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Schistosoma mansoni Egg Antigen-Mediated Modulation of Toll-Like Receptor (TLR)-Induced Activation Occurs Independently of TLR2, TLR4, and MyD88▽

Colleen M. Kane, Euihye Jung, and Edward J. Pearce*

Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

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Unlike most pathogens, helminth parasites and their products induce strong Th2 responses, and dendritic cells (DCs) and macrophages exposed to helminth antigens generally fail to produce interleukin-12. Rather, it has been shown that helminth products such as soluble egg antigens (SEA; a soluble extract from *Schistosoma mansoni* eggs) inhibit the activation of DCs in response to classical Toll-like receptor (TLR) ligands such as lipopolysaccharide or CpG. Nevertheless, recent work has suggested that TLR4 and/or TLR2 plays an important role in the recognition of helminth products by DCs and macrophages and in the development of Th2 responses. Using DCs derived from TLR4$^{-/-}$, TLR2$^{-/-}$, or MyD88$^{-/-}$ mice, we have demonstrated that the ability of SEA to modulate DC activation is MyD88 independent and requires neither TLR4 nor TLR2. Moreover, TLR2 and TLR4 are not required for SEA-pulsed DCs to induce Th2 responses in naïve mice.

Helminth parasites, which colonize organ systems as diverse as the lymphatics, gastrointestinal tract, and vasculature, have evolved multiple immunomodulatory mechanisms to evade host immune responses (20). A delicate balance is required in these chronic infections to establish parasite survival without eliciting lethal immunopathology. This balance is illustrated in schistosomiasis, which is caused by the trematode *Schistosoma mansoni* and causes chronic morbidity in more than 200 million people (24). Following infection, worms migrate to the portal vasculature, where they mature and pair. This early phase of infection is characterized by a Th1 response. After worm pairing, females lay eggs that cross the intestinal barrier to be excreted in the feces. However, some eggs become lodged in the intestinal wall and liver sinusoids, where soluble egg antigens (SEA) induce a polarized Th2 response (23). The Th2 response correlates with the downmodulation of the initial proinflammatory Th1 response to migrating immature worms and results in granuloma formation. Failure to switch to a Th2 response leads to hepatotoxic liver disease and host death (3, 6, 11).

The mechanism by which host innate immune cells recognize SEA remains unclear. Pathogens such as bacteria, viruses, and intracellular parasites express conserved molecular signatures that are shared within classes of pathogens and their free-living relatives. These are recognized by highly conserved pattern recognition receptors (PRRs) expressed by innate defense cells, including dendritic cells (DCs) and macrophages. PRRs include C-type lectins and Toll-like receptors (TLRs) (10, 32). TLRs are the most well-described PRRs, and DC activation by TLR ligation is considered to play a major role in the coordination of innate and adaptive immune responses during infection (22). Typically, the ligation of TLRs initiates a proinflammatory program, which promotes innate defense mechanisms and adaptive Th1 or Th17 immune responses to the invasive pathogens (22). There is evidence, however, that lipopolysaccharide (LPS) activation of TLR4 can induce DCs to support the development of Th2 responses (5, 15).

Emerging data have demonstrated that phospholipids or glycoproteins unique to extracellular helminths ligate TLRs to induce an anti-inflammatory and Th2-inducing antigen-presenting cell phenotype. A phosphorylcholine-containing glycoprotein, ES-62, from the nematode *Acanthocheilonema viteae*, has been shown to induce a polarized Th2 response and to work via TLR4 to modulate antigen-presenting cell activation by a variety of TLR ligands (7, 33). There is also evidence that *S. mansoni* products can stimulate antigen-presenting cells through TLRs. A lipid fraction from *S. mansoni* eggs containing lysophosphatidylserine has been shown, in a TLR2-dependent mechanism, to induce the activation of DCs that promote Th2 and regulatory T-cell development (28), and lacto-*N*-fucopentaose III (LNFPIII), a synthetic copy of a schistosome egg glycan, has been shown to promote Th2 differentiation by DCs via a TLR4-dependent pathway (26).

