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The Pathogenic Th17 Cell Response to Major Schistosome Egg Antigen Is Sequentially Dependent on IL-23 and IL-1β

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CBA/J mice infected with the helminth Schistosoma mansoni develop severe CD4 T cell-mediated hepatic granulomatous inflammation against parasite eggs associated with a robust Th17 cell response. We investigated the requisites for Th17 cell development using novel CD4 T cells expressing a transgenic TCR specific for the major Sm-p40 egg Ag, which produce IL-17 when stimulated with live schistosome eggs. Neutralization of IL-23 or blockade of the IL-1 receptor, but not IL-6 neutralization, abrogated egg-induced IL-17 secretion by transgenic T cells, whereas exogenous IL-23 or IL-1β reconstituted their ability to produce IL-17 when stimulated by syngeneic IL-12p40–deficient dendritic cells. Kinetic analysis demonstrated that IL-17 production was initiated by IL-23 and amplified by IL-1β. Significantly, schistosome-infected IL-12p40–deficient or IL-1R antagonist-treated CBA/J mice developed markedly reduced hepatic immunopathology with a dampened egg Ag-specific IL-17 response. These results demonstrate that the IL-23–IL-1–IL-17 axis has a central role in the development of severe schistosome egg-induced immunopathology. The Journal of Immunology, 2011, 187: 5328–5335.

Schistosomes are blood-dwelling trematode helminths responsible for causing one of the most prevalent tropical parasitic diseases in the world—schistosomiasis. During infection with the species Schistosoma mansoni, adult worm pairs within the mesenteric venous plexus produce an estimated 300 parasite eggs per day, of which a fraction becomes lodged in the liver, precipitating CD4 T cell–mediated granulomatous inflammation and fibrosis (1). The majority of individuals develop a mild intestinal form of schistosomiasis, whereas in 5–10% of infected hosts with hepatosplenic schistosomiasis the disease is severe and life threatening (1). A similar disease heterogeneity is observed in an experimental murine model of schistosomiasis, where C57BL/6 (BL/6) and CBA/J (CBA) mice develop mild and severe perivascular granulomatous inflammation, respectively (2). In BL/6 mice, an initial proinflammatory Th1-type response to schistosome egg Ag (SEA) is gradually superseded by a Th2-dominated environment marked by the rise of IL-4, IL-5, IL-10, and IL-13, whereas in CBA mice, the Th1 response persists for the duration of the infection (2). An effective Th1 to Th2 switch is critical for the modulation of immunopathology and extended survival (3).

Concomitant immunization of typically low-pathology wild type (WT) BL/6 mice with SEA emulsified in CFA (SEA/CFA) results in marked exacerbation of egg-induced granulomatous lesions bearing considerable resemblance to those naturally occurring in CBA mice (4). Significantly, infected SEA/CFA-immunized BL/6 mice deficient in the common IL-12/IL-23 subunit IL-23p40 produced low levels of IL-17 and IFN-γ, and were completely refractory to pathology exacerbation, whereas similarly treated mice deficient in the IL-12–specific subunit IL-12 p35 developed severe lesions associated with high levels of IL-17, despite the absence of IFN-γ (5). Furthermore, infected SEA/CFA-immunized mice deficient for the IL-23–specific subunit IL-23 p19, developed reduced immunopathology and failed to express IL-17 in the granulomatous lesions (6), and in vivo administration of anti–IL-17 Ab significantly reduced immunopathology in both CBA and SEA/CFA-immunized BL/6 mice (5). These data strongly implicate the IL-23/IL-17 axis, and thus Th17 cells, as major factors in the development of severe forms of schistosomiasis.

In addition to the described proinflammatory cytokine milieu, we have found that high pathology in schistosome-infected CBA mice correlates with a significant expansion of CD4 T cells recognizing the immunodominant epitope 234–246 of the major Sm-p40 schistosome egg Ag (Sm-p40234–246) (7). Sm-p40 is one of the most abundant components of the schistosome egg and was found to elicit a strong Th1 polarized response in CBA (H-2b) mice (2, 8). Analysis of the I-Ak–restricted Sm-p40234–246–specific CD4 T cells revealed a clonally restricted population with a TCR expressing Vα11.3Vβ8 (7), which paved the way for the development of a novel CBA mouse expressing a transgenic (Tg) TCR specific for Sm-p40234–246.

