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Helminth Antigens Modulate TLR-Initiated Dendritic Cell Activation

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There is increasing awareness that helminth infections can ameliorate proinflammatory conditions. In part, this is due to their inherent ability to induce Th2 and, perhaps, regulatory T cell responses. However, recent evidence indicates that helminths also have direct anti-inflammatory effects on innate immune responses. In this study, we address this issue and show that soluble molecules from the eggs of the helminth parasite Schistosoma mansoni (SEA) suppress LPS-induced activation of immature murine dendritic cells, including MHC class II, costimulatory molecule expression, and IL-12 production. SEA-augmented LPS-induced production of IL-10 is in part responsible for the observed reduction in LPS-induced IL-12 production. However, analyses of IL-10−/− DC revealed distinct IL-10-independent suppressive effects of SEA. IL-10-independent mechanisms are evident in the suppression of TLR ligand-induced MAPK and NF-κB signaling pathways. Microarray analyses demonstrate that SEA alone uniquely alters the expression of a small subset of genes that are not up-regulated during conventional TLR-induced DC maturation. In contrast, the effects of SEA on TLR ligand-induced DC activation were striking: when mixed with LPS, SEA significantly affects the expression of > 100 LPS-regulated genes. These findings indicate that SEA exerts potent anti-inflammatory effects by directly regulating the ability of DC to respond to TLR ligands.

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5 Abbreviations used in this paper: SEA soluble egg Ag; DC, dendritic cell; HA, hyaluronic acid; HEA, hen egg Ag; ICSBP, IFN consensus-binding protein; DC-SIGN, DC-specific intercellular adhesion molecule-grabbing nonintegrin; WT, wild type.
which are proinflammatory. These genome-wide analyses also revealed that SEA prevents the LPS-induced down-regulation of expression of 37 genes. Moreover, an additional 20 genes not induced by LPS or SEA alone are uniquely up-regulated in SEA/LPS-copulsed DC. Together, these findings indicate that SEA exerts potent control over TLR ligand-induced DC maturation, and possesses anti-inflammatory effects not only by virtue of its ability to induce Th2 responses, but also by directly suppressing the ability of DC to produce proinflammatory mediators.

Materials and Methods

Animals and reagents

Six- to 12-wk-old wild-type (WT) C57BL/6 (B6) or B6 IL-10–/– mice were purchased from The Jackson Laboratory (Bar Harbor, ME). DC activation was induced by 100 ng/ml LPS (Escherichia coli serotype 0111: B4; Sigma-Aldrich, St. Louis, MO), 10 μg/ml CpG 1668 (MWG Biotec, High Point, NC), 10 μg/ml poly(I:C), or 500 μg/ml HA (Sigma-Aldrich). SEA was prepared aseptically, as described previously (13), and used at 50 μg/ml. Hen egg white collected under aseptic conditions provided a source of endotoxin-free control hen egg Ag (HEA) and was used at 50 μg/ml. Ab specific for IL-10R (clone 1B1.3a; BD Pharmingen, San Diego, CA) was used at 5 μg/ml to block endogenous IL-10 signaling.

Characterization of DC cytokine production

Bone marrow cells were aseptically collected from the femurs of WT or IL-10–/– mice and cultured in the presence of 20 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ) for 10 days, as described (13). WT or IL-10–/– DC at 2 × 10⁶/ml were incubated in 96-well plates under the conditions indicated. Cytokine ELISAs were performed using paired mAb in combination with recombinant cytokine standards (BD Pharmingen), as described (13). Intracellular cytokine levels were assessed by flow cytometry, as described previously (18), with PE-conjugated anti-IL-12 mAb (BD Pharmingen; clone C11.5). Samples were collected using a FACS-Calibur flow cytometer (BD Pharmingen), and data were analyzed with FlowJo Software (Tree Star, Ashland, OR).

Characterization of DC surface protein expression

WT DC (2 × 10⁶/ml) were incubated for 6 h at 37°C in medium alone, or in the presence of SEA, LPS, or SEA/LPS. Expression of MHCII, CD80, and CD86 was quantified using FITC-, PE-, or allophycocyanin-conjugated mAb specific for IL-10R (clone 1B1.3a; BD Pharmingen, San Diego, CA) and stained with streptavidin-PE. The fluorescence signal was excited at 488 nm and detected at 509/24 nm.

