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CD209a Expression on Dendritic Cells Is Critical for the Development of Pathogenic Th17 Cell Responses in Murine Schistosomiasis

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In murine schistosomiasis, immunopathology and cytokine production in response to parasite eggs are uneven and strain dependent. CBA/J (CBA) mice develop severe hepatic granulomatous inflammation associated with prominent Th17 cell responses driven by dendritic cell (DC)-derived IL-1β and IL-23. Such Th17 cells fail to develop in low-pathology C57BL/6 (BL/6) mice, and the reasons for these strain-specific differences in APC reactivity to eggs remain unclear. We show by gene profiling that CBA DCs display an 18-fold higher expression of the C-type lectin receptor CD209a, a murine homolog of human DC-specific ICAM-3-grabbing nonintegrin, compared with BL/6 DCs. Higher CD209a expression was observed in CBA splenic and granuloma APC subpopulations, but only DCs induced Th17 cell differentiation in response to schistosome eggs. Gene silencing in CBA DCs and overexpression in BL/6 DCs demonstrated that CD209a is essential for egg-elicited IL-1β and IL-23 production and subsequent Th17 cell development, which is associated with SRC, RAF-1, and ERK1/2 activation. These findings reveal a novel mechanism controlling the development of Th17 cell-mediated severe immunopathology in helminthic disease.

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Schistosoma mansoni is a trematode helminth that causes extensive disease in the developing world, accounting for >200 million infections and 200,000 deaths per year. The principal cause of morbidity and mortality in S. mansoni infection is granulomatous inflammation and subsequent fibrosis around parasite eggs deposited in the liver and intestines (1–5). Most infected individuals develop mild gastrointestinal disease, but 5–10% develop life-threatening hepatosplenic schistosomiasis, which is characterized by severe liver fibrosis, splenomegaly, ascites, and portal hypertension (1–5).

Similar to human disease, heterogeneity of disease severity also is observed in an experimental murine model of schistosomiasis, infected CBA/J (CBA) mice develop severe hepatic pathology characterized by large, poorly circumscribed perioval granulomas (6–8). The severe pathology is mediated largely by T cell IL-17 production induced by egg Ag-stimulated dendritic cell (DC) secretion of IL-1β and IL-23 (9–12). In contrast, infected C57BL/6 (BL/6) mice develop mild pathology, with significantly smaller liver granulomas in a Th2-polarized environment (13). IL-17 is largely the product of Th17 cells, a highly proinflammatory subset of CD4+ effector T cells that also produce IL-22, CSF, CXCL1, CXCL2, and TNF-α (14–17). Presently, the mechanisms underlying the variation in egg-induced immunopathology and selection of dominant CD4+ T cell phenotype are incompletely understood; however, it is noteworthy that a recent study (18) of Schistosoma hematobium infection in humans similarly linked the development of pathology to an increase in Th17 cells.

We now demonstrate that genetic differences in pattern recognition receptor (PRR) expression predispose CBA and BL/6 DCs to develop divergent cytokine responses following stimulation with live schistosome eggs. PRRs are innate sensors used by APCs to recognize conserved pathogen-associated molecular patterns (19, 20). C-type lectin receptors (CLRs) are a family of PRRs capable of binding carbohydrates (21, 22), such as the glycans Lewis X (LeX), GalNAcβ1–4GlcNAc (LacdiNAc [LDN]), and fucosylated LDN (LDN-F) typically expressed by schistosome eggs (23–26). Infected CBA/J (CBA) mice develop severe hepatic pathology characterized by large, poorly circumscribed perioval granulomas (6–8). The severe pathology is mediated largely by T cell IL-17 production induced by egg Ag-stimulated dendritic cell (DC) secretion of IL-1β and IL-23 (9–12). In contrast, infected C57BL/6 (BL/6) mice develop mild pathology, with significantly smaller liver granulomas in a Th2-polarized environment (13). IL-17 is largely the product of Th17 cells, a highly proinflammatory subset of CD4+ effector T cells that also produce IL-22, CSF, CXCL1, CXCL2, and TNF-α (14–17). Presently, the mechanisms underlying the variation in egg-induced immunopathology and selection of dominant CD4+ T cell phenotype are incompletely understood; however, it is noteworthy that a recent study (18) of Schistosoma hematobium infection in humans similarly linked the development of pathology to an increase in Th17 cells.

