialogue between the initiating DCs and a network of responding cells (51). The different cell types recruited into this network affect the final cytokine balance (52), and it is interesting to note that the schistosome-specific IL-17 response that we discuss in this article is not necessarily a product only of CD4<sup>+</sup> Th cells (19, 53). Indeed, several innate populations are known to be potent sources of IL-17, including NK (54), NKT (55), and γδ T cells (56, 57), and these cell types are particularly influential in the gut and liver tissues where *S. mansoni* infection causes immunopathology in vivo.

The transfer of SEA-pulsed DCs in vivo elicited sufficient IL-10 to prevent the emergence of SEA-specific IL-17 in WT C57BL/6 mice (Fig. 2), and yet the essential source of that IL-10 appeared not to be regulatory T cells (Fig. 3) or indeed any T or B cell population (Fig. 4). Many other cell types are capable of secreting IL-10 (58, 59), including mast cells (60), neutrophils (61), and NK cells (62, 63), all of which can interact with DCs and contribute to T cell polarization (52). Recently identified innate lymphocyte populations, termed nuocytes, type 2 multipotent progenitor cells, or natural helper cells, were also shown to be significant sources
of regulatory cytokines during helminth infection (64–66) and could also secrete IL-10. IL-10 can regulate T cell function both directly and indirectly, by altering the activation and cytokine production of APCs (58). In the context of IL-17 induction, IL-10 inhibits Th17 cells by direct signaling and via the heightened activation of Foxp3+ regulatory T cells (67, 68). In schistosomiasis, IL-10 prevents a persistent Th1 response to S. mansoni by restricting IL-12 production; IL-10-deficient mice show increased hepatic inflammation and elevated lymphocyte expression of IFN-γ and TNF-α relative to WT controls (16, 17), and this response is absent in IL-10/IL-12 doubly deficient animals (69). Interestingly, IL-17 was not measured in these studies, and the IL-12p40−/− animals involved were deficient in both IL-12 and IL-23 and, hence, would be predicted to be impaired in both Th1 and Th17 responses (14). We now provide clear evidence that IL-10 suppresses SEA-specific IL-17 in vivo. The association of schistosome-induced IL-17 with hepatic pathology (14) suggests that IL-10–mediated regulation of IL-17 might represent a valuable therapeutic target. IL-10 was reported to limit IL-17 expression during other infectious diseases, including tuberculosis (70) and influenza (71). Indeed, in an autoimmune setting, the induction of IL-10 was reported to be the key factor that distinguishes a nonpathogenic from a pathogenic Th17 response (72).

One particularly interesting aspect of our study is the demonstration that the IL-10–mediated suppression of SEA-induced IL-17 is abrogated by concurrent bacterial stimulation of the initiating DCs. The ability of the host’s microflora to potentiate adaptive-immune responses against coincident Ags is a topic of considerable current research (73, 74). During infection with the protozoan parasite Toxoplasma gondii, commensal bacteria are an essential adjuvant for the induction of antiparasite immunity (75). Microbial interactions can also benefit the parasite: bacterial inhabitants of the host’s large intestine bind to the eggs of the parasitic nematode Trichuris muris and promote hatching (76). In the steady state, commensal bacteria in the gut play a particularly important role in the development of Th17 cells (77, 78). Our data showed that coincident bacterial stimulation can convert the response to SEA-pulsed DCs from one dominated by IL-10 and other Th2 cytokines into one including IL-17 (Fig. 5) and, thus, featuring pathogenic potential (15). A proinflammatory role for intestinal microflora may be particularly relevant in schistosomiasis, an infection in which the parasite eggs breach the gut wall and, hence, provide commensal bacteria with significant opportunity to enter the host. Because distinct bacterial microflora differ in their ability to promote Th17 differentiation (78), it will be interesting to assess whether particular bacteria more potently elicit schistosome-specific IL-17 than others. The association between schistosome-specific IL-17 and schistosome-induced pathology (15) might then provide the opportunity to treat the severe sequelae of schistosomiasis with antibiotics. A similar antibiotic-based therapeutic approach was recently described in a mouse model of IL-17–driven autoimmunity (79).

