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Murine pattern recognition receptor dectin-1 is essential in the development of experimental autoimmune uveoretinitis

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ABSTRACT

Mycobacteria in complete Freund's adjuvant (CFA) are an essential component of immunization protocols in a number of autoimmune disease animal models including experimental autoimmune encephalomyelitis and uveoretinitis (EAE and EAU, respectively). We determined the role in EAU of two C-type lectin receptors on myeloid cells that recognize and respond to mycobacteria. Using receptor-specific antibodies and knockout mice, we demonstrated for the first time that the macrophage mannose receptor delays disease development but does not affect severity. In contrast, dectin-1 is critically involved in the development of CFA-mediated EAU. Disease severity is reduced in dectin-1 knockout mice and antibody blockade of dectin-1 during the induction, but not the effector phase, prevents EAU development. Significantly, similar blockade of dectin-1 in vivo has no effect in non-CFA-mediated, spontaneously induced or adoptive transfer models of EAU. Thus dectin-1 plays a critical role in the ability of complete Freund's adjuvant to induce EAU in mice.

1. Introduction

Autoimmune diseases, including sight-threatening posterior uveitis generate considerable morbidity with increasing incidence in industrialized countries. It is widely accepted that such diseases result from a breakdown in tolerance to self-antigens, and a range of organ-specific and non-organ specific experimental models have been developed using immunogenic peptides derived from known sequences of purified autoantigens. For sight-threatening uveitis, the most frequently employed model is EAU induced in C57Bl/6 mice by subcutaneous injection of peptides from the retina-specific antigen (interphotoreceptor retinal binding protein, IRBP) with complete Freund's adjuvant (CFA) and pertussis toxin. EAU is also inducible without pertussis toxin in the more susceptible B10RIII mouse strain. IRBP peptides are presented on activated innate immune antigen presenting cells, especially dendritic cells (DCs) (Forrester et al., 2010) and this immunization-induced model of EAU results in a predominantly $CD4^+$ Th17 cell-mediated disease. However, EAU could also be induced by adoptive transfer of IRBP-specific Th1 effector cells into syngeneic recipient mice without CFA (Luger et al., 2008). Furthermore spontaneous models of EAU which do not require adjuvants have been developed by crossing T cell receptor (TCR) mice specific for IRBP or for a neo-antigen, hen egg lysozyme (HEL) with the corresponding transgenic (Tg) mice expressing IRBP or HEL in the retina (Horai et al., 2013; Lambe et al., 2007). These models have been used extensively in determining the contribution of autoreactive T effector cells in EAU, but

Abbreviations: CARD, caspase recruitment domain; CLR, C-type lectin receptor; DC, dendritic cells; EAE, experimental autoimmune encephalitis; EAU, experimental autoimmune uveoretinitis; HEL, hen egg lysozyme; IRBP, interphotoreceptor retinal binding protein; MR, macrophage mannose receptor; MyD88, myeloid differentiation primary response gene 88; NLR, nuclear oligomerization domain receptor; PAMP, pathogen-associated molecular pattern; p.i, post immunization; PRR, pattern recognition receptor; RLR, retinoic acid inducible gene receptor; Syk, spleen tyrosine kinase; Tg, transgenic; WT, wild type.

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less so in defining innate recognition and signalling pathways that are involved in the generation of uveitogenic T cells.

Autoimmune diseases in humans are thought to be triggered by infectious diseases in which several types of innate immune cells, including antigen presenting DCs, are activated by pattern recognition receptors (PRRs) that bind conserved pathogen-associated molecular patterns (PAMPs) (Mills, 2011). Indeed commonly employed experimental models of autoimmune diseases such as EAU are not inducible without activation of the innate immune system by way of adjuvants, with killed Mycobacterium tuberculosis (MTB) in CFA being especially effective. Additional adjuvants such as pertussis toxin, which affects immune responses in a variety of ways (Racke et al., 2005), are also required to induce EAU in less susceptible mouse strains such as C57Bl/6 (Agarwal et al., 2012). Activation of PRRs belonging to the Toll-like receptor (TLR), C-type lectin receptor (CLR), nuclear oligomerization domain receptor (NLR) and retinoic acid inducible gene receptor (RLR) families generally induce the secretion of pro-inflammatory and T effector cell-polarising cytokines, such as IL-1β, IL-6, IL-12, IL-23 and TNFα (Kawai and Akira, 2011; Osorio and Reis e Sousa, 2011). It is unclear as to how MTB in CFA induces autoimmune diseases in experimental models, but a number of PRRs are known to recognise and respond to MTB. These include TLRs such as TLR-2, -4 and -9; CLRs such as dectin-1, DC-SIGN and MR; and NLRs such as NOD2 (Kleinnijenhuis et al., 2011).