Western blots

A total of 5 × 10⁶ IL-10–/– DC were solubilized in 50 μl of 2× NuPage sample buffer (Invitrogen Life Technologies, Carlsbad, CA), 1 mM sodium vanadate, and 10 mM sodium fluoride (Sigma-Aldrich). Equivalent amounts of cellular extracts were resolved on 10% Bis-Tris NuPage gels (Invitrogen Life Technologies) and transferred to Immobilon membrane (Millipore, Billerica, MA). Blots were incubated with primary Ab against phosphorylated p38, ERK1/2, and JNK (Cell Signaling Technology, Beverly, MA), and bound Ab was visualized using secondary HRP-conjugated Ab and LumigenOLO (Cell Signaling Technology). Data were acquired with an Image Reader Las-1000 Lite (Fuji Film, Stamford, CT).

EMSA

IL-10–/– DC were incubated, as indicated, and supernatants were removed and whole cell extracts were prepared, as described previously (19). EMSAs were performed with the double-stranded oligodeoxynucleotides corresponding to the palindromic B site (5′-GGGAATTCCC-3′), as previously described (20); equivalent free probe was detected in all samples, confirming that probe was added in excess to each. Complexes were generated using the Superscript Choice System (Invitrogen Life Technologies), and cRNA was made with BioArray HighYield RNA Transcription kit (Enzo Biochem, Farmington, NY). Biotin-labeled, fragmented (200 nt or less) cRNA was hybridized for 16 h at 45°C to MOE430A arrays (Affymetrix, Santa Clara, CA) by the Microarray Facility at the University of Pennsylvania (www.med.upenn.edu/microarr). The arrays were washed and stained with streptavidin-PE. The fluorescence signal was excited at 570 nm, and data were collected on a confocal scanner at 3 μm resolution. Initial data analysis was performed by the Microarray Facility using Affymetrix Microarray Suite 5.0 to determine gene expression levels. The individual probe signals were multiplied by a scaling factor specific for the chip, which was determined by adjusting the chip average to the arbitrary target of 150. Two fully independent experiments were compared by Principal Component Analysis in GeneSpring 6.2 (Silicon Genetics, Redwood, CA) and subjectively determined to be similar and treated as duplicate hybridizations. Genes that were not detected in at least two of the eight samples examined were excluded from further analysis. Pairwise ANOVA analyses were performed by PartekPro 5.1 (Partek, St. Charles, MO) on log-transformed values. Gene trees and expression tables were generated in GeneSpring using Pearson Correlation as the similarity metrics.

Results

SEA suppresses TLR ligand-induced DC activation

As anticipated, LPS activated DC to increase surface expression of MHCII, CD80, and CD86 (Fig. 1A) and to produce IL-12 p40/p70 (Fig. 1B). In contrast, SEA alone did not affect these parameters, but markedly suppressed LPS-induced MHCII, CD80, or CD86 up-regulation and IL-12 production (Fig. 1, A and B). To investigate whether the suppression of DC activation by SEA was specific to LPS or common to multiple TLR ligands, we pulsed DC with SEA plus or minus the TLR3, 4, and 9 ligands; poly(I:C); and SEA/LPS (S/L). Surface expression levels of MHCII, CD80, and CD86 were quantified using FITC-, PE-, or allophycocyanin-conjugated mAb specific for IL-10R (clone 1B1.3a; BD Pharmingen, San Diego, CA) and stained with streptavidin-PE. The fluorescence signal was excited at 488 nm and detected at 509/24 nm.

FIGURE 1. SEA inhibits TLR ligand-induced IL-12 production. A, DC were pulsed for 4 h with medium alone (−), SEA, and LPS, or copulsed with SEA/LPS (S/L). Surface expression levels of MHCII, CD80, and CD86 were measured by ELISA. B, DC were pulsed with medium alone (−), SEA, and LPS, or copulsed with SEA/LPS (S/L). C, DC were pulsed with CpG, poly(I:C), or HA alone or with SEA. D, DC were pulsed with LPS, SEA/LPS (S/L), or HEA/LPS (H/L). IL-12 p40 and p70 levels in 24-h culture supernatants were measured by ELISA. *, Significant differences by Student’s t test (p < 0.05). Data points represent means ± SEM of data from three or more experiments.
SEA augments TLR ligand-induced IL-10 production

IL-10 is a known regulator of IL-12 production (21) and plays a key role in the Th2 polarization of the anti-egg Ag response (2). Consequently, we examined whether SEA-stimulated production of IL-10 could account for the SEA-mediated inhibition of LPS-induced DC activation. Although SEA alone failed to directly induce IL-10 production by DC, it significantly augmented LPS-induced production of this cytokine (Fig. 2A, and data not shown). SEA also promoted IL-10 production in response to CpG and HA, but not to poly(I:C) (Fig. 2B). Again, the effects observed appeared to be due to specific properties of SEA, because HEA had no effect on LPS-induced IL-10 production (Fig. 2C).