We now demonstrate that genetic differences in pattern recognition receptor (PRR) expression predispose CBA and BL/6 DCs to develop divergent cytokine responses following stimulation with live schistosome eggs. PRRs are innate sensors used by APCs to recognize conserved pathogen-associated molecular patterns (19, 20). C-type lectin receptors (CLRs) are a family of PRRs capable of binding carbohydrates (21, 22), such as the glycans Lewis X (LeX), GalNAcβ1–4GlcNAc (LacdiNAc [LDN]), and fucosylated LDN (LDN-F) typically expressed by schistosome eggs (23–26). We found overall CLR expression to be higher in CBA cells than in BL/6 cells, and in CBA DCs, there was a striking overexpression of the CLR CD209a, a murine homolog of human DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN; CD209). CD209a was shown to facilitate the induction of egg-induced Th17 cells responsible for causing severe immunopathology.

Materials and Methods

Mice, parasites, and infection

Five- to six-week-old female CBA and BL/6 mice were obtained from The Jackson Laboratory. Swiss Webster mice were obtained from Charles River.
Laboratories. A CBA mouse expressing a transgenic (Tg) TCR specific for the Sm-p40 schistosome egg Ag was made in-house, as previously described (12). All mice were maintained at the Tufts University School of Medicine Animal Facility in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines. For some experiments, CBA and BL/6 mice were infected with 85 S. mansoni cercariae (Puerto Rico strain) by i.p. injection. Cercariae were shed from infected Biomphalaria glabrata snails provided to us by BEI Resources (Manassas, VA). All Swiss Webster mice in infected in an identical fashion for the purpose of isolating schistosome eggs. Eggs were isolated from livers of 7–8-Wk infected mice under sterile conditions by a series of blending and staining techniques, as described previously (11).

**Cells**

Bone marrow–derived DCs. Bone marrow was flushed from femurs and tibias of normal CBA and BL/6 mice. RBCs were lysed with Tris ammonium chloride buffer, and cells were cultured in complete RPMI 1640 medium (Lonza) containing 10% FBS (Alegken Biologicals) and rGM-CSF at 15 ng/ml (PeproTech; AF-315-03) or GM-CSF–containing supernatant from the J558L transfectant B cell hybridoma. The medium was changed on days 3 and 5, and cells were harvested on day 7. CD11c+ DC purity was >85% by flow cytometric analysis.

**Gene expression profiling**

CBA and BL/6 bone marrow–derived DCs (BMDCs) prepared from individual mice were plated in replicate at 1 × 10^6 cells/ml in 48-well tissue culture plates (BD Falcon). Replicates were pooled after 4 h, and total RNA was obtained by TRizol reagent (Invitrogen) extraction, according to the manufacturer’s instructions. Amplified and labeled cRNA was assessed with a Mouse Gene 1.0 ST Array (Affymetrix, Santa Clara, CA), following the manufacturer’s instructions. Affymetrix Expression Console 1.1 software was used to generate annotated NetAffx CSV files for analysis. Microarray data were deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under accession number GSE55307 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE55307).

**Gene ontology analysis**

Genes with ≥2-fold difference in expression between CBA and BL/6 DCs and known biological function based on Ensembl release 54 gene archive (http://may2009.archive.ensembl.org/index.html) were defined as differentially expressed. Differentially expressed genes characterized to have putative immunological functions were further selected for gene ontology (GO) and pathway analysis using the public Web server, g:Profiler (27) (Fig. 1B, above).

**Cocultures**

Purified naive CD4+ T cells from normal spleen and syngeneic BMDCs were cultured at an 8:1 ratio in complete RPMI 1640 medium together with anti-CD3/CD28–coated beads (2 × 10^5 beads/ml, Dynabeads Invitrogen) and freshly isolated live schistosome eggs for 96 h.

**Cytokine analysis**

ELISA. Supernatants from 96 h cocultures were assayed for IL-1β, IL-23, and IL-17A using ELISA kits from R&D Systems.

Quantitative RT-PCR. RNA from cocultures with TRizol reagent or a TurboCapture 96 mRNA Kit (QIAGEN), and cDNA was synthesized with a High Capacity Reverse Transcription Kit (Invitrogen) or a SensiScript RT kit (QIAGEN). TaqMan probes for Il17a (Mm00439618) and Gapdh (4352339E) were used in combination with TaqMan Gene Expression Master Mix (all from Applied Biosystems).