The role of TLRs in EAU is not clear. Activation of TLR-2, -3, -4 or -9 has not been found to be essential for the induction of EAU, although the principal TLR signalling pathway-associated adaptor molecule MyD88 is required (Su et al., 2005; Fang et al., 2010). The role of CLRs in EAU induction is also currently unknown, but they are likely to be important because Th1 and Th17 adaptive immune responses are also entrained in the overall response to some CLR ligation including dectin-1, dectin-2, MR and Mincle (Hardison and Brown, 2012; van de Veerdonk et al., 2009). Dectin-1 and MR are best known for their induction of innate immune responses to fungal and bacterial carbohydrates including β-glucans and α-mannans (Brown and Gordon, 2001; Levitz, 2010), but they also recognise and respond to MTB (Kleinnijenhuis et al., 2011; Yadav and Schorey, 2006). This study investigates the role, if any, of MR and dectin-1 in CFA-mediated IRBP peptide-induced EAU.

2. Materials and methods

2.1. Reagents

Freund's adjuvant and purified *Bordetella pertussis* toxin were purchased from Sigma–Aldrich. *Mycobacterium tuberculosis* strain H37Ra was purchased from Thomas Scientific. Human IRBP-derived peptide 161–180 (SGIPYIISYLHPGNTILHVD) was synthesized by Fmoc chemistry (model 432A peptide synthesizer; Applied Biosystems). Monoclonal antibodies for the treatment of mice with immunization-induced EAU which included rat anti-mouse dectin-1 (IgG2b, clone 2A11), mannose receptor (IgG2b, clone MR5D3) or an isotype control (IgG2b, YTH913) were purified from hybridoma cell supernatants using Protein G column chromatography. The hybridomas for 2A11 and MR5D3 were produced as described previously (Brown et al., 2002; Martinez-Pomares et al., 2003) and YTH913 was a generous gift of Prof. Herman Waldmann, University of Oxford, U.K.

2.2. Mice

All mice were housed under specific pathogen-free conditions provided by the Medical Research Facility, University of Aberdeen and all procedures were carried out in compliance with the Home Office Animals Act (Scientific Procedures), 1986. Dectin-1$^{-/-}$ and MR$^{-/-}$ (CD206$^{-/-}$) knockout mice were generated by Taylor et al. (2007) and Lee et al. (2002), respectively. Wild type (WT) C57Bl/6, dectin-1$^{-/-}$ and MR$^{-/-}$ knockout mice backcrossed to the C57Bl/6 background were used at 6-8 weeks of age, groups in single experiments were age and gender matched. Gene knock out status of the breeders was confirmed via PCR as described previously (Taylor et al., 2007; Lee et al., 2002). B10RIII and B10BR mice were also used as described below.

2.3. Immunization-induced EAU model

For antibody blockade studies, B10RIII mice were immunized s.c. with 50 mg of the IRBP peptide 161–180 (SGIPYIISYLHPGNTILHVD; purity >95%; Sigma Genosys Co., Cambridge, U.K.) in CFA. Monoclonal antibodies were administered at various times during the course of EAU at 0.9 mg in PBS per mouse, as a single i.p. or i.v. injection. For determining the role if any of dectin-1 and MR in EAU, wild type C57Bl/6 mice and receptor knockout mice on this background were immunized s.c. in both hind limbs with 500 μg of the IRBP peptide 1–20 (GPTHLFQPSLVLDMAKVLLD; purity >95%) emulsified in CFA supplemented with a further 2.5 mg/mL of heat killed MTB (H37Ra, Difco, Detroit, MI). One μg of purified pertussis toxin was also administered i.p. at the time of immunization. In order to assess EAU during the peak phase of immunization-induced disease in the two strains, B10RIII mice were sacrificed at 14 days and C57Bl/6 at 21–28 days. Eyes and lymphoid organs were collected for further analysis.

2.4. Spontaneous uveoretinitis model

The mouse model of spontaneous uveoretinitis used here has been described: briefly single transgenic mice expressing HEL in the retina under control of the promoter for IRBP are crossed with TCR transgenic mice specific for HEL to produce double transgenic mice, 100% of which develop spontaneous uveitis with onset at day 21 post natal and peak of disease between days 35–40 as seen by histology (Lambe et al., 2007). This model does not rely on MTB as an innate immune system stimulator. Anti-dectin-1 (2A11) antibody was administered s.c. 50–100 μg per mouse 3 days prior to the onset of disease (day 18 post natal).

2.5. Adoptive transfer EAU model

The adoptive transfer model was chosen to test the effect of dectin-1 blockade on the effector phase of EAU since the HEL-specific T cells from 3A9 TCR Tg mice can be obtained in large numbers for adoptive transfer into naïve single Tg retinal HEL mice. EAU was induced following i.p. injection of 1×10^6 cells/mouse of cells derived from 3A9HEL-TCR Tg donor lymph node and spleen that had been cultured for six days in the presence of HEL (30 μg/ml). After 6-8 days, when peak disease was reached, the mice were sacrificed and EAU scored histopathologically.

2.6. EAU evaluation by clinical scoring and histopathology

In experiments that required the progress of EAU to be followed clinically, disease was scored by means of fundoscopy at days 12, 15 and 21 post immunization (p.i.) as previously described (Xu et al., 2008). Mice were sacrificed at the peak of disease and freshly enucleated eyes were embedded and frozen in Tissue-Tek O.C.T. (Sakura, U.K.); 5–6 μm cryosections, paraformaldehyde fixed and haematoxylin stained, were histopathologically graded from 0 (no disease) to 4 (severe disease, where no normal retina remains) as previously described (Caspi et al., 1988).

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