SEA suppresses IL-12 production by IL-10-dependent and -independent mechanisms

The augmentation of LPS-induced production of IL-10 by SEA suggested that this cytokine was involved in the suppression of IL-12 production. To examine this issue, we pulsed DC with SEA, LPS, and SEA/LPS in the presence or absence of anti-IL-10R Ab and measured IL-12 production. The presence of anti-IL-10R resulted in greatly increased levels of IL-10 in the supernatants of LPS and SEA/LPS-pulsed DC, consistent with effective blockade of the IL-10R (Fig. 3A), but did not reveal otherwise undetectable levels of IL-10 or IL-12 production by DC pulsed with SEA alone (data not shown). However, blocking endogenous IL-10 signaling did result in increased IL-12p40/p70 production by DC pulsed with LPS or with SEA/LPS (Fig. 3A). Nevertheless, even when the IL-10R was blocked, DC stimulated with SEA/LPS continued to produce significantly less IL-12p40/p70 than did DC pulsed with LPS alone (Fig. 3A). The IL-10-independent effects of SEA on TLR ligand-induced DC activation were strikingly illustrated by intracellular cytokine analyses, which revealed that SEA caused a 4-fold reduction in the percentage of IL-10−/− DC making IL-12 in response to LPS (Fig. 3B). Together, these data demonstrate that while IL-10 plays a role in the inhibition of IL-12 production by SEA, there are clearly SEA-dependent, IL-10-independent components of the suppressive mechanism. This is consistent with the observation that SEA coordinately inhibited both IL-12 and IL-10 production by poly(I:C) (Fig. 2B).

FIGURE 2. SEA augments TLR ligand-induced IL-10 production. A, DC were pulsed for 24 h with medium alone (−), SEA, LPS, or SEA/LPS (S/L). B, DC were pulsed, as in A, with CpG, poly(I:C), or HA alone or with SEA. C, DC were pulsed, as in A, with LPS, SEA/LPS (S/L), or HEA/LPS (H/L). IL-10 levels in culture supernatants were measured by ELISA. *, Significant differences by Student’s t test (p < 0.05). Data points represent means ± SEM of data from three or more experiments.

FIGURE 3. IL-10-independent SEA-mediated suppression of TLR ligand-induced DC activation. A, DC were pulsed for 24 h with medium alone (−), SEA, LPS, or SEA/LPS (S/L) with or without anti-IL-10R, as indicated, to block signaling by endogenous IL-10. IL-10 and IL-12 in 24-h culture supernatants were measured by ELISA. *, Significant differences by Student’s t test (p < 0.05). Data points represent means ± SEM of data from three or more experiments. B, IL-10−/− DC pulsed for 8 h with medium (−), SEA, LPS, or S/L were stained for CD11c and intracellular IL-12 and analyzed by flow cytometry. Data from one experiment are representative of three independent experiments. Numbers represent percentage of total cells lying within quadrants.

FIGURE 4. IL-10-independent SEA-mediated alterations in LPS-induced signaling and gene expression

To begin to characterize the IL-10-independent modification of TLR ligand-induced DC maturation by SEA, we examined these cells for changes in the MAPK and NF-κB signaling pathways that are crucial for TLR-initiated activation and IL-12 production (22). As expected, LPS induced phosphorylation of the MAPK family members, p38, JNK, and ERK within 10 min (Fig. 4A), and activation of NF-κB within 30 min (Fig. 4B). SEA alone induced transient low-level phosphorylation of p38 and ERK, and had no measurable effect on JNK. However, SEA dramatically reduced LPS-stimulated phosphorylation of p38, JNK, and ERK, as well as...
induced changes in gene expression. We reasoned that this ap-
strated significant differences. Our initial analysis focused on SEA-
Expression tables were generated using only genes that demon-
tistically significant changes in gene expression between groups.
different experimental conditions were performed to identify sta-
gene expression caused by SEA. Results from two experiments
broadly for IL-10-independent changes in TLR ligand-induced
ation. To examine this issue, we used microarray analyses to search
for IL-10-independent changes in TLR ligand-induced gene expression caused by SEA. Results from two experiments
contrast with LPS, which induced changes in the expression of
of 551 genes (data not shown). The expression of the majority of
genes affected by SEA, including Trif-pending, Tgm2 (transglu-
taminase 2), Pkry (protein kinase inhibitor γ), and the transcription
factor, Sp1, was up-regulated, whereas that of a small group, in-
cluding Skk4 (serine/threonine kinase 4), was down-regulated (Fig. 5).
The expression of the majority of the genes directly affected by
SEA was either unaltered, or repressed, by LPS (Fig. 5).