In this study, we demonstrate that naive schistosome egg Ag–specific Tg CD4 T cells produce IL-17 and IFN-γ when stimulated with peptide Sm-p40234–246, or with live schistosome eggs, which allowed us to investigate the requirements for egg Ag–specific Th17 cell development and function in the high pathology CBA strain. We found optimal IL-17 production by Sm-p40234–246–specific TCR Tg T cells stimulated by syngeneic dendritic cells (DCs) to be dependent on IL-23, which in turn, induces IL-1β secretion in the presence of live schistosome eggs.
Importantly, the central in vivo roles of IL-23 and IL-1β were demonstrated by a significant reduction in hepatic immunopathology and in IL-17 production in infected IL-12p40-deficient CBA mice, and CBA mice in which IL-1 signaling was blocked by IL-1Ra. These results identify IL-23 and IL-1β as critical factors in the generation of egg Ag-specific Th17 cells involved in the pathogenesis of severe schistosomiasis.

Materials and Methods

Mice, infections, and treatments

Schistosome egg Ag-specific TCR Tg mice were generated following the protocol described by Waldner et al. (9). The Va11.3 and VB6 TCR chains were isolated from genomic DNA purified from an Sm-p40 234–246–specific T cell hybridoma and cloned, respectively, into pTrCass and pTBcass expression cassettes (provided by Dr. Diane Mathis, Harvard Medical School, Boston, MA). Linearized pTrCass and pTBcass vectors were introduced into BL/6 (H-2b) oocytes by microinjection to produce Tg founders, which were subsequently backcrossed for >10 generations to CBA (H-2b) mice, resulting in a TCR Tg mouse on the pathology-prone CBA background capable of presenting the I-Aβ-restricted immunodominant peptide Sm-p40324–336. Mice were genotyped for the presence of the Va11.3VB6 TCR gene rearrangement using standard PCR genotyping techniques on genomic DNA obtained from tail biopsies. The primer sequences for these PCRs were:

*Va11.3 forward: 5′-GCCCTGATATCTTTTTACTCCTGG-3′
*Va11.3 reverse: 5′-TAGCCAGGTTGAGAACCATTGCCC-3′
*VB6 forward: 5′-CACCCCAAGGCCCAGAAACAGGT-3′
*VB6 reverse: 5′-ATCCITGACGGGAATGTCCTC-3′

Female CBA (CBA), C57BL/6 (BL/6), and IL-12p40–deficient B6.129S1-PtgSb/-Tg (Tg) mice were purchased from The Jackson Laboratory (Bar Harbor, ME), and Swiss Webster mice were obtained from Charles River (Wilmington, MA). To generate IL-12p40–deficient IL-12p40−/− mice on the H-2k background, the Sm-p40 egg Ag-coding vector was introduced into B6.129S1-Ii12b/112mIj Tg mice backcrossed to CBA mice for >10 generations. Bone marrow-derived DCs from the IL-12p40−/− mice failed to produce bioactive IL-12p40 protein following stimulation with schistosome eggs or LPS (Fig. 3A).

In some experiments, age-matched female CBA, BL/6, and IL-12p40−/− mice were infected by ip injection with 85 cercariae of *S. mansoni* (Puerto Rico strain), which were obtained from infected Biomphalaria glabrata snails (provided by Dr. Fred Lewis, Biomedical Research Institute Rockville, MD), through National Institutes of Health–National Institute of Allergy and Infectious Diseases contract N01AI-55270. In addition, some mice were treated daily with ip injection with 300 μg of recombinant IL-1Ra for the last 10 d of a 7-wk infection (10). Swiss Webster mice were inoculated in an identical fashion for the purpose of isolating schistosome eggs.

To examine expression of the Va8β TCR chain, bulk splenocytes from uninfected TCR Tg mice were cultured in 1 ml of medium together with 10 μg/ml of SEA for 48 h, after which culture supernatants were analyzed by a His Fusion Protein Purification Kit (Pierce). To eliminate any potential contaminating endotoxin, the Sm-p40 preparation was treated using the Endotoxin Removal Maxi Kit (Norgen Biotek, Thorold, Ontario, Canada) and purification was verified using the Limulus amebocyte lysate assay (Cambrex, East Rutherford, NJ). Protein concentrations were assessed with the Bradford Protein Assay Kit (Pierce). The 30-mer peptide (QVAVRPSDNSQIKPASQLAVAKGIVHGLS), which harbors the immunodominant epitope Sm-p40324–336 (in bold), as well as peptide Sm-p40304–313, was synthesized and purified with HPLC at the Tufts University Core Facility.