Here, using gene-targeted mice, we demonstrate conclusively that the anti-inflammatory and Th2-inducing characteristics of SEA are MyD88 independent and require neither TLR2 nor TLR4.

MATERIALS AND METHODS

Animals and reagents. C57BL/6 (B6), TLR2$^{-/-}$ (B6.129-*Tlr2tm1Kir*/J), C57BL/10 (B10), and TLR4$^{-/-}$ (C57BL/10ScNJ) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). MyD88$^{-/-}$ mice were bred and maintained under specific-pathogen-free conditions at the University of Pennsylvania and were a gift from Larry Turka. DC activation was induced by the following TLR ligands: Pam-3Cys (5 μg/ml; EMC Microcollections GmbH, Tübingen, Germany), poly(I:C) (10 μg/ml; InvivoGen, San Diego, CA), LPS (100 ng/ml; *Escherichia coli* serotype 0111:B4; Sigma-Aldrich, St. Louis, MO), or CpG ODN 1826 (10 μg/ml; Coley Pharmaceutical Group, Ottawa, ON, Canada). SEA was prepared aseptically as previously described and used at 50 μg/ml (19). Antibody (Ab) specific for the interleukin-10

* Corresponding author. Mailing address: Department of Pathobiology, School of Veterinary Medicine, 318 Hill Pavilion, 380 S. University Ave., Philadelphia, PA 19104. Phone: (215) 573-3493. Fax: (215) 746-2295. E-mail: ejpearce@mail.med.upenn.edu.

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receptor (IL-10R) (clone 1B1.3a; BD Pharmingen, San Diego, CA) was used at 10 μg/ml to block endogenous IL-10 consumption.

Generation of DCs. Bone marrow cells were aseptically collected from the femurs and tibias of wild-type (WT), TLR2$^{-/-}$, TLR4$^{-/-}$, or MyD88$^{-/-}$ mice, and 1×10^6 cells were cultured in untreated six-well plates for 8 to 10 days in 3 ml of complete DC medium consisting of RPMI (Mediatech Inc., Herndon, VA), 100 U/ml penicillin-streptomycin (Mediatech), 2 mM L-glutamine (Mediatech), 10% fetal bovine serum (HyClone, Logan, UT), and 0.05 mM 2-mercaptoethanol (Sigma) and in the presence of 20 ng/ml granulocyte-macrophage colony-stimulating factor (PeproTech, Rocky Hill, NJ). On day 3 of culture, 3 ml of fresh medium was added, and on days 6 and 8, 3 ml of medium was removed and replaced with fresh medium. Nonadherent cells, which were routinely between 80 to 85% CD11c$^+$, were collected and incubated at 4×10^5 cells/well in round-bottom 96-well plates under the conditions indicated.

Measurement of cytokine production by DCs. Cytokine enzyme-linked immunosorbent assays (ELISAs) were performed on 24-h cell-free supernatants by use of paired monoclonal Abs (MAbs) in combination with recombinant cytokine standards (BD Pharmingen) as described previously (19). Statistical significance was determined by a two-tailed Student *t* test with equal variance. Intracellular cytokine levels were assessed by the addition of brefeldin A (BD Pharmingen) to the culture for the final 4 h. Cells were surface stained with fluorescein isothiocyanate-conjugated anti-CD11c, washed, and fixed overnight in 1% formaldehyde (Sigma). The following day, cells were permeabilized with 0.1% saponin (Sigma) and incubated with phycoerythrin-conjugated anti-IL-12 MAb (clone C11.5; BD Pharmingen). Samples were washed twice and collected using a FACSCalibur flow cytometer (BD Pharmingen). Data were analyzed using FlowJo Software (Tree Star, Ashland, OR).