More striking effects of SEA were evident in a comparison of the
effects on gene expression of LPS vs LPS plus SEA (Fig. 6). Again using a stringent statistical cutoff of \( p < 0.003 \), we found that SEA
had a significant effect on the expression of 103 LPS-stimulated
genes. The effects of SEA could be assigned to one of three groups.
First, consistent with our finding that SEA suppresses the produc-
tion of IL-12 by LPS, we found that SEA had a negative effect on
the LPS-induced expression of 46 genes, among which were Icsbp
(IFN consensus-binding protein, IC5B), Cdl3, the chemokine
Ccl3 (MIP1α), Tnfsf7 (OX40L), Map2k1 (MAP kinase 1, or
MEK1), and Bcl10 (B cell lymphoma 10), as well as Il-12b (IL-
12p40), (Fig. 6A). Second, SEA prevented the LPS-induced down-
regulation of 37 genes, including Casp9 (caspase 9), Pip5k1c
(phosphatidylinositol-4-phosphate 5 kinase type 1), Dgkz (diacy-
glycerol kinase ζ), Dapk (death-associated protein kinase 1), and
Sp1 (Sp1) (Fig. 6B). Third, in conjunction with LPS, SEA pro-
moted the increased expression of a small group of genes normally
down-regulated and/or unaffected by LPS or SEA alone (Fig. 6C).
This group includes Anpep (alanyl aminopeptidase, or CD13),
Ar16ip (ADP ribosylation factor-like 6 interacting protein 1), and
Ipr1 (type 1 inositol-3-phosphate receptor).

Discussion
We have shown that the schistosome Ag, SEA, significantly alters
TLR ligand-induced activation of DC. The effect of SEA on DC is
consistent with a requirement for helminths to prevent inflamma-
tion, especially as mediated by the IL-12/IFN-γ axis (12). We
noted that SEA inhibited IL-12 production induced by endogenous
(HA) as well as pathogen-derived (LPS) TLR4 ligands, and patho-
gen-derived TLR3 and TLR9 ligands (poly(I:C) and CpG, respec-
tively), indicating the anti-inflammatory effects of SEA could op-
erate at sites of tissue damage or exposure to microbial pathogens.

The abilities of SEA to suppress innate inflammation by enhancing
the production of IL-10 (Fig. 2) and by directly inhibiting DC
activation (Figs. 3, 4, and 6), as well as its potential to induce Th2
responses (12), suggest that schistosomes have multiple mecha-
nisms for preventing proinflammatory responses.

Although SEA promotes LPS- and CpG-induced IL-10 produc-
tion, it inhibits poly(I:C)-induced production of this cytokine.
Poly(I:C) differs from LPS and CpG in uniquely activating DC via
a TLR-dependent, MyD88-independent pathway (23). Conse-
sequently, one interpretation of our data is that SEA is able to inhibit
MyD88-independent, but not -dependent, pathways leading to
IL-10 production. However, both MyD88-dependent and -inde-
dendent pathways leading to IL-12 production can be blocked by
SEA, because CpG-, LPS-, and poly(I:C)-induced production of
this cytokine are inhibited by SEA. Thus, SEA appears to be able
to regulate multiple pathways downstream of TLRs, suggesting
that its effect could be proximal to signaling initiation.

How SEA suppresses DC activation remains unclear at this
time. Recent reports have indicated that ligation of the mannos
receptor or DC-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN) results in significant inhibition of the ability of DC to make IL-12 in response to TLR ligands (24, 25). DC-SIGN ligation was shown to lead to IL-10 production, which was implicated in, but not demonstrated to be responsible for, the observed suppression of DC function (25). This situation bears similarity to our findings. SEA has been reported to bind to DC-SIGN (26), and while we did not address a link between this event and suppression of DC activation, we do show that SEA significantly augments LPS-induced IL-10 production by DC, which