In vitro cocultures and cytokine analysis

DCs derived from CBA or IL-12p40−/− mice (1 × 10^6 cells/ml) were stimulated with 100 or 500 live eggs or LPS (Sigma, St. Louis, MO). After 24 h, culture supernatants were collected, sterile filtered, and assayed by ELISA to measure the levels of IL-12p40, IL-10, and TGF-β (using mAb, standards, and protocols from BD Pharmingen) and for IL-6 and TNF-α (using mAb, standards, and protocols from R&D Systems). DCs (1.25 × 10^5) plus CD4 T cells (1 × 10^6) purified from the spleens of uninfected TCR Tg mice were cultured in 1 ml of medium together with Sm-p40 egg Ag, Sm-p40324–336 (both 2 μg/ml), or 50 or 200 eggs. After 4 d, culture supernatants were removed, sterile filtered, and assayed by ELISA for IL-17, IL-6, and IFN-γ (using mAb, standards, and protocols from R&D Systems). Sm-p40 egg Ag, Sm-p40324–336 (both 2 μg/ml), or 50 or 200 eggs. After 4 d, culture supernatants were removed, sterile filtered, and assayed by ELISA for IL-17, IL-6, and IFN-γ (using mAb, standards, and protocols from R&D Systems). In some experiments, neutralizing mAb against IL-12p40, IL-6 (both 2 μg/ml; BD Pharmingen), and IL-23 p19 (10 μg/ml; eBioscience, San Diego, CA), IL-1α, at indicated concentrations, IL-6 (1 and 10 μg/ml; R&D Systems), and IL-23 (1 and 10 μg/ml; R&D Systems) were added either individually or in combinations to the DC-CD4 T-cell egg cocultures, as indicated.

Cell preparations and cytokine analysis from infected mice

After a 7-wk infection, mesenteric lymph node cells (MLNC) were isolated from CBA, BL/6, IL-12p40−/−, and CBA IL-1α–treated mice, as described previously (4). Cell preparations were >95% viable by trypan blue exclusion. Bulk MLNC suspensions (5 × 10^6) were cultured in the presence or absence of 15 μg/ml of SEA for 48 h, after which culture supernatants were tested for the presence of IL-17 and IFN-γ by ELISA (R&D Systems).

Real-time quantitative RT-PCR

Total RNA was isolated from DC–T cell cocultures in the presence or absence of schistosome eggs using the TRIzol reagent according to the manufacturer’s instructions (Invitrogen, Carlsbad, CA). RNA (1–5 μg) was
subjected to DNase I treatment (Roche Molecular Biochemicals, Indianapolis, IN) and reverse-transcribed using an RT2 first-strand kit (C-03). Real-time quantitative RT-PCR on 10 ng of cDNA from each sample was performed by Taqman analysis. All reactions were performed using an ABI 7300 instrument. GAPDH levels were measured in a separate reaction and used to normalize the data. Reagents and protocols for Taqman real-time quantitative PCR were obtained from Applied Biosystems (Foster City, CA). Using the average mean cycle threshold (Ct) value for GAPDH and the gene of interest for each sample, the following equation was used to obtain normalized values (11):

$$\frac{Ct\text{ GAPDH} - Ct\text{ gene of interest}}{Ct\text{ GAPDH}} \times 10^6.$$

**Hepatic immunopathology**

Liver samples obtained from infected CBA, BL/6, IL-12p40−/−, and CBA IL-1RA–treated mice were fixed in 10% buffered formalin, processed and sectioned by routine histopathologic technique, stained with H&E, and examined by optic microscopy. The size of the granulomatous lesions was measured by computer-assisted morphometric analysis (Image-ProPlus Software; Media Cybernetics, Bethesda, MD), as described elsewhere (4). For each liver, 8–15 granulomas with single eggs were analyzed.

**Statistical analysis**

ANOVA and Student t tests were used to determine the statistical analysis of the differences between groups. A p value < 0.05 was considered significant; values were calculated with GraphPad Prism.