**CD209a analysis**

Flow cytometry and cell sorting. Spleen cells from individual normal and infected CBA and BL/6 spleens were isolated, and RBCs were lysed. Cells were blocked with rat IgG Ab and stained with fluorescently labeled Abs specific for CD11c (BD Pharmingen; 553802), CD19 (BD Pharmingen; 553786), Gr-1 (BD Pharmingen; 553127), or F4/80 (Abd Serotec; MCA497APC) in combination with biotin-conjugated anti-CD209a (BD Pharmingen; 558073). Subsequently, cells were stained with Alexa Fluor 647-conjugated streptavidin (Invitrogen; S-21374). Data were acquired with a FACSCalibur flow cytometer and CellQuest software version 3.2.1 (Becton Dickinson) and analyzed with Summit Software. Spleen and granuloma cells (GCs) were gated for viability based on forward scatter and side scatter parameters, as well as propidium iodide exclusion. Normal CBA splenocytes were sorted at the Tufts Flow Cytometry Core Facility using the Abs listed above.

Quantitative RT-PCR. Normal and 7-wk infected CBA and BL/6 spleen or liver tissue was homogenized in TRizol reagent, and cDNA was synthesized, as described above. A TaqMan probe for Cd209a (Applied Biosystems; Mm00460067) was used in combination with TaqMan Gene Expression Master Mix.

**Immunohistochemistry.** Ten-micrometer OCT-embedded liver and spleen cryostat sections were fixed in acetone. Liver sections were stained with anti-CD209a Ab (BMD10) for 18 h at 4°C, followed by mouse anti-rat IgG2a-HRP for 30 min and Tyramide signal amplification (Invitrogen), or with anti-CD209a plus anti-CD11c (N418), followed by streptavidin-HRP and Tyramide signal amplification. Spleen sections also were stained with anti-B220–Alexa Fluor 647. Fluorescent staining was observed using an LSM 710 confocal microscope (Zeiss).

**RNA interference**

Knockdown. CBA BMDCs derived with rGM-CSF were infected with lentivirus containing CD209a- or GFP-targeted short hairpin RNA (shRNA) (RNAi Platform of the Broad Institute of MIT and Harvard). Puromycin was used to select for infected cells, and BMDCs were harvested for coculture experiments on day 10.

**Overexpression.** The open reading frame sequence for CD209a (28, 29) was inserted into a lentiviral plasmid and packaged into viral particles in HEK293T cells with X-tremeGENE 9 DNA Transfection Reagent (Roche). Concentrated virus was used to infect BMDCs, as described above.

**Signaling protein analysis**

**Intracellular staining.** BMDCs were washed, fixed with 3% paraformaldehyde, blocked with rat IgG, and stained with a fluorescently labeled Ab specific for CD11c. Subsequently, cells were washed, permeabilized with methanol, and stained with a primary Ab specific for phospho-ERK (Cell Signaling; 4695), followed by a fluorescently labeled secondary anti-rabbit IgG Ab (Invitrogen; A1034). Data were acquired with the FACSCalibur flow cytometer and CellQuest software version 3.2.1 (Becton Dickinson) and analyzed with Summit Software.

**Western blot.** BMDCs were washed, lysed, and prepared with Laemmli (SDS-Sample) Buffer (Boston BioProducts). Samples were run on an SDS-PAGE gel and transferred to an Immobilon-P polyvinylidene difluoride (Millipore) membrane, which was blocked in 5% BSA. The activation of MAPKs was detected with Abs specific for phospho-Src Tyr416 (2101), phospho-RAF-1 Ser338 (9427), phospho-ERK1/2 Thr202/Tyr204 (4695P), phospho-JNK Thr183/Tyr185 (9251S), and phospho-p38 Thr180/Tyr182 (9215S; all from Cell Signaling). Total kinase expression was detected with Abs specific for S6c (236G), RAF-1 (9422P), ERK1/2 (4695), JNK (9252), p38 (9212), and GAPDH (2118S; all from Cell Signaling).

**Statistical analysis**

ANOVA and the Student t test were used to statistically analyze differences between groups. The p values < 0.05 were considered significant.