T-cell response priming in vivo. Immature B6, TLR2$^{-/-}$, B10, and TLR4$^{-/-}$ DCs were harvested on day 9 or 10 and pulsed overnight in medium alone or with 50 μg/ml SEA. Subsequently, DCs were washed twice in Hanks' balanced salt solution (Mediatech) to remove excess antigen, resuspended in Hanks' balanced salt solution, and injected intraperitoneally with a sterile 25-gauge needle into naïve mice (5×10^5 DC/mouse). After 7 days, single-cell suspensions (1×10^7/ml) of splenocytes from immunized mice were restimulated in vitro with SEA (50 μg/ml) as described previously (19). After 3 days, supernatants were collected for the determination of antigen-specific cytokine production by ELISA. Significance was established by a paired, two-tailed Student *t* test.

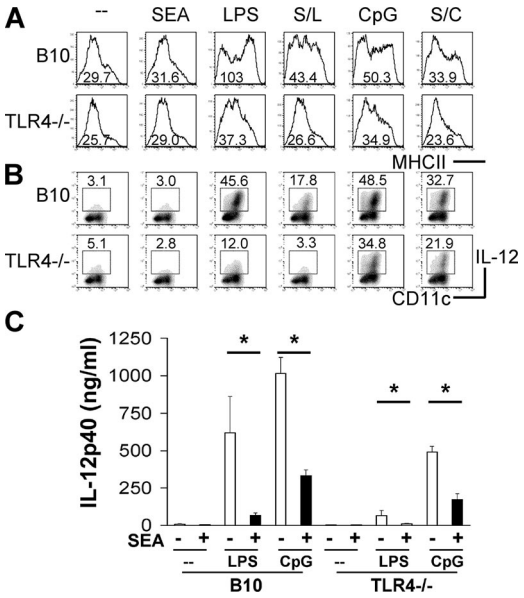

FIG. 1. SEA-mediated suppression of TLR-induced DC activation is TLR4 independent. (A) Immature DCs derived from B10 and TLR4$^{-/-}$ mice were pulsed in vitro with the TLR4 and TLR9 ligands LPS and CpG, respectively, either alone or in the presence of SEA (S/L and S/C, respectively, for LPS and CpG). MHC-II surface expression at 4 h poststimulation was measured by flow cytometry. Data are representative of three experiments. (B) LPS- and CpG-induced production of IL-12 in the presence or absence of SEA. Intracellular IL-12 at 6 h poststimulation was measured by flow cytometry. Data are representative of three experiments. (C) IL-12p40 production, measured by ELISA; DCs were pulsed for 24 h with TLR ligand LPS or CpG plus or minus SEA. Data points represent mean values ± standard errors (SE) from three experiments.

RESULTS

SEA-mediated suppression of CpG-induced DC activation is TLR4 independent. Previous work has demonstrated that SEA can suppress TLR ligand-induced DC activation by modulating the production of proinflammatory cytokines like IL-12 and preventing the maximal upregulation of DC maturation markers, such as major histocompatibility complex type II (MHC-II), CD80, and CD86 (4, 16, 34). Furthermore, other helminth products have been shown to act directly through TLR4 to alter DC maturation in response to inflammatory TLR ligands (7, 26). To determine whether the modulation of DC activation by SEA is TLR4 dependent, DCs were generated from the bone marrow of TLR4-deficient mice and congenic control WT B10 mice and pulsed with LPS and, as a control, the TLR9 ligand CpG alone or in the presence of SEA. DC maturation was assessed by MHC-II surface expression at 4 h, intracellular IL-12 production at 6 h, and secreted IL-12 protein levels at 24 h poststimulation. As expected based on previous findings, SEA alone did not alter MHC-II expression on, or induce IL-12 production by, WT or TLR4$^{-/-}$ DCs (Fig. 1) (16, 18). In contrast, WT DCs upregulated MHC-II and IL-12 production in response to LPS and CpG (Fig. 1). When WT DCs were pulsed simultaneously with SEA and either LPS or CpG, the upregulation of MHC-II was significantly inhibited (Fig. 1A). Moreover, SEA suppressed CpG-induced MHC-II upregulation in the TLR4$^{-/-}$ DCs (Fig. 1A). In WT DCs, SEA was capable of suppressing IL-12 production induced by either LPS or CpG. LPS stimulation of TLR4$^{-/-}$ DCs induced only low levels of MHC-II and IL-12p40 expression (this is consistent with previous reports demonstrating that commercially available LPS contains TLR2 ligands [12]). Nonetheless, TLR4-independent LPS-induced IL-12 production was suppressed by SEA (Fig. 1B). In addition, CpG-induced IL-12 production was inhibited by SEA in the TLR4$^{-/-}$ DCs (Fig. 1B). IL-12p40 was also measured in the 24-h culture supernatant DCs pulsed with TLR ligands either alone or in the presence of SEA (Fig. 1C). Consistent with the intracellular staining data, SEA suppressed LPS- and CpG-induced IL-12p40 production in both B10 and TLR4$^{-/-}$ DCs. These results demonstrate that the anti-inflammatory effects of SEA are TLR4 independent.