**Results**

**Characterization of the Sm-p40 egg Ag-specific TCR Tg CD4 T cell response**

The discovery of an expanded and remarkably restricted CD4 T cell response against the major Sm-p40 egg Ag in schistosome-infected high-pathology CBA mice (7) enabled us to develop a mouse transgenically expressing a Vα11.3Vβ8 TCR specific for the immunodominant peptide Sm-p40234–246. Indeed, 96% of splenic CD4 T cells from Tg mice expressed Vβ8 with only 9% of T cells from WT controls (Fig. 1A, 1B). Naïve Tg splenocytes exhibited strong proliferative responses to Sm-p40 and peptide Sm-p40234–246, but not to irrelevant peptide Sm-p40304–333; none of these Ags stimulated non-Tg cells (Fig. 1C, 1D). Strikingly, stimulation with peptide Sm-p40234–246 induced robust production of IL-17 and IFN-γ (Fig. 1E, 1F), but only elicited a mild IL-5 and IL-10 response (Fig. 1G, 1H). Sm-p40, Sm-p40234–246, or live schistosome eggs serving as a natural source of Sm-p40 elicited robust IL-17 production by TCR Tg CD4 T cells in cultures with syngeneic DCs (Fig. 1I). TCR Tg CD4 T cells also secreted IFN-γ, but no detectable IL-5 or IL-10 (data not shown), suggesting that they are biased toward a proinflammatory cytokine response. These findings demonstrate the successful development of the first mouse TCR Tg T cell system specific for a schistosome egg-derived or any other helminth-derived Ag.

**IL-23 and IL-1β are required for IL-17 production by schistosome egg Ag-specific TCR Tg CD4 T cells**

Although initially shown to depend on IL-23, the differentiation of the Th17 lineage was subsequently demonstrated in various experimental systems to depend on IL-6 and TGF-β, as well as IL-1β and IL-21 (12). We previously demonstrated that schistosome egg-stimulated CBA, but not BL/6, DCs induce T cell IL-17 production following nonspecific TCR engagement via IL-23 and IL-1β (11). The development of Sm-p40–specific TCR Tg mice enabled us to analyze the differentiation and effector functions of schistosome egg Ag-specific T cells. When these TCR Tg CD4 T cells expressed as means of triplicate ELISA determinations ± SD. Data are from one representative experiment of three.
were cocultured with DCs and live schistosome eggs, they produced significant amounts of IL-17. Abs against IL-12p40 and IL-23p19 significantly inhibited IL-17 production; IL-1Ra was equally effective (Fig. 2). In contrast, anti–IL-6 Ab, used at a concentration capable of neutralizing 10 ng of recombinant IL-6, which far exceeds the amount naturally produced by egg-stimulated CBA DCs (Fig. 3B) and data not shown), failed to significantly affect IL-17 production (Fig. 2).

To further examine the cytokine requirements for Th17 cell differentiation, we made use of DCs from novel IL-12p40−/− (IL-12p40−/−) mice, which we developed by backcrossing IL-12p40−/− BL/6 mice to the CBA background for >10 generations. DCs from the IL-12p40−/− CBA mice failed to produce detectable levels of IL-12p40 upon stimulation with either eggs or LPS (Fig. 3A), whereas their IL-6 (Fig. 3B) and TNF-α (Fig. 3C) responses were similar to those of WT controls. Production of the immunomodulatory cytokine TGF-β was similar between unstimulated WT CBA and IL-12p40−/− DCs, but was not further enhanced upon stimulation with eggs or LPS (Fig. 3D). Both DC populations demonstrated similar IL-10 responses after stimulation with LPS, but not in the presence of live eggs (Fig. 3E). Egg-stimulated DCs from IL-12p40−/− mice induced significantly less IL-17 secretion by TCR Tg CD4 T cells (Fig. 4A) and also elicited a reduced IFN-γ response (Fig. 4B). However, egg-stimulated IL-6 production was similar in IL-12p40−/− and WT DC–CD4 T cell cocultures (Fig. 4C). These findings demonstrate that IL-23 and IL-1β are crucial for inducing robust egg Ag-specific Th17 responses.