**Results**

Gene profiling reveals elevated CLR expression by CBA versus BL/6 DCs

CBA and BL/6 DCs were previously shown to induce divergent schistosome egg-specific cytokine profiles (9–12). To identify genetic differences intrinsic to CBA and BL/6 DCs that may influence the cytokine response to schistosomes, we used Affymetrix microarray technology to assess baseline expression of >22,000 genes in CBA and BL/6 BMDCs (Fig. 1A). Genes of known biological function with a ≥2-fold difference in expression between CBA and BL/6 BMDCs were functionally categorized by GO analysis using the public Web server, g:Profiler (27) (Fig. 1B, Supplemental Table I). Of 180 biologically characterized genes that were elevated ≥2-fold in CBA versus BL/6 BMDCs, 34 had known
immunological functions (Supplemental Table II). GO analysis revealed elevated expression of genes with predicted roles in cell membrane–intrinsic immune defense response to external stimuli, carbohydrate binding, and molecular-transducer activity (Fig. 1B, Supplemental Tables I, II). Of 157 genes that were elevated ≥2-fold in BL/6 BMDCs versus CBA BMDCs, 17 had known immunological function (Supplemental Table III). GO analysis revealed that BL/6 BMDCs exhibited a markedly different expression profile, characterized by genes involved in the regulation of immune effector processes and various metabolic processes (Fig. 1B, Supplemental Table I).

Among all of the genes assessed by microarray technology, the most apparent difference between CBA and BL/6 BMDCs was in PRR expression. In CBA DCs, the majority of overexpressed PRR genes belonged to the CLR family, among which there was a striking 18-fold increase in expression of the CLR $CD209a$ (Fig. 1C). $CD209a$, also known as mouse DC-SIGN and SIGNR5, is one of eight murine homologs of human DC-SIGN, a CLR known to bind to schistosome egg glycans (25, 28, 30). Elevated expression of $CD209a$ by CBA DCs was confirmed in BMDCs from individual CBA and BL/6 mice by quantitative RT-PCR (qRT-PCR) (Fig. 1D). These results denote a significant difference in baseline CLR gene expression between CBA and BL/6 BMDCs.

**CD209a expression is elevated in splenocyte subpopulations from normal and schistosome-infected CBA mice**

$CD209a$ expression was significantly higher in the spleens of both normal and infected CBA mice versus BL/6 mice (Fig. 2A). The overall relative expression of $CD209a$ decreased in infected spleen because of the development of pronounced splenomegaly after 7 wk of infection, primarily caused by clonal T and B cell expansion; however, elevation of $CD209a$ in CBA spleen compared with BL/6 spleen remained intact (Fig. 2A, 2B).

Several studies (22, 31–33) demonstrated that human DC-SIGN is primarily expressed on DCs, but little is known about $CD209a$ expression by APC subpopulations in mice. $CD11c^+$, $CD19^+$, $Gr-1^+$, and F4/80$^+$ splenocytes from normal and infected CBA and BL/6 mice, largely representative of DCs, B cells, granulocytes, and macrophages, respectively, were assessed for $CD209a$ expression by flow cytometric analysis. The percentage of $CD209a$-expressing $CD11c^+$ cells within normal and infected splenocytes ($CD209a^{+}CD11c^+/bulk$ spleen and subpopulation-specific–gated $CD209a^{+}CD11c^+/total$ $CD11c^+$) was higher in CBA mice. Moreover, $CD209a$-expressing $CD11c^+$ cells increased after 7 wk of infection in CBA spleen but remained unchanged in BL/6 spleen (Fig. 2C, 2G). Similarly elevated $CD209a$ expression was seen on CD19$^+$, Gr-1$^+$, and F4/80$^+$ cells in infected CBA spleen versus BL/6 spleen (Fig. 2D–F). Subpopulation-specific gating revealed that a higher percentage of CD19$^+$ cells ($CD209a^{+}CD19^{+}/total$ CD19$^+$) and Gr-1$^+$ cells ($CD209a^{+}Gr-1^+/total$ Gr-1$^+$) expressed $CD209a$ in normal and infected CBA spleen versus BL/6 spleen (Fig. 2H, 2I). There was no difference in the percentage of $CD209a$-expressing F4/80$^+$ cells by subpopulation-specific gating (Fig. 2J). These data indicate that $CD209a$ is expressed by various APC subpopulations, and elevated $CD209a$ expression is apparent in normal and schistosome-infected CBA APCs versus BL/6 APCs.

**CD209a expression is higher in GCs from infected CBA mice versus BL/6 mice**

As the liver is the principal site of egg-induced granulomatous pathology in *S. mansoni* infection, we investigated $CD209a$ expression in livers from normal and infected CBA and BL/6 mice. Although there was no difference in $CD209a$ expression in normal livers from these strains when assessed by qRT-PCR, after 7 wk of infection we detected a significant increase in $CD209a$ expression in CBA liver (Fig. 3A). Additionally, elevated $CD209a$ expression was confirmed in bulk CBA liver GCs by flow cytometric analysis (Fig. 3B).

