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Dendritic cell expression of the C-type lectin receptor CD209a: A novel innate parasite-sensing mechanism inducing Th17 cells that drive severe immunopathology in murine schistosome infection

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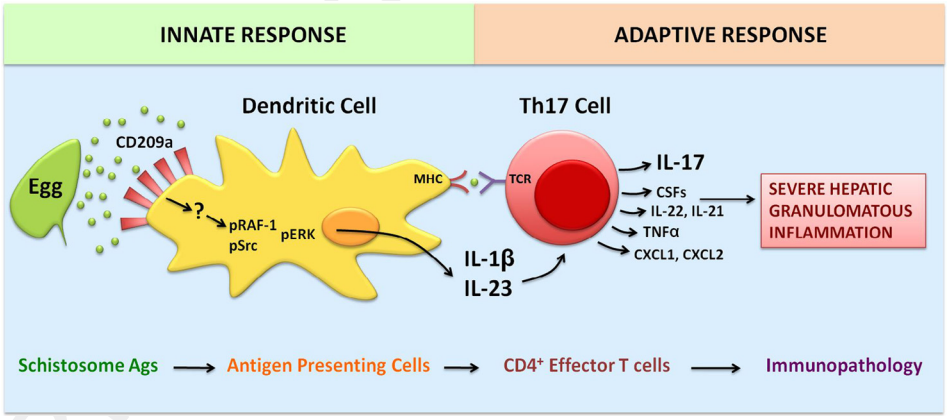
HIGHLIGHTS

- CBA mice develop severe Th17 cell-mediated pathology in schistosomiasis.
- The Th17 cells are induced by parasite egg-stimulated dendritic cells (DCs).
- CBA DCs express high levels of the c-type lectin receptor CD209a.
- Th17 cell development depends on DC CD209a expression.
- DC CD209a expression represents a novel proinflammatory parasite sensing mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

Following infection with the trematode helminth *Schistosoma mansoni*, CBA mice develop severe parasite egg-induced hepatic granulomatous inflammation as well as prominent CD4+ T helper 17 (Th17) cell responses driven by dendritic cell (DC)-derived IL-1β and IL-23. By comparison, C57BL/6 mice develop mild hepatic immunopathology, egg stimulation of DCs does not result in IL-1β and IL-23 production, and Th17 cells fail to develop. To investigate the reasons for strain-specific differences in antigen presenting cell (APC) reactivity to eggs, we performed a comparative gene profiling analysis of normal bone marrow-derived DCs (BMDCs) and found that CBA DCs display markedly elevated expression of C-type lectin receptors (CLRs). In particular, expression of CD209a, a murine homologue of human DC-specific ICAM-3-grabbing non-integrin (DC-SIGN, CD209), was strikingly higher in CBA than BL/6 DCs. High CD209a surface expression was observed in various CBA splenic and granuloma APC subpopulations; however, only DCs, and not macrophages, B cells or neutrophils, were able to induce Th17 cell differentiation in

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response to schistosome eggs. Lentiviral gene silencing in CBA DCs, and over-expression in BL/6 DCs, demonstrated CD209a to be critical for egg-induced DC IL-1β and IL-23 production necessary for Th17 cell differentiation and expansion. These findings reveal a novel innate parasite-sensing mechanism promoting CD4+ Th17 cells that mediate severe immunopathology in schistosomiasis.

1. Schistosomiasis: the human disease and the experimental murine model

Schistosomes are trematode helminths that cause extensive disease in the developing world, accounting for over 200 million infections and more than 200,000 deaths per year. In infection with the species *Schistosoma mansoni (S. mansoni)*, the principal causes of morbidity and mortality are granulomatous inflammation and subsequent fibrosis around parasite eggs deposited in the liver and intestines (Bica et al., 2000; Fallon, 2000; Larkin et al., 2012; Pearce and MacDonald, 2002; Wilson et al., 2007). Most infected individuals develop mild gastrointestinal disease, but 5–10% develop life-threatening hepatosplenic schistosomiasis, characterized by severe liver fibrosis, portal hypertension, splenomegaly, ascites, gastrointestinal bleeding and death (Bica et al., 2000; Fallon, 2000; Larkin et al., 2012; Pearce and MacDonald, 2002; Wilson et al., 2007). The granulomatous inflammation in schistosomiasis is precipitated and orchestrated by CD4+ T lymphocytes sensitized to schistosome eggs, which are highly immunogenic structures capable of secreting a vast array of highly fucosylated glycoproteins (Cass et al., 2007; Cummings and Nyame, 1999; Hokke and Yazdanbakhsh, 2005; Prasanphanich et al., 2013). Analogous to humans, dissimilar disease severity is also observed in an experimental murine model of schistosomiasis. Infected CBA/J (CBA) mice develop severe hepatic pathology characterized by large poorly circumscribed perioval granulomas (Cheever et al., 1987; Fanning et al., 1981; Hernandez et al., 1997). Such severe immunopathology in CBA and other high-pathology strains, including C3H, SJL and MOLF (Cheever et al., 1987; Smith et al., 2009, 2011), is largely mediated by Th17 cells (Rutitzky et al., 2005, 2008; Shainheit et al., 2008, 2011), which are a highly proinflammatory CD4+ T cell subset characterized by their production of IL-17A (henceforth termed IL-17), as well as IL-22, IL-21, colony stimulating factors (CSFs), CXCL1, CXCL2, and TNF-α (Bettelli et al., 2007; Dong, 2006; Iwakura et al., 2008; Stockinger and Veldhoen, 2007). Importantly, Th17 cells were also found to be key mediators of pathology in many autoimmune and infectious diseases (Curtis and Way, 2009; Di Cesare et al., 2009; Huang et al., 2004; Iwakura et al., 2008; Langrish et al., 2005; Murphy et al., 2003). Unlike CBA mice, infected C57BL/6 (BL/6) mice develop mild hepatic pathology with considerably smaller, well-circumscribed liver granulomas and little parenchymal inflammation that arise in a Th2 cell-dominated environment characterized by the presence of IL-4, IL-5, and IL-13 (Pearce et al., 1991). In both the severe and mild forms of disease, the egg-induced granulomatous inflammation in schistosomiasis is a true example of T cell-mediated "adaptive immunopathology", as it fails to materialize in the absence of CD4+ T cells expressing rearranged αβ T cell receptors (TCR) (Iacomini et al., 1995).