Exogenous IL-23 and IL-1β sequentially reconstitute the ability of IL-12p40−/− DCs to stimulate egg-induced IL-17 production by TCR Tg CD4 T cells

We further investigated the role of IL-23 and IL-1β in egg Ag-specific Th17 cell development using egg-stimulated DC–Tg T cell cocultures in vitro. In this system, live eggs stimulate WT CBA DCs to induce robust IL-17 production by TCR Tg CD4 T cells, whereas IL-12p40−/− DCs, which cannot make IL-23, are completely unable to do so (Fig. 5A). The addition of IL-23 and IL-1β to IL-12p40−/− DC–T cell cocultures leads to expected IL-17 production, even in the absence of eggs, suggesting that these two cytokines are sufficient to induce IL-17 without further stimulation from the schistosome eggs (Fig. 5A). Accordingly, the addition of exogenous IL-23 fully reconstitutes egg-stimulated IL-17

FIGURE 2. Neutralization of IL-23 or IL-1 receptor blockade inhibits egg-induced IL-17 production by TCR Tg CD4 T cells. WT CBA DCs (from two mice) and TCR Tg CD4 T cells (from five mice) were cultured in the presence or absence of 200 eggs together with the indicated blocking reagents used at the following concentrations: anti–IL-12p40 (2 μg/ml), anti–IL-23p19 (10 μg/ml), anti–IL-6 (2 μg/ml), and IL-1Ra (50 or 500 ng/ml). IL-17 levels in 48-h supernatants were measured by ELISA. Cytokine levels are expressed as means of triplicate ELISA determinations ± SD. Data are from one representative experiment of three. Results from a culture stimulated with 2 μg/ml Sm-p40 are shown for comparison. ***p < 0.001 compared with egg-stimulated cultures without blocking reagent.

FIGURE 3. Cytokine production profile of egg-stimulated WT CBA versus CBA IL-12p40−/− DC. DCs from WT CBA and CBA IL-12p40−/− mice (two mice per group) were cultured with eggs or control LPS for 24 h, and culture supernatants were assayed for IL-12p40 (A), IL-6 (B), TNF-α (C), TGF-β (D), and IL-10 (E) by ELISA. Cytokine levels are expressed as means of triplicate ELISA determinations ± SD; where appropriate, background cytokine levels from unstimulated controls were subtracted. Data are from one representative experiment of three. nd, not detectable.
expression and secretion by Tg CD4 T cells in cultures with IL-12–p40−/− DCs (Fig. 5A, 5B), implying that IL-1 is contributed by the egg-stimulated DCs. This was demonstrated to be the case because IL-17 levels were significantly reduced in egg-stimulated cocultures treated with IL-1Ra (Fig. 5A, 5B). Interestingly, the addition of IL-1β alone induced some IL-17 production, which was enhanced in the presence of eggs. However, the addition of IL-6 failed to elicit significant levels of IL-17 in cocultures with DCs from IL-12p40−/− mice (Fig. 5A). Furthermore, exogenous IL-23 in egg-stimulated cultures augmented the expression of the Th17-associated effector cytokine IL-22 and chemokine CXCL1, as well as IL-23R and the lineage-specific transcription factor Rorc (Rorc; Fig. 5C–F) (12). The enhanced expression of these factors was also strongly dependent on intact IL-1 signaling, as the addition of IL-1Ra completely abrogated the IL-23-induced effects. These findings suggest that the TCR Tg Th17 phenotype can be attained by cognate interaction via specific Ag or an appropriate cytokine combination independent of antigenic stimulation.

A kinetic analysis of IL-23 and IL-1β secretion involved in the differentiation of TCR Tg Th17 cells in cocultures with cytokine-sufficient WT DCs revealed that stimulation with 50 schistosome eggs first elicited IL-23 production, which reached peak levels at 12 h and then declined, but was followed by a steady increase in IL-1β (Fig. 6A). Most importantly, only with increasing levels of IL-1β was there effective IL-17 production (Fig. 6A). A similar profile was observed when 200 eggs were used for stimulation, except that IL-23 levels were slightly higher at 36 h and absolute cytokine levels were correspondingly increased (Fig. 6B).