Within GCs, the percentage of $CD209a$-expressing $CD11c^+$ cells ($CD209a^{+}CD11c^+/bulk$ GCs and subpopulation-specific–gated $CD209a^{+}CD11c^+/total$ $CD11c^+$) was elevated in infected CBA versus BL/6 mice (Fig. 3C, 3F). Similarly, $CD209a$ expression also was higher in Gr-1$^+$ and F4/80$^+$ bulk CBA GCs (Fig. 3D, 3E). Subpopulation-specific gating revealed an increase in the...
percentage of CD209a-expressing granuloma Gr-1+ cells (CD209a+ Gr-1+/total Gr-1+) and F4/80+ cells (CD209a+F4/80+/total F4/80+) within CBA GCs but not BL/6 GCs (Fig. 3F–H). Thus, CD209a expression by CBA GCs, including populations largely representative of DCs, granulocytes, and macrophages, is elevated in comparison with BL/6 GCs.

CD209a+ cells are abundant in infected CBA spleen and liver sections

To assess CD209a+ cell localization and frequency in CBA and BL/6 tissues, infected spleen and liver cryostat sections were stained for CD209a and visualized by confocal microscopy (Fig. 4). In infected CBA spleen, CD209a+ cells were abundant in CD11c-rich interfollicular T cell areas compared with BL/6 spleen (Fig. 4A–D); fewer CD209a+ cells also were present within B220+ B cell-rich follicles in CBA spleen but not BL/6 spleen (Fig. 4A, 4B). In the liver sections, marked infiltration of CD209a+ cells was apparent mainly in the egg-induced granulomas (Fig. 4E, 4F). In contrast, minimal amounts of egg-specific IL-17 was produced by cocultures containing CD19+, Gr-1+, or F4/80+ cells, representing the APC subpopulation that most efficiently induces egg-specific IL-17 secretion by CD4+ T cells.

Egg-simulated CD11c+ cells, but not CD19+, Gr-1+, or F4/80+ cells, elicit Th17 responses in vitro

To determine which CD209a-expressing APC subpopulation induces schistosome-specific Th17 cell responses, splenocytes from normal CBA mice were sorted and cocultured with naive CD4+ T cells, together with anti-CD3/CD28–coated beads in the presence or absence of eggs. CBA CD11c+ cells induced robust IL-17 production by T cells in response to eggs (Fig. 5A); in contrast, minimal amounts of egg-specific IL-17 was produced by cocultures containing CD19+, Gr-1+, or F4/80+ cells (Fig. 5B–D). These results indicate that CD11c+ cells, but not CD19+, Gr-1+, or F4/80+ cells, represent the APC subpopulation that most efficiently induces egg-specific IL-17 secretion by CD4+ T cells.

CD209a expression on DCs is necessary for schistosome egg–induced Th17 cell responses

Given the association of CD11c expression with DCs, we examined the role of CD209a on DCs in eliciting Th17 cell responses to eggs. CD209a-targeted shRNA was delivered into CBA BMDCs via lentiviral infection, resulting in an 86% knockdown, as assessed by flow cytometric analysis and qRT-PCR (Fig. 6A–C). Subsequently, BMDCs in which CD209a expression was knocked down (CD209a-targeted shRNA [shCD209a]) or control BMDCs (normal; GFP-targeted shRNA [shCTRL]) were cocultured with naive CD4+ T cells and anti-CD3/CD28–coated beads in the presence or absence of live eggs, and Th17 cytokine production was assessed.
by ELISA and qRT-PCR. In response to eggs, cocultures containing shCD209a DCs produced significantly less IL-1β and IL-23 compared with normal or shCTRL DCs (Fig. 6D, 6E). Accordingly, there was a substantial decrease in egg-stimulated T cell IL-17 secretion and mRNA expression in cocultures containing shCD209a DCs compared with cocultures containing control DCs (Fig. 6F, 6G). Significantly, shCD209a DCs also elicited lower IL-17 production in egg-stimulated TCR-Tg CD4+ T cells specific for the immunodominant peptide of the Sm-p40 major schistosome egg Ag (Fig. 6H) (12).

Further assessment of transcription factors required for lineage commitment of CD4+ T cells revealed that egg-stimulated cocultures containing T cells and shCD209a DCs exhibited significantly lower expression of the Th17-associated transcription factor Rorc. In contrast, the Th1- and Th2-associated transcription factors, Tbx21 and Gata3, were unchanged, regardless of the DC population present in the cultures (Fig. 6I–K). Collectively, these data indicate that CD209a expression on CBA DCs is necessary for egg-stimulated IL-23 and IL-1β production, which induces Th17 cell differentiation.

