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CD200 receptor and macrophage function in the intestine

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ABSTRACT

CD200 receptor 1 is an inhibitory receptor expressed by myeloid cells which has inhibitory effects on macrophage function after binding its ubiquitously expressed ligand CD200. Recent evidence suggests that this is important in controlling inflammatory reactions in the lung and here we have explored if the CD200R1-CD200 axis plays a similar role in other mucosal surfaces such as the intestine. We show for the first time that CD200R1 is expressed selectively by resident macrophages in normal mouse colon and that CD200 is present on many haematopoietic and non-haematopoietic cells in the intestine. Although acute colitis induced by feeding dextran sodium sulphate is associated with an influx of CD200R1^{neg} macrophages, CD200R1 KO mice have normal macrophage function in the colon and they do not develop spontaneous intestinal inflammation, nor are they more susceptible to DSS colitis. CD200 KO mice also develop experimental colitis normally and we conclude that CD200R1 does not play an essential role in macrophage homeostasis in the colon, indicating that these molecules may have distinct functions in different mucosal tissues.

Introduction

Macrophages are one of the most abundant leucocytes in the normal large intestine, where they play a number of essential roles in maintaining local homeostasis in the face of the enormous burden of commensal bacteria and other insults (Bain and Mowat 2011). This is through their ability to ingest and kill any organisms that penetrate the surface epithelium (Smythies et al. 2005), as well as by the production of mediators such as prostaglandin E2 that promote epithelial renewal and integrity (Pull et al. 2005). In addition, recent evidence indicates that IL10 produced by mucosal mϕ is required to sustain the generation of the regulatory T cells that normally prevent inflammatory reactions to food proteins and commensal microbes (Denning et al. 2007). Paradoxically however, colonic mϕ are also important components of the pathology in inflammatory bowel diseases such as Crohn's disease caused by inappropriate immune responses to commensal bacteria (Smith et al. 2011). Thus it is essential that mϕ function is tightly regulated in the intestine to ensure that their physiological roles are permitted without overt inflammation developing.

There is considerable evidence that this balance is maintained by active mechanisms that limit how resident colonic mϕ respond to local stimuli. In many ways these mϕ have an activated appearance, expressing high levels of class II MHC and being avidly phagocytic and bactericidal. However unlike mϕ in other tissues, they do not produce pro-inflammatory mediators in response to stimuli such as TLR ligation or phagocytosis of particles (Bain and Mowat 2011; Platt et al. 2010; Smith et al. 2011). As a result, colonic mϕ can ingest any commensal bacteria that might cross the epithelial barrier without causing inflammation. Elucidating the basis of this selective inertia would be important for understanding the pathogenesis of IBD and could allow mϕ to be targeted selectively in therapy.

One mechanism that has received recent attention as a means of regulating mϕ function at mucosal surfaces is the CD200-CD200R1 axis. CD200R1 is expressed at high levels on myeloid cells and binding of CD200R1 to its ubiquitously expressed ligand CD200 induces negative signalling in mϕ (Mihrshahi et al. 2009). As a result, knockout or neutralisation of CD200 or CD200R1 *in vivo* leads to mϕ hyperactivity and autoimmune disease (Wright et al. 2000) and recent work shows that it also causes increased susceptibility to lung inflammation during infection with influenza virus (Snelgrove et al. 2008). This was correlated with the strong expression of CD200R1 by resident alveolar mϕ in the lung, a population which has many similarities to resident intestinal mϕ. Therefore

Abbreviations: 7-AAD, 7-amino-actinomycin D; BLP, bacterial lipopeptide; BMM, bone marrow macrophage; CD200R1, CD200 receptor 1; CLP, colonic lamina propria; CMF, calcium magnesium free; DSS, dextran sodium sulphate; FCS, foetal calf serum; H&E, haematoxylin and eosin; HBSS, Hank's balanced salt solution; IBD, inflammatory bowel disease; KO, knockout; LP, lamina propria; LPS, lipopolysaccharide; M-CSF, macrophage colony stimulating factor; mϕ, macrophage; PBS, phosphate buffered saline; SIRPα, signal regulatory protein α; SPF, specified pathogen free; SSC, side scatter; TLR, toll like receptor; WT, wild type.

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here we have investigated the role of the CD200-CD200R1 axis in regulating intestinal mϕ function and show for the first time that colonic mϕ in normal mice express CD200R1. However absence of this molecule does not influence mϕ behaviour in the intestine or predispose to mucosal inflammation, indicating that distinct mechanisms may be responsible for mϕ inertia in different mucosal surfaces.

Materials and methods

Mice

Wild type C57Bl/6 mice were purchased from Harlan Olac (Bicester, UK), while CD200R1$^{-/-}$ mice on the B6 background (Boudakov et al. 2007) (obtained originally from Prof. Tracy Hussell, Imperial College, London, UK) were bred in house. CD200$^{-/-}$ mice on the B6 background (Hoek et al. 2000) were obtained from Prof. Andrew Dick, University of Bristol, UK. All mice were maintained under specific pathogen free (SPF) conditions at the Central Research Facility at the University of Glasgow and were used between 6 and 12 weeks of age, unless otherwise indicated. All procedures were carried out in accordance with UK Home Office regulations.

Isolation of peritoneal and alveolar macrophages

To obtain resting peritoneal mϕ, mice were injected intraperitoneally with 8–10 ml of ice-cold PBS (Gibco, Invitrogen, Paisley, Scotland)/1 mM EDTA (Sigma–Aldrich, St. Louis, MO) and the peritoneal exudate cells were then harvested by lavage. Alveolar mϕ were obtained by broncho-alveolar lavage of anaesthetised mice with 2× 1.5 ml of PBS/1 mM EDTA via an intra-tracheal cannula.