FIGURE 6. IL-10-independent SEA-mediated alterations in LPS-induced gene expression. Gene expression profiles of IL-10−/− DC pulsed for 6 h with medium alone (−), SEA, LPS, or SEA/LPS (S/L). Figure shows expression profile clustering of genes for which LPS induction is inhibited by SEA (A), LPS-induced inhibition of expression is prevented by SEA (B), and SEA and LPS synergize to promote gene expression (C). Normalized expression levels relative to median are displayed in yellow (median expression), red (increased expression), or blue (decreased expression), according to the expression/color bar. The gene trees were generated based on a pairwise ANOVA analysis of DC pulsed with LPS vs S/L-pulsed DC. Genes displayed are those for which the p value for the difference in expression between these two groups was <0.003. Data from two independent experiments (E1 and E2) are shown side by side. Raw data from experiment 1 are available at National Center for Biotechnology Information GenBank (GEO accession GSE1382).
contributed to the regulation of IL-12 production. Thus, it is feasible that SEA is inhibiting DC function by ligating DC-SIGN or functionally related lectins. Another possibility is that SEA contains a ligand(s) for G protein-coupled receptors, which in other systems have been shown to have profound effects on IL-12 production. For example, cholera toxin, PGE2, histamine, and C5a all suppress IL-12 production (27). Strikingly, as is the case for SEA, some of these ligands additionally augment TLR-initiated IL-10 production. In this vein, it was reported that larval schistosomes, and therefore perhaps schistosome eggs, produce PGE2, which significantly inhibits DC activation (28). Finally, we have also considered the possibility that SEA contains a molecule that is capable of interacting with FCyR, because the effects of SEA on LPS-stimulated DC are reminiscent of the modulatory effects of FCyR ligation on macrophage activation (29, 30). However, we found that DC generated from FCyRIIα−/− mice exhibit similar sensitivity as WT DC to SEA-mediated suppression of LPS-induced IL-12 production, making it clear that FCyR-mediated effects are not responsible for the inhibitory effects of SEA in our system (data not shown).

Although IL-10 clearly plays a role in the SEA-mediated suppression of TLR-initiated DC activation, it is apparent that SEA additionally induces a potent IL-10-independent mechanism(s) for suppressing MAPK and NF-κB activation and altering the expression of genes, including those encoding IL-12. Interestingly, the suppression of TLR signaling by SEA could itself be TLR mediated. Recent analyses of signaling pathways induced in DC by SEA or by a multivalent array of lacto-N-fucopentaose III, a common carbohydrate modification of schistosome egg glycoconjugates, have indicated preferential activation of ERK1/2 in the absence of the activation of p38 (31, 32), and have implicated TLR2 and TLR4 (31) in these pathways. ERK signaling without p38 involvement was shown to lead to c-Fos stabilization and the suppression of IL-12 production (32). However, our analyses of signaling in DC pulsed with SEA alone revealed phosphorylation of ERK and p38 that was slight in comparison with that seen in LPS-stimulated cells. Moreover, these effects seemed minor compared with the potent SEA-mediated suppression of LPS-induced phosphorylation of MAPK, including ERK, and of NF-κB activation.

We used microarray analyses to broadly examine the IL-10-independent effects of SEA on TLR-initiated DC activation. This approach confirmed the observed suppression of LPS-induced IL-12 production by SEA, and revealed multiple additional SEA-mediated changes in the response to LPS. Many of the LPS-induced genes suppressed by SEA were proinflammatory in nature (Fig. 6A). Of particular interest among this group is Icshp1, which has been shown to be important for IL-12 production and for the up-regulation of MHCI and CD80 in CD8α+ DC (the type used in this study) in response to microbial stimuli (33, 34). Other genes of interest in this group are Map2k1, which is partially responsible for ERK1/2 activation (35); Bcl10, which has been recently shown to play a role in NF-κB activation in response to LPS in macrophages (36); Cdl3, an important DC activation marker (37); Ccl3 (MIP1α), which encodes a proinflammatory chemokine; and Tnfrsf1a, TNF receptor 1, which is important for DC maturation in response to TNF-α. In addition to inhibiting expression of LPS-induced genes, SEA also prevented the down-regulation of a series of genes constitutively expressed by DC and suppressed by LPS, some of which encode signaling proteins, and others of which encode transcriptional regulators that may play a role in the observed changes in DC function precipitated by SEA. Among these are genes encoding Cdc42, a member of the Ras superfamily of GTP-binding proteins, and two members of the phosphoinositide production pathway, phosphatidylinositol-4-phosphate 5 kinase type 1, a type 1 phosphatidylinositol phosphate kinase, and diacylglycerol kinase ζ, which would support the generation of phosphoinositide-3 kinase, an enzyme known to down-regulate IL-12 production by negative feedback inhibition (38, 39). SEA also prevented the down-regulation of the ubiquitous transcription factor, Sp1, which has been shown to be important in IL-10 transcriptional regulation (40), and which may have diverse effects on DC activation. SEA and LPS were additionally found to be able to synergize to promote the expression of a series of genes that were unaffected by exposure of DC to either SEA or LPS alone. These data provide insights into the mode of action of SEA, and suggest candidate genes for future detailed analysis of the molecular basis of the effects of SEA on DC activation.