**CD209a overexpression confers on BL/6 DCs the capacity to induce Th17 cell responses**

CD209a expression is significantly lower in BL/6 DCs than in CBA DCs (Figs. 1–3), and BL/6 DCs fail to induce Th17 cell responses to eggs in vitro (9–12). Thus, we investigated whether CD209a might confer on BL/6 DCs the capacity to induce egg-specific Th17 cell responses. A lentiviral vector was used to overexpress CD209a in BL/6 BMDCs, which was accomplished successfully, as determined by flow cytometric analysis and qRT-PCR (Fig. 7A–C).

BL/6 CD209a-expressing BMDCs were cocultured with naive BL/6 CD4+ T cells and anti-CD3/CD28–coated beads in the presence or absence of live eggs, and Th17-associated cytokine production was assessed by ELISA and qRT-PCR. Egg-stimulated CD209a-expressing BMDCs produced higher levels of IL-1β and IL-23 in comparison with control BMDCs (Fig. 7D, 7E); IL-17 secretion and mRNA expression also were enhanced in CD209a BMDC cocultures compared with cocultures containing control BMDCs (Fig. 7F, 7G). Additionally, Rorc expression was elevated in CD209a BMDC cocultures compared with control BMDCs (Fig. 7H), but there was no significant change in Tbx21 or Gata3 expression (Fig. 7I, 7J). Overall, these results confirm that CD209a expression enables egg-stimulated DCs to induce IL-1β and IL-23 secretion, leading to Th17 cell development.

**CD209a expression is associated with schistosome egg–stimulated MAPK activation in DCs**

Although signaling pathways associated with CD209a are unknown, recent studies demonstrated that the MAPKs RAF-1 and ERK1/2 are activated in DCs following ligation of human DC-SIGN (34–37). Therefore, we investigated MAPK activation in CBA and BL/6 BMDCs. Following stimulation with live eggs,
there was a steady increase in phospho-ERK1/2 in CBA BMDCs, as determined by intracellular staining, which was not apparent in BL/6 BMDCs (Fig. 8A, 8B). This striking increase in phospho-ERK1/2 was still clearly evident at 24 h after egg stimulation in CBA BMDCs by Western blot, at which time little or no phospho-ERK was detectable in BL/6 BMDCs (Fig. 8E). An increase in the

FIGURE 4. CD209a+ cells are abundant in infected CBA spleen and liver sections. CBA and BL/6 mice were infected for 7 wk. OCT-embedded frozen spleen (A–D) and liver (E and F) cryostat sections were stained for CD209a (red) and imaged by confocal microscopy. CBA (A and C) and BL/6 (B and D) spleen sections were counterstained for CD11c (green) and B220 (blue). (A–D) CD209a localization in spleens from CBA and BL/6 mice. Scale bars, 200 µm (A–D, E and F, upper panels); 500 µm (E and F, lower panels). (G and H) Average CD209a fluorescence intensity in whole liver (E and F) and granulomas (E1, E2, F1, and F2) was quantified by Velocity 6.0 Software (PerkinElmer). (I) A CBA liver section was stained for CD209a and CD11c to demonstrate colocalization in a granuloma after merge. Scale bars, 200 µm. Images are representative of five mice examined per strain.

FIGURE 5. CD11c+, but not CD19+, Gr-1+, or F4/80+ cells, elicit Th17 cell responses. Splenocytes from normal CBA mice were stained for CD11c, CD19, Gr-1, or F4/80 subpopulation markers and separated by FACS. The indicated concentrations of CD11c+ (A), CD19+ (B), Gr-1+ (C), and F4/80+ (D) cells were individually cocultured with 1 × 10^6 CD4+ T cells and anti-CD3/CD28-coated beads, with or without schistosome eggs. IL-17 production in 96-h culture supernatants was assessed by ELISA. Additional single-cell controls are shown. Data are from one representative experiment of two.
phosphorylation of the upstream MAPK RAF-1, as well as SRC kinase, also was detected in CBA BMDCs compared with BL/6 BMDCs (Fig. 8C, 8D). In contrast, there was no significant activation of the MAPKs p38 or JNK (Fig. 8F, 8G). These results are consistent with the notion that egg-stimulated CD209a expression on CBA DCs is associated with SRC, RAF-1, and ERK1/2 MAPK activation.