Schistosome-infected IL-12p40−/− CBA mice or CBA mice treated with IL-1Ra develop milder hepatic immunopathology and produce reduced levels of IL-17

We have demonstrated the importance of IL-23 and IL-1β in the development of Sm-p40–specific Th17 cells in vitro. To lend in vivo relevance to these observations, we examined the egg-induced hepatic immunopathology and Th17 responses in infected IL-12p40−/− mice and WT CBA mice treated in vivo with IL-1Ra during the last 10 d of a 7-wk infection. Schistosome-infected IL-12p40−/− mice exhibited a significant reduction of granulomatous inflammation in comparison with WT CBA mice (Fig. 7A), which was paralleled by a significant drop in serum levels of aspartate aminotransferase, an enzyme that reflects liver cell damage (not shown). SEA-stimulated MLNC from infected IL-12p40−/− mice produced significantly lower amounts of IL-17 than did the CBA controls (Fig. 7B). Moreover, IFN-γ levels were also markedly reduced (Fig. 7C) as the production of this cytokine is largely dependent on IL-12p70, of which IL-12p40 is a subunit (13). In addition, there was also significantly less granulomatous inflammation (Fig. 7D), as well as lower IL-17 (Fig. 7E) and IFN-γ production (Fig. 7F) in IL-1Ra–treated mice than in untreated controls. These findings imply that the loss of IL-23 or IL-1 causes a significant reduction of immunopathology and in the levels of IL-17, thus supporting their role in mediating disease severity in vivo.

Discussion

The development of a novel CBA mouse transgenically expressing a TCR recognizing the immunodominant peptide Sm-p40234–246 allowed us to directly assess the cytokine requirements for the development of schistosome egg Ag-specific Th17 cells. The naive TCR Tg CD4 T cells are highly sensitive to stimulation with Sm-p40, either in recombinant form or naturally delivered by schistosome eggs—the pathogenic form of the S. mansoni life cycle. In the CBA mice, these I-Ak–restricted TCR Tg T cells develop into IL-17− and IFN-γ−producing cells, representing the Th17 phenotype, and produce reduced levels of IL-17 and IL-1β (Fig. 7A, 7B) (14). The importance of both IL-23 and IL-1β is further highlighted by the lack of IL-17 production in cultures using IL-12p40−/− DCs, or in IL-23 sufficient cultures in which IL-1β function was blocked by IL-1Ra. Significantly, both IL-23 and IL-1β were also effective in vivo, as mice lacking the IL-12p40 subunit or treated with IL-1Ra produced lower levels of IL-17 and exhibited milder egg-induced hepatic immunopathology. Several other pathogen-based and autoimmune models such as Candida albicans, Klebsiella pneumoniae, experimental autoimmune encephalomyelitis, collagen induced arthritis, and inflammatory bowel disease similarly emphasize the importance of IL-23 and IL-1β as critical Th17 cell inducers (16–19). Of im-
The importance is that IL-1β has been widely implicated as an essential factor in human Th17 cell development, either independently or in cooperation with IL-23, IL-6, and TGF-β (20).

The molecular mechanisms leading to IL-23 and IL-1 production for Th17 cell development vary greatly according to the structure of pathogen-derived ligands, the type of host innate receptors that recognize them, and the ensuing downstream signaling circuits resulting in cytokine-specific gene activation (20, 21). For example, the yeast form of C. albicans engages TLR2, TLR4, and dectin-1, resulting in the secretion of both IL-12p70 and IL-23, whereas the hyphal form is recognized by TLR2 and dectin-2 leading to the sole secretion of IL-23 (20). Moreover, a Syk-CARD9-dependent signaling cascade initiated following β-glucan binding by dectin-1 or dectin-2 is essential for the development of Th17 responses (19). Although DCs stimulated with the β-glucan-containing molecule zymosan elicited the production of TNF-α, IL-6, and IL-23, the differentiation of Th17 cells was shown to be completely dependent on IL-23 (19). In human T cells, co-recognition of bacterial peptidoglycan by TLR2 and nucleotide-binding oligomerization domain 2 elicited the production of IL-23 and IL-1, resulting in the differentiation of Th17 cells (22). An additional layer of complexity concerns the transcription factors that control the expression of cytokines critical for Th17 cell development, as well as those expressed by Th17 cells themselves. Thus, mice deficient for the NF-κB family member c-Rel exhibited a significant decrease in IL-23p19 expression (23), a defect in the differentiation of Th17 cells, and consequent resistance to immunization-induced experimental autoimmune encephalomyelitis (24). STAT3 was also demonstrated to influence IL-23–induced IL-17 production (25). Specifically, IL-23, in tandem with IL-1β, augmented the expression of IL-23r and modified the IL-17 and Rorc loci, emphasizing the importance of IL-23 in the differentiation of Th17 cells (26). In addition to the original master regulator of the Th17 lineage, RoRγt, other transcription factors...
factors such as RORα and IFN regulatory factor 4 (IRF4) were also shown to contribute to the development of Th17 cells (12).