SEA-mediated suppression of CpG-induced DC activation is TLR2 independent. Previous work demonstrated that TLR2 ligands induce the phosphorylation of the mitogen-activated protein kinase family member extracellular signal-regulated kinase, which results in the activation of the transcription factor c-Fos and the suppression of IL-12 production, thereby biasing the immune response toward a Th2 phenotype (1). Of interest in this context is that a TLR2 ligand has been identified in the lipid fractions of extracts from egg and adult stages of *S. mansoni* (28). To determine if the suppression of TLR ligand-induced DC activation by SEA is dependent on TLR2, WT B6 and TLR2$^{-/-}$ B6 DCs were pulsed with the TLR2 and TLR9 ligands Pam3Cys (P3C) and CpG, respectively. Again,

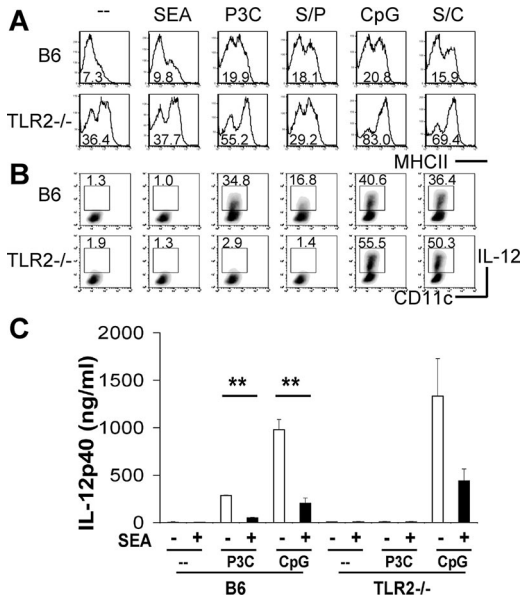

FIG. 2. SEA-mediated suppression of TLR-induced DC activation is TLR2 independent. (A) Immature DCs derived from B6 and TLR2$^{-/-}$ mice were pulsed in vitro with the TLR2 and TLR9 ligands P3C and CpG, respectively, either alone or in the presence of SEA (S/L and S/C, respectively, for LPS and CpG). MHC-II surface expression at 4 h poststimulation was measured by flow cytometry. Data are representative of three experiments. (B) LPS- and CpG-induced production of IL-12 in the presence or absence of SEA. Intracellular IL-12 at 6 h poststimulation was measured by flow cytometry. Data are representative of three experiments. (C) IL-12p40 production, measured by ELISA; DCs were pulsed for 24 h with TLR ligand LPS or CpG plus or minus SEA. Data points represent mean values ± SE from three experiments.