Discussion

DCs are potent APCs that sense foreign Ag through PRRs and induce the differentiation of naive CD4+ T cells into various effector T cell populations. Diverse cellular, environmental, and genetic factors may influence DCs to bias Th cell differentiation toward Th1, Th2, Th17, or regulatory T cell lineages (4, 20, 22, 31, 38). In murine S. mansoni infection, the development of a Th17 cell response is detrimental to the host, rather than protective, and leads to the development of severe pathology (5, 9–12).

In the current study, comprehensive gene profiling revealed major differences in the expression of PRRs between CBA and BL/6 mice. We found a strikingly higher expression of the CLR CD209a on CBA DCs compared with BL/6 BMDCs (Fig. 8C, 8D). In contrast, there was no significant activation of the MAPKs p38 or JNK (Fig. 8F, 8G). These results are consistent with the notion that egg-stimulated CD209a expression on CBA DCs is associated with SRC, RAF-1, and ERK1/2 MAPK activation.

Proinflammatory cytokine responses to various bacterial, viral, fungal, and parasitic Ag have been characterized downstream of PRR–Ag recognition by APCs (19–22, 39, 40). Of the various families of PRRs, CLRs are the best characterized in the context of schistosome Ag recognition (25, 26, 41–44). CLRs are a large family of calcium-dependent receptors that bind glycans on both pathogen and host cell surfaces, facilitating recognition of a wide range of glycosylation patterns (21, 22). Schistosome-specific glycans, both O- and N-linked, are rich in fucose and include LeX, polyLeX, pseudo–Lewis Y, LDN, LDN-F, CAA, F-LDN, F-LDN-F, and HexNAc-DF (23, 24, 26). Such glycans are heavily expressed on the surface of S. mansoni eggs and are actively secreted in the form of soluble glycoproteins (45). Human CLRs, such as DC-SIGN, DC-SIGNR, macrophage galactose-type lectin, and the mannose receptor, bind to schistosome-expressed glycan LeX, polyLeX, pseudo–Lewis Y, LDN, LDN-F, CAA, F-LDN, F-LDN-F, and HexNAc-DF (23, 24, 26). Such glycans are heavily expressed on the surface of S. mansoni eggs and are actively secreted in the form of soluble glycoproteins (45). Human CLRs, such as DC-SIGN, DC-SIGNR, macrophage galactose-type lectin, and the mannose receptor, bind to schistosome egg glycans, such as LeX, polyLeX, pseudo–Lewis Y, LDN, LDN-F, CAA, F-LDN, F-LDN-F, and HexNAc-DF (23, 24, 26). Such glycans are heavily expressed on the surface of S. mansoni eggs and are actively secreted in the form of soluble glycoproteins (45).

Proinflammatory cytokine responses to various bacterial, viral, fungal, and parasitic Ag have been characterized downstream of PRR–Ag recognition by APCs (19–22, 39, 40). Of the various families of PRRs, CLRs are the best characterized in the context of schistosome Ag recognition (25, 26, 41–44). CLRs are a large family of calcium-dependent receptors that bind glycans on both pathogen and host cell surfaces, facilitating recognition of a wide range of glycosylation patterns (21, 22). Schistosome-specific glycans, both O- and N-linked, are rich in fucose and include LeX, polyLeX, pseudo–Lewis Y, LDN, LDN-F, CAA, F-LDN, F-LDN-F, and HexNAc-DF (23, 24, 26). Such glycans are heavily expressed on the surface of S. mansoni eggs and are actively secreted in the form of soluble glycoproteins (45). Human CLRs, such as DC-SIGN, DC-SIGNR, macrophage galactose-type lectin, and the mannose receptor, bind to schistosome egg glycans, such as LeX, polyLeX, pseudo–Lewis Y, LDN, LDN-F, CAA, F-LDN, F-LDN-F, and HexNAc-DF (23, 24, 26). Such glycans are heavily expressed on the surface of S. mansoni eggs and are actively secreted in the form of soluble glycoproteins (45).
novel findings suggest that CD209a plays a critical role in precipitating Th17 cell–mediated inflammation following stimulation with egg glycans.

