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The Mannose Receptor (CD206) is an important pattern recognition receptor (PRR) in the detection of the infective stage of the helminth *Schistosoma mansoni* and modulates IFNγ production

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ABSTRACT

In this study, infective larvae of the parasitic helminth *Schistosoma mansoni* were shown to contain a large number of glycosylated components specific for the Mannose Receptor (MR; CD206), which is an important pattern recognition receptor (PRR) of the innate immune system. MR ligands were particularly rich in excretory/secretory (E/S) material released during transformation of cercariae into schistosomula, a process critical for infection of the host. E/S material from carboxyfluorescein diacetate succinimidyl ester (CFDA-SE)-labelled cercariae showed enhanced binding by cells lines that over-express the MR. Conversely, uptake was significantly lower by bone marrow-derived macrophages (MΦ) from $MR^{-/-}$ mice, although they were more active as judged by enhanced pro-inflammatory cytokine production and CD40 expression. After natural percutaneous infection of $MR^{-/-}$ mice with CFDA-SE-labelled parasites, there were fewer cells in the skin and draining lymph nodes that were $CFDA\text{-}SE^{+}$ compared with wild-type mice, implying reduced uptake and presentation of larval parasite antigen. However, antigen-specific proliferation of skin draining lymph node cells was significantly enhanced and they secreted markedly elevated levels of IFNγ but decreased levels of IL-4. In conclusion, we show that the MR on mononuclear phagocytic cells, which are plentiful in the skin, plays a significant role in internalising E/S material released by the invasive stages of the parasite which in turn modulates their production of pro-inflammatory cytokines. In the absence of the MR, antigen-specific $CD4^{+}$ cells are Th1 biased, suggesting that ligation of the MR by glycosylated E/S material released by schistosome larvae modulates the production of $CD4^{+}$ cell specific IFNγ.

1. Introduction

Cercariae of the parasitic helminth *Schistosoma mansoni* gain entry to the mammalian host via the skin which provides the initial site for stimulation of the innate immune response. Schistosome larvae can take several days to migrate through the epidermis and dermis of the skin, facilitated by the secretion of proteases from the acetabular glands (Knudsen et al., 2005; Curwen et al., 2006), before exiting via vascular and lymphatic vessels (Wheater and Wilson, 1979). Using an amine-reactive tracer, we have labelled live cercariae to visualise the release of excretory/secretory (E/S) material after skin penetration and detect its uptake by host cells such as macrophages (MΦs) and dendritic cells (DCs) (Paveley et al., 2009). Both MΦs and DCs are activated after exposure to E/S products from schistosome larvae (Jenkins et al., 2005; Jenkins and Mountford, 2005; Ferret-Bernard et al., 2008; Paveley et al., 2009), although they have a 'limited' maturation phenotype compared with cells matured with classical pathogen-associated molecular patterns (PAMPs) such as bacterial lipopolysaccharide (LPS) (Ferret-Bernard et al., 2008), and favour the induction of Th2 rather than Th1 lymphocytes (Jenkins and Mountford, 2005). However, although cytokine production by MΦs is MyD88-dependent (Jenkins et al., 2005), indicating an important role for non-phagocytic Toll-like receptors (TLRs), it is not known which pattern recognition receptors (PRRs) are involved in the uptake or phagocytosis of E/S material by host cells.

Phagocytic PRRs include scavenger receptors, complement receptors and C-type lectins (CLRs) such as the MΦ Mannose Receptor (MR; CD206) (Underhill and Ozinsky, 2002). CLRs are involved in the uptake of numerous pathogens through binding of glycans and have a diverse range of functions (Gazi and Martinez-Pomares, 2009; Kerrigan and Brown, 2009). The MR in particular can recognise sulphated sugars, collagen and sugars

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terminating in D-mannose, L-fucose or *N*-acetylglucosamine (Taylor et al., 2005; Martinez-Pomares et al., 2006). It is expressed amongst others on tissue MΦs and DCs (Engering et al., 1997; Linehan et al., 1999; McKenzie et al., 2007) and is mainly intracellular with only 10–30% being expressed at the cell surface under steady state conditions (Schweizer et al., 2000). Furthermore, the MR can be up-regulated by cytokines such as IL-4, IL-13 and IL-10, or down-regulated with IFNγ (Harris et al., 1992; Doyle et al., 1994; Martinez-Pomares et al., 2003; Dewals et al., 2010).

Parasitic helminths express various carbohydrate containing glycoproteins on their surface and release glycan-rich E/S products that can potentially bind to various CLRs. The MR in particular binds to *Trichinella spiralis* muscle larvae (Gruden-Movsesijan and Milosavljevic Lj, 2006), *Trichuris muris* E/S (deSchoolmeester et al., 2009), and soluble schistosome egg antigen (SEA) from *S. mansoni* (Linehan et al., 2003; van Liempt et al., 2007). As MΦs are common in murine skin (Dupasquier et al., 2004; McKenzie et al., 2007), and the MR is highly expressed on dermal MΦs in murine and human skin (McKenzie et al., 2007; Ochoa et al., 2008), we suggest that they may play a major role in sensing invasive schistosome cercariae during infection of the skin and play a critical role in the development of the acquired immune response.

Here, we focus on the role of the MR in recognising and internalising E/S products released by infectious *S. mansoni* cercariae, and in the activation of the ensuing immune response. The presence of MR ligands in the E/S products released by transforming cercariae (termed 0–3hRP) was determined, while carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) was used to label live cercariae in order to track uptake of E/S products by murine cells either in the presence or absence of the MR. We show that not only is the MR an important receptor in the uptake of E/S products by phagocytic cells, its absence leads to an increase in the ability of $CD4^+$ lymphocytes in the skin draining lymph nodes (sdLN) to secrete antigen-specific IFNγ and reduce the production of IL-4.