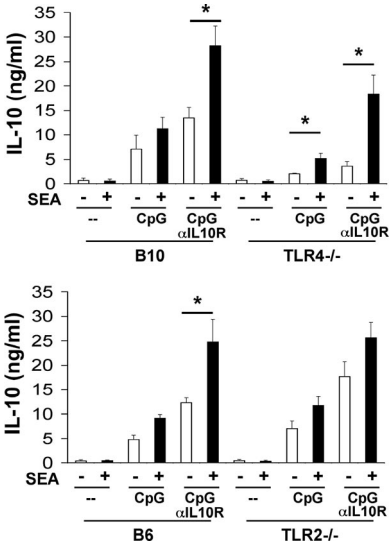

FIG. 3. SEA augments CpG-stimulated IL-10 production independently of TLR4 and TLR2. WT, TLR4$^{-/-}$, and TLR2$^{-/-}$ DCs were pulsed in vitro with the TLR9 ligand CpG, with or without SEA and the IL-10R-blocking Ab (αIL10R) for 24 h, and cytokine production was measured by ELISA. SEA augments the production of CpG-induced IL-10, which is more evident when endogenous consumption of IL-10 is blocked. Data points represent mean values ± SE from three experiments.

we observed no signs of DC activation following pulsing with SEA (Fig. 2). As expected, in WT DCs both P3C and CpG induced increased MHC-II expression (Fig. 2A) and production of IL-12 as measured by intracellular staining at 6 h (Fig. 2B) and by ELISA at 24 h (Fig. 2C). The ability of SEA to block activation was particularly apparent when the levels of IL-12p40 in DC supernatants were measured (Fig. 2C). For TLR2$^{-/-}$ DCs, background levels of MHC-II expression were high but nevertheless increased when CpG was added (Fig. 2A). MHC-II levels, although not IL-12 production, also increased in these cells in response to P3C (Fig. 2A), which was unexpected and presumably reflects the contamination of P3C with another TLR ligand, such as endotoxin. Nevertheless, SEA was able to suppress TLR2$^{-/-}$ DC activation by CpG, and again this was most apparent as reduced levels of IL-12p40 in the supernatants of DCs cultured with CpG plus SEA versus those cultured with CpG alone (Fig. 2C). These data indicate that the suppressive effects of SEA are not mediated by TLR2.

SEA augments CpG-stimulated IL-10 production independently of TLR4 and TLR2. Previous data have demonstrated that the SEA-mediated suppression of TLR ligand-induced IL-12 production by DCs is accompanied by a corresponding augmentation of IL-10 production (16). The TLR9 ligand CpG was used to examine whether this augmentation of IL-10 production by SEA occurs in the absence of TLR4. SEA alone does not induce IL-10 production (Fig. 3). However, as expected, B10 WT and TLR4$^{-/-}$ DCs and B6 WT and TLR2$^{-/-}$ DCs pulsed with CpG produced IL-10 (Fig. 3). IL-10 accumulation in the culture supernatants was increased when a MAb that blocks the IL-10R was included in the cultures to inhibit endogenous consumption of this cytokine (Fig. 3). When WT, TLR2$^{-/-}$, or TLR4$^{-/-}$ DCs were copulsed with CpG and SEA, the TLR ligand-induced production of IL-10 was increased, and this was particularly evident when the IL-10R was blocked (Fig. 3). These data provide further support for the ability of SEA to modulate TLR ligand-induced DC activation independently of TLR2 or TLR4.

SEA pulsed TLR$^{-/-}$ DCs induce an antigen-specific Th2 response in naïve mice. We and others have shown that immature bone marrow-derived DCs pulsed with SEA induce an antigen-specific Th2 response in naïve mice (4, 19). Previously, it has been shown that the *S. mansoni* carbohydrate LNFPIII produces a Th2-inducing DC phenotype in a TLR4-dependent manner (26). In contrast, the TLR2 ligand from the lipid portion of SEA has been shown to induce a tolerogenic DC phenotype in humans (29). To specifically ask whether SEA-pulsed DCs could induce a Th2 response despite the lack of TLR2 or TLR4, we pulsed immature DCs from WT and TLR$^{-/-}$ mice with SEA and adoptively transferred them into naïve syngeneic WT mice. We found that splenocytes from mice injected with SEA-pulsed DCs from either WT or TLR4$^{-/-}$ mice made IL-5 upon restimulation with SEA (Fig. 4A). Similarly, splenocytes from mice injected with SEA-pulsed DCs derived from WT or TLR2$^{-/-}$ mice made IL-5 upon restimulation with SEA (Fig. 4B). We routinely measured more IL-5 when using the B10 strain of mice (Fig. 4B) than when using the B6 strain (Fig. 4A), perhaps indicating a strain-dependent propensity of B10 mice to mount stronger Th2 responses. We were unable to