CD209a is one of eight murine homologs of human DC-SIGN, a CLR capable of binding various mannose- and fucose-containing bacterial and viral glycans, as well as facilitating endocytosis and cytokine production (22, 28, 29, 50). The specific glycan ligand for CD209a has not been identified (29), but some studies (29, 44) suggested that it may play a role in Ag internalization and presentation. Peptide sequence analysis showed that CD209a retains many structural properties of human DC-SIGN, including one carbohydrate- and calcium-binding domain in the extracellular peptide sequence, one conserved 23-aa sequence in the neck region, and triacidic cluster and dileucine motifs in the cytoplasmic tail (28, 29, 51). Such cytoplasmic tail motifs promote receptor internalization and may function in receptor–Ag uptake (51). As such, DC-SIGN expressed on human DCs was shown to internalize and target soluble egg Ag to lysosomal compartments containing MHC class II molecules (44). Along these same lines, CD209a+ monocyte-derived DCs were shown to possess powerful Ag-presenting capability (29). A more recent study (52) on bacterial sepsis was the first to reveal that leukocyte cell–derived chemotaxin-2 interacts with CD209a on murine macrophages to promote endocytosis, bacterial killing, and cytokine production.

Signaling events proximal to ligand binding by CD209a have not been characterized; however, various human studies (34–37) documented MAPK activation downstream of DC-SIGN. MAPKs are evolutionarily conserved signaling molecules essential for mediating rapid communication of extracellular signals to the nucleus during diverse cellular processes (53, 54). Gringhuis et al. (35, 36) showed that, in the presence of TLR4 stimulation, DC-SIGN ligation by ManLam stimulates the recruitment of a signalingosome of scaffolding and signaling proteins that leads to MAPK activation, modification of NF-κB, and IL-12p40, IL-10, IL-12p35, and IL-6 production. Caparros et al. (34) revealed that ERK1/2 and PI3K, but not p38, are activated following engagement of DC-SIGN, which also coprecipitates with tyrosine kinases Lyn and Syk in human DCs. A similar study (37) documenting interactions between DC-SIGN and syncytial virus glycoprotein G also demonstrated ERK1/2 stimulation post–DC-SIGN ligation. Although MAPK activation was reported following cross-linking of CD209b (SIGNR1) (55), as well as CD209d (SIGNR3) (56), the signaling mechanisms critical for Th17 cell–associated cytokine production downstream of murine DC-SIGN homologs, particularly in response to schistosome products, are largely unknown.

Our findings demonstrate that CD209a expression on DCs is essential for the induction of egg-stimulated Th17 responses associated with DC ERK1/2 activation. Although little is known
of the relationship between ERK1/2 activation and Th17 cell responses, signaling through ERK1/2 was shown to promote IL-23p19 and IL-1β production, leading to Th17 cell differentiation in a study of primary human fibroblasts (57). More relevant to immunity in schistosome infection, ERK1/2 activation, in the absence of significant JNK or p38 activation, also was reported in response to lacto-N-fucopentaose III and ES62 from Acanthocheilonema vitaeae; however, these studies (58–62) focused on the anti-inflammatory properties of helminth-related molecules and the induction of Th2 cell responses. Indeed, numerous studies (61–66) established the immunomodulatory Th2-promoting properties of helminth-related molecules that may signal through receptors, such as human DC-SIGN and the mannose receptor. Prior to this study, the only CLRs known to facilitate Th17 cell responses were Dectin-1 and Dectin-2, which signal via Syk kinase and CARD9 in response to fungal Ags, resulting in IL-23 production (21, 67, 68). Our finding that a novel CLR, CD209a, mediates proinflammatory Th17 cell responses indicates that the eightmurine homologs of DC-SIGN, each of which retains slightly different structural properties of human DC-SIGN, may have evolved to mediate divergent functions. As reflected in the nomenclature of DC-SIGN, its natural ligand is ICAM-3, one of several transmembrane glycoproteins and ICAMs that facilitate DC–T cell interactions (32). However, it is unknown whether murine DC-SIGN homologs also bind adhesion molecules to promote cell-cell interaction. Immune synapse formation between DCs and T cells is required for efficient Ag presentation and DC–T cell intercellular feedback that leads to cytokine production. Future assessment of the ICAM-binding capacity of CD209a will clarify whether murine DC-SIGN homologs also have the ability to promote cell adhesion and interaction. In sum, severe hepatic granulomatous pathology in murine schistosomiasis, as seen in infected CBA mice, is dependent on CD4+ Th17 cell responses. In this article, we show that pathogenic Th17 cell cytokine responses to live S. mansoni eggs are largely dependent on the expression of CD209a by DCs. Future work investigating the outcome of pathology in S. mansoni-infected CD209a-deficient CBA mice will clarify the function of CD209a in murine schistosomiasis in vivo.

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References