Of note is the recent report that in human infection with *S. haematobium*, a related species that is the causative agent of urinary schistosomiasis, the development of bladder pathology has been similarly linked to an increase in peripheral Th17 cells together with a decrease in Foxp3-expressing regulatory T cells, a pattern akin to that observed in *S. mansoni*-infected CBA mice (Mbow et al., 2013). These findings imply that Th17 cell responses also occur in human pathology and thus validate the experimental murine model.

2. Distinct strain-dependent responses to schistosome egg antigens result in divergent CD4+ T cell differentiation programs and severity of immunopathology

Presently, the mechanisms underlying the striking strain-dependent differences in egg-induced immunopathology and the selection of opposite dominant CD4+ T cell subsets still remain incompletely understood. It is known that the activation of pathogenic Th17 cells in the high-pathology CBA strain is dependent on egg Ag-stimulated DCs that produce the Th17-stimulatory cytokines IL-1β and IL-23, as well as IL-6 and TGF-β (Rutitzky et al., 2008; Shainheit et al., 2008). However, in BL/6 mice, egg stimulation of DCs does not result in IL-1β or IL-23 secretion, and, consequently, Th17 cells fail to develop despite the fact that BL/6 T cells are wholly capable of differentiating into Th17 cells *in vivo* in a surrogate model of severe schistosomiasis that ensues upon concurrent immunization with soluble schistosome egg antigens (SEA) in complete Freund's adjuvant (SEA/CFA) (Rutitzky et al., 2001, 2005), or *in vitro*, when BL/6 T cells are stimulated exogenously with the Th17 cell-stimulatory cytokines IL-6, TGF-β and IL-23 (Shainheit et al., 2008). These findings suggest that dissimilar Ag recognition and handling by DCs can result in disparate cytokine profiles and in the differentiation of T effector cell subsets of variable pathogenicity.

To address the aforementioned paradigm, we set out to elucidate the molecular means by which genetically diverse DCs could instruct distinct T cell differentiation programs following encounter with schistosome products. The initial findings from our studies were presented at the 8th conference on Molecular and Cellular Biology of Helminth Parasites, held on September 1–6, 2014, in Hydra, Greece. These findings constitute the essence of the present article; more details about this work with primary data are provided in a recent publication (Ponichtera et al., 2014). Additional information about the murine model of schistosomiasis and the biology of the parasite is available in previous review articles (Bica et al., 2000; Fallon, 2000; Larkin et al., 2012; Pearce and MacDonald, 2002; Wilson et al., 2007).

3. DCs from CBA and BL/6 mice exhibit different C-type lectin receptor expression; the lectin CD209a is significantly elevated in CBA DCs

To evaluate possible intrinsic differences between APCs that instruct divergent T cell differentiation programs in CBA vs. BL/6 mice following encounter with schistosome egg Ags, we subjected normal BMDCs from each strain to comprehensive baseline gene profiling using Affymetrix microarray technology (Ponichtera et al., 2014; Reimand et al., 2011). Surprisingly, among the genes with immunological function that were significantly overexpressed in CBA vs. BL/6 DCs were pattern recognition receptors (PRRs), the majority of which belonging to the C-type lectin receptor (CLR) family. CLRs are a large family of calcium-dependent receptors that bind glycans on both pathogen and host cell surfaces, affording recognition of a wide range of glycosylation patterns (Geijtenbeek and Gringhuis, 2009; Robinson et al., 2006). Prominent among these are fucose-rich schistosome glycans, both O- and N- linked, including Lewis (Le^x), poly Le^x, pseudo Lewis Y (Le^y), GalNAcβ1–4GlcNAc (LacdiNAc, LDN), and diversely fucosylated LDN (F-LDN, LDN-F, F-LDN-F) (Cummings and Nyame, 1999; Hokke and Deelder, 2001; Hokke and

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