As a component of the studies described in this work, we examined the direct effect of SEA on the DC transcriptional program. In previous reports, based on analyses of a limited set of >20 maturation markers, we argued that SEA does not directly induce DC maturation (13). This finding was consistent with previous analyses of the effects of other helminths on DC (41). In this study, we report that SEA does, however, induce changes in expression of a small panel of genes, the majority of which are not up-regulated during LPS-induced DC maturation (Fig. 5). Based on the reported functions of these genes, it is currently unclear how any of them might confer upon DC the properties known to accompany exposure to SEA, such as the ability to induce Th2 responses, or to respond in a highly modified manner to TLR ligands. Nevertheless, the regulation of expression of a set of 29 genes in response to SEA provides a framework with which to begin to dissect the molecular basis of the effect of SEA on DC. In this context, it should be noted that our data contrast significantly with those recently reported from a microarray analysis of the effect of whole schistosome eggs on DC. This report indicated that eggs promote the expression of inflammatory genes, including MIP-1α and IL-12p40, and activate autocrine/paracrine signaling through the type 1 IFN receptor, leading to the induction of IFN-responsive genes (42). A possible explanation to account for the differences between these previous findings and those reported in this work is that we used a soluble extract of eggs (SEA), rather than whole eggs, to stimulate DC. In preliminary studies using DC made from type 1 IFN−/− mice, we have found that the inhibitory effects of SEA on TLR-mediated signaling are type 1 IFN independent (C. Kane and E. Pearce, unpublished observations).

We have shown that SEA has profound effects on TLR-initiated signaling. The overall picture emerging is one of SEA exerting anti-inflammatory effects on innate responses. Although we have shown that IL-10 plays a significant role in this process, it is clear that additional IL-10-independent inhibitory mechanisms are induced by SEA. It is appealing to imagine that the effects of SEA on DC reported in this work play a role in the intrinsic ability of SEA to induce Th2 responses, but this has yet to be shown experimentally. It is clear that TLR-induced DC activation via MyD88 is important for Th1 response induction (23), and the absence of MyD88 allows classic Th1-inducing Ag to induce Th2 responses (43). Consequently, failure of SEA to stimulate activation of signaling pathways downstream of MyD88, and its ability to strongly inhibit these pathways, are consistent with its ability to condition DC to induce Th2 responses.

Schistosomes have evolved to allow their eggs to move from the intravascular space to the external environment. In the case of _S. mansoni_, which lives in the portal vasculature, this passage compromises the integrity of the intestinal epithelial layer, allowing for the translocation of bacteria from the gut into the tissues and bloodstream (12). Moreover, many eggs fail to traverse the gut and rather are carried by the blood flow to the liver, where they become
trapped in large numbers in the sinuses. These eggs initiate granulomatous pathological changes, which render the liver acutely susceptible to experimentally elevated levels of LPS or IL-12 (44, 45). Perhaps to control this type of inflammation during infection, eggs inhibit TLR-mediated DC activation, and activate innate and adaptive immune responses that result in the production of the anti-inflammatory cytokines IL-10 and IL-4 (12). These cytokines act in concert to regulate the development of potentially life-threatening inflammation that can be induced by conventional TLR ligands and Th1 cells in infected animals.

Our work has focused on a murine system, but a recent report has indicated that anti-inflammatory effects of schistosomiasis are evident in people, because PBMC from schistosome-infected patients exhibit reduced responsiveness to LPS (46). Consistent with the view that the inhibition of inflammatory processes is a feature of helminth parasites in general, ES-62, a secreted glycoprotein from the nematode *Acanthocheilonema viteae*, has been reported to have similar immunomodulatory properties to SEA, being able to inhibit LPS-induced inflammatory cytokine secretion by macrophages (47). The extent to which the anti-inflammatory properties of helminths are effective is evident in settings in which helminth infection and/or Ag prevent severe Th1-mediated autoimmune pathologies (17, 48, 49). We are currently working to understand the molecular basis for the anti-inflammatory properties of SEA.

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