2. Materials and methods

2.1. Animals

C57BL/6 mice were bred and maintained at the University of York, UK and housed alongside MR deficient ($MR^{-/-}$) mice on a C57BL/6 background (a kind gift from Prof. Michel C. Nussenzweig, Rockefeller University, New York, USA). All experimental procedures were undertaken within the guidelines of the United Kingdom Animals Scientific Procedures Act 1986 and approved by the University of York Ethics Committee.

2.2. Parasite material

Cercariae from a Puerto Rican strain of *S. mansoni* were shed from snails by exposure to light for 2 h. The live cercariae were then used immediately for labelling with CFDA-SE (see below) and/or infection. Alternatively, they were used for the production of a soluble cercarial antigen preparation (SCAP), or prepared for the collection of larval E/S products (0–3hRP) (Mountford et al., 1995; Jenkins et al., 2005; Jenkins and Mountford, 2005; Paveley et al., 2009) and soluble schistosomula antigen preparation (SSAP) (Mountford et al., 1995). Briefly, 1×10^6 cercariae were induced to be shed in 1,000 ml 'aged tap water' (ATW), then concentrated on ice and washed extensively (>5×) with sterile ATW prior to mechanical transformation in sterile RPMI medium. Parasite bodies were then incubated for 3 h in RPMI to induce the release of 0–3hRP (Paveley et al., 2009). All antigen preparations were prepared following sonication at 21 kHz at 6.5 μm amplitude for 90 s and centrifugation at 100,000*g* for 1 h (Mountford et al., 1995).

2.3. Parasite labelling

Live cercariae were cultured in the presence of 20 μM CFDA-SE (Invitrogen Ltd., Paisley, UK) at 28 °C for 1 h, washed, then incubated for a further 1 h to remove unconjugated tracer as previously described (Paveley et al., 2009). As reported previously, labelling of cercariae with CFDA-SE had no effect upon the viability of the cercariae to penetrate the skin or mature into egg-laying worms (Paveley et al., 2009). CFDA-SE-labelled cercariae were then used to infect mice or in the preparation of 0–3hRP. Alternatively, live parasites were labelled with 1 μg/ml of a recombinant fusion protein CTLD4-7-Fc (Linehan et al., 2001) which contains the MR CRD 4-7 fused to the Fc portion of human IgG_1, for 30 min at 28 °C, washed and incubated with anti-human IgG FITC. Labelled parasites were then fixed and imaged on a Zeiss LSM 510 meta confocal microscope (Carl-Zeiss Ltd., Welwyn Garden City, UK) at 488 nm/520 nm excitation/emission.

2.4. Detection of glycans within schistosome preparations

Components 0–3hRP, SCAP and SSAP were separated by 4–12% one-dimensional (1D) SDS-PAGE and stained with Coomassie® Blue. Gels were then oxidised with sodium meta-periodate (Perbo Science Ltd., Cheshire, UK) and stained with Pro-Q® Emerald before imaging on a Bio-Rad Molecular Imager FX Pro (Bio-Rad Laboratories, Hemel Hempstead, UK) at 280/530 excitation/emission. Glycoprotein content in the various preparations was determined with a glycoprotein estimation kit (Perbio Science Ltd.) against a D-mannose standard curve.

2.5. Analysis of carbohydrate ligands

Using CTLD4-7-Fc and CR-FNIII-CTLD1-3-Fc (Linehan et al., 2001), the specificity of mannose and galactose ligands present in 0–3hRP was determined using lectin-specific ELISAs according to the protocol of Zamze et al. (2002). Plates were coated with diluted schistosome antigen preparations, (10 μg/ml), mannan (1 μg/ml) and SO_4-3-gal (5 μg/ml) in PBS pH 7.6 and left overnight at 4 °C. Plates were washed and probed with CTLD4-7-Fc or CR-FNIII-CTLD1-3-Fc for 1.5 h at room temperature (RT) in the presence or absence of 10 mM mannose or galactose. After washing, plates were incubated with anti-human IgG alkaline phosphate conjugate (Sigma–Aldrich Ltd., Poole, UK) for 1 h and then developed with *p*-nitrophenyl phosphate substrate (Sigma–Aldrich Ltd.). Absorbance was measured at 405 nm.

To detect the presence of MR ligands by western blot, samples of 0–3hRP, SCAP and SSAP were separated by 1D SDS–PAGE, transferred to polyvinyl difluoride (PVDF) membranes (Invitrogen Ltd.) for 75 min at 30 V in Blot-cell (Bio-Rad Laboratories) and blocked with 5% non-fat milk. Membranes were probed with 1 μg/ml of CRD4-7Fc and then horseradish peroxidase (HRP)-conjugated anti-human IgG (Amersham Pharmacia Biotech Ltd., UK). Binding was revealed using ECL Plus™ reagent (Perbio Science Ltd.). Post-imaging, membranes were stripped of antibody, incubated with SYPRO® Ruby protein blot stain (Invitrogen Ltd.) and imaged at 488 nm/640 nm excitation/emission.

2.6. Production and stimulation of cell lines and bone marrow (BM) MΦs

Wild type CHO (WT; 85050302), MR-expressing CHO cells (Martinez-Pomares et al., 2003) and the MΦ cell lines J774A.1 and J774E, were a kind gift from Professor S. Gordon, University of Oxford, UK. MΦs derived from BMMΦs were generated from WT and $MR^{-/-}$ mice as follows. BM cells (femurs) were resuspended in DMEM (Invitrogen Ltd.) supplemented with 10%