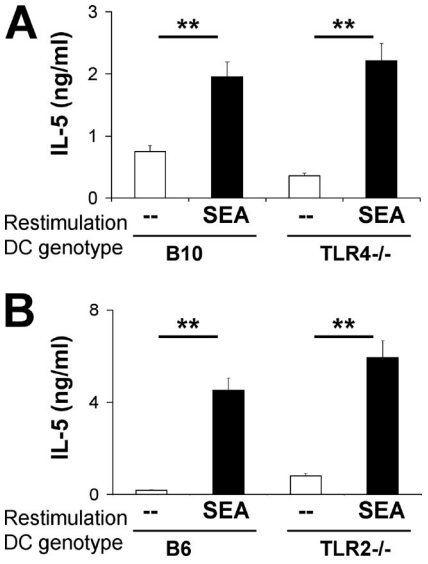

FIG. 4. SEA-pulsed TLR4$^{-/-}$ and TLR2$^{-/-}$ DCs induce antigen-specific Th2 responses in naïve mice. WT and TLR4$^{-/-}$ (A) and TLR2$^{-/-}$ (B) DCs were pulsed overnight with SEA, washed, and injected into naïve mice. Seven days later, splenocytes from immunized mice were recovered and cultured with or without SEA for 3 days. Supernatants were collected and antigen-specific T-cell production of IL-5 was measured by ELISA. SEA-pulsed DCs from WT and TLR$^{-/-}$ mice were equivalently capable of inducing antigen-specific responses in naïve mice. Data represent the mean values ± SE for nine mice per group ($P < 0.01$).

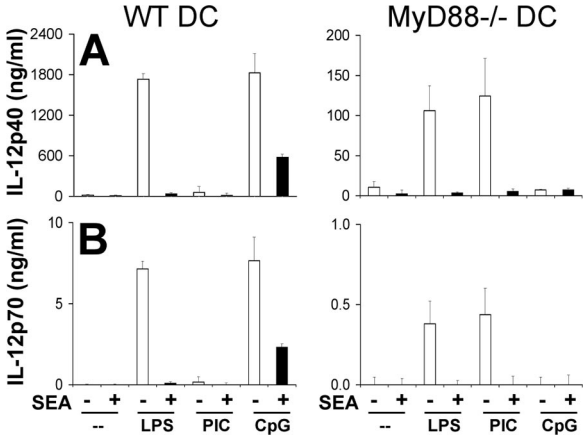

FIG. 5. SEA suppresses TLR ligand-induced IL-12 production in MyD88$^{-/-}$ DCs. WT and MyD88$^{-/-}$ DCs were pulsed with the TLR3, TLR4, and TLR9 ligands poly(I:C), LPS, and CpG, respectively, for 24 h. IL-12p40 (A) and IL-12p70 (B) levels were measured by ELISA. SEA suppressed the MyD88-dependent IL-12p40 and IL-12p70 production induced by LPS and CpG. poly(I:C)- and LPS-induced TRIF-dependent IL-12p40, and IL-12p70 production was also suppressed in a MyD88-independent manner. Data represent the mean ± SE from duplicate wells. The experiment was performed three times with similar results.

measure gamma interferon in antigen-restimulated cultures (data not shown). These results demonstrate that neither TLR2 nor TLR4 is required for SEA to condition DCs to polarize T-cell responses in a Th2 direction.