We demonstrated previously that stimulation with schistosome eggs is under genetic control, resulting in a Th17 response in high pathology-prone CBA mice, but not in low-pathology BL/6 mice (11). However, it is still unclear whether this is a consequence of differential recognition of egg-derived ligands by a distinct display of innate receptors or whether the divergence occurs at the level of intracellular signaling. As egg components are heavily glycosylated (27), glycans and glycoconjugates are some of the more intensively investigated schistosome-derived ligands. For example, glycans terminating in Lewis (Le) Le^x_ or pseudo-Le^x_ were shown to interact with TLR4 or the C-type lectin receptor DC-SIGN (CD209), respectively, whereas LacdiNAc was identified as a putative ligand for the S-type lectin galectin-3 (27). By comparison, the egg glycoprotein Omega-1 was shown to induce Th2-biased immune responses in a MyD88/TRIF-independent fashion, suggesting that TLRs were not the primary PRRs involved in its recognition (28). Thus, the precise ligands, receptors, and signaling pathways involved in the development of immunopathogenic responses in schistosomiasis still need to be elucidated.

A somewhat controversial issue concerns the role of IL-6 in the generation of Th17 cells. In our hands, IL-6 had no apparent role in inducing IL-17 responses by schistosome egg Ag-specific CD4 T cells, a finding that contrasts with the notion that IL-6, in combination with TGF-β, is required for the differentiation of the

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**FIGURE 6.** Kinetic analysis of IL-23, IL-1β, and IL-17 production in WT CBA DCs and TCR Tg CD4 T cell cocultures following stimulation with eggs. WT CBA DCs plus TCR Tg CD4 T cells were stimulated with 50 (A) or 200 (B) eggs. At the indicated times, culture supernatants were tested for IL-23, IL-1β, and IL-17 by ELISA, DCs were obtained from two mice; CD4 T cells were obtained from three to five mice. Cytokine levels are expressed as means of triplicate ELISA determinations ± SD. Data are from one representative experiment of two.

**FIGURE 7.** Reduced hepatic immunopathology and proinflammatory cytokine production in schistosome-infected CBA IL-12p40^-/- mice and WT CBA mice treated with IL-1Ra. WT CBA and IL-12p40^-/- mice (A–C) or WT CBA mice treated or not with IL-1Ra (D–F) were infected with 85 cercariae of *S. mansoni*. After 7 wk (A, D), granulomatous lesions were measured on H&E-stained liver sections by computer-assisted morphometric analysis, MLNC were stimulated with 15 μg/ml of SEA for 48 h, and IL-17 (B, E) and IFN-γ (C, F) in culture supernatants were measured by ELISA. Cytokine levels are expressed as means of triplicate determinations ± SD; background levels from unstimulated MLNC were subtracted. Each group consisted of three to five mice. Data are from one experiment representative of five for A–C and two for D–F. *p < 0.05, **p < 0.01.
Th17 cell subset (29, 30). This difference may be explained by the nature of the T cells used in each experimental system. The requirement for IL-6 and TGF-β for inducing Th17 cell differentiation was demonstrated using FACs-sorted CD4+CD45+ or CD4+CD62L+ naive lymphocytes (12). In contrast, the CD4 T cells used in our study were purified from a normal, unselected spleen cell population. As such, these T cells had been previously exposed to IL-6, leading to enhanced IL-23R expression (31) and consequent increased susceptibility to stimulation with DC-derived IL-23 and IL-1β for Th17 cell differentiation without the need for additional exogenous IL-6. This scenario is more compatible with one encountered by a normal mature immune system following exposure to a new Ag. Further evidence of shifting paradigms is presented in a recent study demonstrating that TGF-β is dispensable for Th17 cell development and, in agreement with our findings, that Th17 cells with the most pathogenic potential are dependent on IL-23 and are amplified by IL-1β (26). Nevertheless, it is possible that IL-6 could have a role in Th17 responses to certain microbial PRR ligands, which in contrast to schistosome egg Ags result in greater DC activation (32, 33). Our observations provide novel insight into the cytokine circuits involved in the development of egg Ag-specific Th17 cells that mediate high-pathology schistosomiasis and identify possible targets for intervention to curtail severe disease.

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Disclosures

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References


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