Our data illustrate the capacity of coincident bacterial stimulation to elicit SEA-specific IL-17, but not IFN-γ, induction from SEA-activated DCs. DCs direct P. acnes and SEA into distinct Ag-processing compartments and retain the ability to generate concurrent, intact P. acnes–specific Th1 and SEA-specific Th2 responses (48). One explanation for the oppositely polarized immune responses directed against each Ag is the different densities of Ag presented on the DC surface (48). An alternative explanation is that, during Ag presentation, DCs can spatially segregate the stimulatory molecules and cytokines that polarize the responding T cell populations. Directional release of cytokines across the immunological synapse has been observed in vitro (80), but it is not common to all cytokines (80, 81). We identified IL-6 as the critical factor elicited by coincident bacterial stimulation of SEA-pulsed DCs that results in SEA-specific IL-17 induction (Fig. 5). These data suggest that IL-6 is promiscuously released from DCs, affecting not just T cells engaged in synapses formed around bacterial Ags but also T cells contacting the DC elsewhere on its surface. IL-6 was shown to promote both Th17 differentiation (27, 82) and IL-17 release from other lymphocyte populations (54). In the context of schistosome egg immunization, IL-6 is antagonized by the action of IL-10 (83), and our data provided further evidence of an inhibitory relationship between these two cytokines (Fig. 5). Therefore, the ability of bacterial microflora to enhance schistosome-specific IL-17 responses may be particularly important in patients with naturally low IL-10 expression (84). Interestingly, our data also suggest that DC-derived IL-6 can restrain IL-5 and IL-13 production in vivo (Fig. 5D). Because Th2 cytokines can be inhibitory to Th17 development in vitro (19, 53), it is possible that bacterial stimulation of DCs can also promote a schistosome-specific IL-17 response indirectly, through IL-6–mediated suppression of the Th2 response.

Host genetics has a powerful influence on the extent of immunopathology during schistosomiasis, as illustrated by the varying disease susceptibilities of different mouse strains to this pathogen (13). In high-pathology CBA mice, DC-derived IL-23 and IL-1β were shown to be critical for the IL-17 response that characterizes severe disease in this strain, whereas IL-6 was dispensable (15, 35, 85). In contrast, we demonstrated in this study that DC-derived IL-6 is essential for schistosome-specific IL-17 induction in C57BL/6 mice (Fig. 5). Together, these data highlighted that different cytokine networks may dominate in different hosts, with our study revealing that coincident bacterial exposure can be an additional risk factor in schistosomiasis through its promotion of an IL-6–driven IL-17 response.

Our data demonstrate that C57BL/6 DCs can elicit IL-17 responses directed against schistosome Ags but that this IL-17 is normally inhibited by the concomitant induction of IL-10. Such IL-10–mediated regulation is abrogated if the priming DC receives concurrent bacterial stimulation. In humans, the immune response to schistosome infection is affected by multiple factors, including parasite exposure and host variability (86). Our study suggests that bacterial infection or commensal microbiota may also be an influential factor.

Acknowledgments

We thank Nico Ghilardi (Genentech, South San Francisco, CA) for provision of p19−/− mice, Prof. I. Paxton and R. Brown (University of Edinburgh, Edinburgh, U.K.) for preparation of heat-killed P. acnes, and Dr. Maurice Gallagher (University of Edinburgh) for provision of S. typhimurium.

Disclosures

The authors have no financial conflicts of interest.

References

mainly retinoic acid receptor-related orphan receptor (gamma)t+ and respond preferentially under inflammatory conditions. J. Immunol. 183: 2142–2149.

plurinmunffective IL-17+ and regulatory IL-10+ RORgammat+ T cells. J. Exp. Med. 205: 1381–1393.


tivation of Syk kinase and MyD88 adaptor protein pathways by bacteria pro-
motes regulatory properties of neutrophils. Immunity 31: 761–771.