SEA suppresses TLR ligand-induced IL-12 production in MyD88$^{-/-}$ DCs. The adaptor molecule MyD88 associates with the cytoplasmic domains of most TLRs, except for TLR3, after ligand binding and recruits the IRAKs and TRAF6, resulting in their activation and the subsequent downstream activation of NF-κB and mitogen-activated protein kinases. Another adaptor molecule, TRIF, is responsible for TLR3- and TLR4-MyD88-independent activation of interferon regulatory factor 3 (IRF3) and NF-κB (25). We examined whether SEA could suppress DC activation in a MyD88-independent manner. WT and MyD88$^{-/-}$ DC were pulsed with LPS (the TLR4 ligand that activates both MyD88- and TRIF-dependent pathways) or with the TRIF-dependent TLR3 ligand poly(I:C) or the MyD88-dependent TLR9 ligand CpG. We found that WT DCs pulsed with LPS or CpG produced high levels of IL-12p40 and IL-12p70, but when DCs were copulsed with LPS or CpG plus SEA, cytokine production was dramatically suppressed (Fig. 5A and B). As expected, MyD88$^{-/-}$ DCs did not make IL-12p40 or IL-12p70 in response to the TLR9 ligand CpG (Fig. 5A and B). However, MyD88$^{-/-}$ DCs pulsed with LPS or poly(I:C) made low levels of IL-12p40 and IL-12 p70 (Fig. 5A and B), presumably via the TRIF-dependent pathway. The MyD88-independent production of IL-12 was potently suppressed by SEA (Fig. 5A and B). These findings indicate that the SEA-mediated suppression of TLR-induced DC activation is MyD88 independent.

DISCUSSION

We have demonstrated that the SEA-mediated modulation of TLR ligand-induced DC activation occurs independently of TLR2, TLR4, and, most importantly, MyD88. These data suggest that SEA binds to an as-yet-unknown PRR that antagonizes the proinflammatory response initiated by TLR ligands. Importantly, SEA-pulsed TLR2$^{-/-}$ and TLR4$^{-/-}$ DCs are capable of inducing a polarized Th2 response in naïve mice. These data, together with the recently published report that schistosome infection of MyD88$^{-/-}$ mice leads to a normal Th2 response (18), indicate that SEA does not depend on novel signaling pathways activated by TLR2 or TLR4 to elicit Th2 responses, as has been suggested by some (27).

To establish chronic infections, many helminths have evolved immunomodulatory mechanisms to subvert the host immune system. The filarial nematode *A. viteae* secretes the immunomodulatory molecule ES-62, which, similar to SEA, has been shown to promote Th2 polarization through its interaction with antigen-presenting cells. Importantly, ES-62 also suppresses TLR ligand-induced DC activation (7–9, 33). ES-62 modulates TLR-induced DC activation through a nonclassical interaction with TLR4 (21). Additionally, the synthetic *S. mansoni*-related glycan LNFPIII has been reported to condition DCs, in a TLR4-dependent manner, to become capable of inducing Th2 responses (26). Therefore, we assessed the role of TLR4 in the SEA-mediated modulation of TLR ligand-induced DC activation. We found that the presence of TLR4 was not required for SEA to suppress the TLR-induced upregulation of MHC-II and the production of the proinflammatory cytokine IL-12. Similarly, the SEA-mediated augmentation of CpG-induced IL-10 production was intact in TLR4-deficient DCs, as was the ability of SEA-pulsed TLR4$^{-/-}$ DCs to induce a polarized Th2 response. The data presented here illustrate that the important interactions between SEA and

DCs do not require the expression of TLR4 by the latter. In this context, it is important to note that *S. mansoni* products have not been documented to possess the phosphorylcholine moieties that are responsible for the immunomodulatory activity of ES-62, nor does SEA alone induce low levels of proinflammatory cytokine production as does ES-62, suggesting that this latter activity is unique to helminths that express phosphorylcholine-containing molecules and may reflect an evolutionary divergence of trematodes and filarial nematodes. The differences between our data using SEA and findings reported for LNFPIII may be due to the presence of multiple ligands and antigens in SEA versus the specific synthetic composition of LNFPIII. Furthermore, LNFPIII has not been shown to alter TLR-induced DC activation, suggesting that other SEA components are responsible for this anti-inflammatory property.