Isolation of colonic lamina propria cells

The large intestines of mice were excised and soaked in PBS. After removing all excess fat and faeces, the intestines were opened longitudinally, washed in Hank's balanced salt solution (Invitrogen) 2% FCS, and cut into 0.5 cm sections. The tissue was then shaken vigorously in 10 ml HBSS/2% FCS, and the supernatant was discarded. To remove the epithelial layer, 10 ml fresh calcium/magnesium-free HBSS containing 2 mM EDTA was then added, the tube placed in a shaking incubator for 15mins at 37 °C, before being shaken vigorously and the supernatant discarded. The intestinal tissue was washed by adding 10 ml fresh CMF HBSS, shaking the tube vigorously and discarding the supernatant. After a second incubation in CMF HBSS/2 mM EDTA, the wash step was repeated and the remaining tissue was digested with pre-warmed 1.25 mg/ml collagenase D (Roche), 0.85 mg/ml collagenase V (Sigma–Aldrich), 1 mg dispase (Gibco, Invitrogen), and 30 U/ml DNase (Roche Diagnostics GmbH, Mannheim, Germany) in complete RPMI 1640 containing 2 mM L-glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, 1.25 μg/ml Fungizone, and 10% FCS (all Gibco, Invitrogen) for 30–45 min in a shaking incubator at 37 °C. The resulting cell suspension was passed through a 40 μm cell strainer (BD Falcon) and washed twice in complete RPMI. Cells were counted and kept on ice until use.

Generation of bone marrow derived macrophages

BM was flushed out of the femurs and tibias of adult mice in RPMI 1640. The BM cells were passed through Nitex mesh (Cadisch and Sons, London, UK) and counted. To generate mϕ, 1 ml of cells were added to 90 cm Petri dishes (Sterilin, UK) at 3×10^6 cells/ml with 8 ml complete medium containing 20% FCS, 1 mM sodium pyruvate (Gibco, Invitrogen) and 20% L929 fibroblast supernatant as a source of M-CSF at 37 °C in 5% CO_2. After 3 days, the medium was supplemented with 5 ml complete medium and 20% M-CSF. Adherent cells were used on day 6/7 of culture by adding ice cold PBS/1 mM EDTA for 5 min and then displacing them with cell scrapers (Costar). The resulting cells were typically >90% F4/80 positive.

In vitro stimulation of macrophages

Mϕ were plated out at 1×10^6 cells/ml and whole colonic lamina propria digest cell suspensions were plated at 2×10^6 cells per well in 1 ml, both in ultra low adherence, 24-well tissue culture plates (Costar). Cells were incubated either in medium alone, or with 1 μg/ml lipopolysaccharide from *Salmonella typhimurium* (Sigma–Aldrich), 1 μg/ml bacterial lipoprotein (BLP; Pam3CSK4) (Invivogen, San Diego, CA), and/or 100 U/ml recombinant mouse interferon γ (BioSource, Invitrogen) at 37 °C in 5% CO_2. In some experiments BMM were pre-incubated with 2.5 μg/ml CD200-Fc fusion protein (R&D Systems) from 1 h before and then throughout stimulation with LPS and/or IFNγ.

Flow cytometry

2–3 × 10^6 cells were added to polystyrene tubes (BD Falcon), washed in ice cold FACS buffer (PBS containing calcium and magnesium + 4% FCS) and then incubated with purified anti-CD16/CD32 (BD Biosciences) to reduce non-specific binding via Fc receptors. Cells were washed once with ice cold FACS buffer and then incubated with the relevant primary antibodies (Table 1) or appropriate isotype controls. Cells were then washed three times in ice cold FACS buffer and if required, incubated for a further 10–15 min with fluorochrome-conjugated streptavidin. All incubations were carried out at 4 °C in the dark and cells were washed in ice cold FACS buffer before analysis on a FACSAria I or LSRII flow cytometer (BD Biosciences). Dead cells were excluded by including 7-amino-actinomycin D (BD Biosciences) in all staining panels. All data generated was analysed using FlowJo software (Tree Star Inc, OR, USA).

To detect intracellular cytokines, 2×10^6 cells were incubated with or without TLR ligands for 4.5 h in presence of 1 μM monensin and 10 μg/ml Brefeldin A (both Sigma–Aldrich). Cells were then

Table 1
Monoclonal antibodies used for flow cytometry.

Antibody	Clone	Isotype	Source
CD3	17A2	Rat IgG2b	BD Biosciences
CD11b	M1/70	Rat IgG2b	BD Biosciences
CD11c	HL3	Hamster IgG1	BD Biosciences
CD16/32	2.4G2	Rat IgG2b	BD Biosciences
CD31	MEC 13.3	Rat IgG2a	BD Biosciences
CD40	3/23	Rat IgG2a	BD Biosciences
CD45	30-F11	Rat IgG2b	BD Biosciences
CD45R (B220)	RA3-6B2	Rat IgG2a	BD Biosciences
CD80	16-10A1	Hamster IgG1	BD Biosciences
CD86	GL1	Rat IgG2a	BD Biosciences
CD200	OX-90	Rat IgG2a	AbD Serotec, Kidlington, UK
CD200R	OX-110	Rat IgG2a	AbD Serotec
F4/80	BM8	Rat IgG2a	eBioscience, San Diego, CA
Ly6C	AL-21	Rat IgM	BD Biosciences
Ly6G	1A8	Rat IgG2a	BD Biosciences
MHC II (IA-IE)	M5/114.15.2	Rat IgG2b	eBioscience
SiglecF	E50-2440	Rat IgG2a	BD Biosciences
TLR2	6C2	Rat IgG2b	eBioscience
TLR4	MTS510	Rat IgG2a	eBioscience
TNFα	MP-6XT22	Rat IgG1	BD Biosciences