TLR2 was implicated as a possible receptor for the immunomodulatory activity of SEA by the findings that schistosome-derived lipids activate human DCs via TLR2 to elicit a tolerogenic phenotype (28). Moreover, during experimental infections of mice, the absence of TLR2 was associated with a defect in T-regulatory-cell response induction, which resulted in stronger, more prolonged Th1 responses and reduced Th2 immune responses, leading to increased immunopathology (17). In other studies, TLR2 ligands have been shown to elicit a DC maturation status that differs from that induced by TLR4 and TLR5 (1). This difference is characterized by enhanced extracellular signal-regulated kinase phosphorylation, suppressed IL-12p70 production, and the induction of a Th2 rather than a Th1 polarized immune response (1). Nevertheless, in our hands, the immunomodulatory characteristics of SEA were intact in TLR2$^{-/-}$ DCs, and SEA-pulsed TLR2$^{-/-}$ DCs were capable of inducing an antigen-specific Th2 response in naïve mice, suggesting that TLR2 is not the receptor through which SEA achieves these effects. However, it should be stressed that SEA, a phosphate-buffered saline-soluble extract of eggs, is unlikely to contain the immunomodulatory lipid described by van der Kleij and colleagues (28), and thus our results cannot exclude a role for this schistosome molecule, or TLR2, on DCs, or on cells other than DCs, in the overall response of the host to infection.

To more broadly address the question of a role for TLRs in the immunomodulatory activity of SEA, we used MyD88$^{-/-}$ DCs, which eliminates the possibility that SEA is signaling through TLR1, TLR2, TLR5, TLR6, TLR7, TLR8, or TLR9 in a conventional manner. We found that SEA suppresses the LPS- and poly(I:C)-induced production of IL-12p40 and IL-12p70 by MyD88$^{-/-}$ DCs, demonstrating not only the MyD88-independent immunomodulatory activity of SEA but also that SEA suppresses TLR ligand-induced, TRIF-dependent DC activation.

Although there is evidence that double-stranded RNA in SEA ligates TLR3, thereby resulting in STAT1 activation (2), it is unlikely that the immunomodulatory components of SEA bind to TLR3 for two reasons: first, in our hands, poly(I:C) did not inhibit LPS-induced IL-12 production (unpublished observations); and second, schistosome double-stranded RNA has been shown to result in the production of type I interferons rather than to inhibit DC activation (2). This suggests that the SEA-mediated modulation of DC activation occurs in a TRIF-independent fashion. The exclusion of a direct role for TLRs and their adaptor proteins in the SEA-mediated modulation of DC function is further supported by reports that the ability of SEA to condition DCs to prime Th2 responses in vitro is MyD88 and TLR4 independent (13) and that Th2 responses are intact in SEA-injected MyD88$^{-/-}$ mice (14). Moreover, Th2 responses do develop in MyD88$^{-/-}$ (18), TLR2$^{-/-}$ (17), and TLR3$^{-/-}$ (30) mice infected with *S. mansoni*. Thus, although it remains possible that SEA modulates DC activation via TLRs in a nontraditional manner, the data suggest that PRRs other than TLRs are responsible for this activity. Likely candidates for such PRRs are C-type lectins. Recent reports have implicated C-type lectins in the ability of antigen-presenting cells to respond to SEA and specifically in the ability of these receptors to limit DC activation (31).

We have shown here that SEA does not utilize TLR2, TLR4, or MyD88 to exert its immunomodulatory activity on TLR-induced DC activation. In addition, the expression of neither TLR2 nor TLR4 on DCs is required for SEA to induce a polarized Th2 response. The data support the view that PRRs other than TLRs are crucial in the recognition of SEA components and the initiation of signaling events that intersect with, and modulate, TLR signaling in DCs: C-type lectins are prime candidates for the PRRs that play this role (32). This is logical, as pathogens are encountered as complex mixtures of molecules that may interact with multiple receptors to elicit a specific response or manipulate the host's response to benefit the pathogens themselves. In the case of schistosomes, antigen secretions, such as those represented in SEA, with many possible immunomodulatory ligands, may have evolved to suppress IL-12 production and promote a Th2 response, favoring the survival of both pathogen and host.

ACKNOWLEDGMENTS

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