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Research paper

Modulation of human macrophage activity by *Ascaris* antigens is dependent on macrophage polarization state

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ABSTRACT

Parasitic worms (helminths) are known to actively modulate host immune responses and inflammation. The aim of this study was to investigate if adult body fluid (ABF) from the helminth *Ascaris suum* has immunomodulatory effects on different subtypes of human monocyte-derived macrophages (Mϕ) in vitro. Mϕs were exposed to *A. suum* ABF at different stages of their differentiation and/or polarization. Mϕ were first differentiated from monocytes into either uncommitted (M-), classically activated (M(GM-CSF)) or alternatively activated (M(M-CSF)) phenotypes and then stimulated with lipopolysaccharide (LPS). ABF strongly suppressed LPS-induced TNF-α, IL-6 and IL-10 secretion in M(GM-CSF)s, however in M(M-CSF)s only TNF-α was suppressed, with these cells secreting high levels of IL-10 which was not affected by ABF treatment. To determine if ABF modulated the differentiation of previously uncommitted Mϕ to either type 1 or type 2 Mϕ, monocytes were differentiated with human serum into (M-)s and then polarized by IFN-γ/LPS or IL-4 treatment in the presence of ABF. Under these conditions, ABF did not modulate cytokine secretion but did reduce CD80 expression in IFNγ/LPS-polarized cells but not IL-4-polarized cells. Finally, we demonstrate that when monocytes are differentiated into M(GMCSF)s in the presence of ABF, subsequent inflammatory responses are markedly suppressed. Our data suggest that ABF inhibits cytokine secretion and co-stimulatory molecule expression in classically activated Mϕ but not in alternatively activated Mϕ, indicating selective action of ABF depending on Mϕ subtype. Moreover, ABF appears to exert stronger activity when acting upon Mϕ that have already been polarized to the type 1 phenotype, rather than influencing the polarization process per se.

1. Introduction

Parasitic worms (helminths) are known to be strong regulators of the host immune response, minimizing immune attacks and creating an anti-inflammatory environment favorable to their survival and maintenance, persisting in the host for many years. These parasites generally skew the immune response towards T helper type 2 (Th2)-type responses, educating the immune system to avoid excessive T helper 1 (Th1)-type responses that are central in several autoimmune and inflammatory disorders (Ottow et al., 2014; van Riet et al., 2007). The incidence and prevalence of autoimmune and allergic diseases, such as inflammatory bowel disease, type 1 diabetes (T1D) and multiple sclerosis (MS) is growing in Western countries (Okada et al., 2010), which in part is thought to be due to increased hygiene and less exposure to parasites early in life. Consequently, controlled infection with helminths has been pursued as a novel therapy for autoimmune disorders, with mixed results (Summers et al., 2005; Voldsgaard et al., 2015). The identification of the mechanisms responsible for the pronounced immunomodulation observed in helminth infections may be a major step towards the identification of helminth-derived molecules which may be developed as novel anti-inflammatory agents. In addition, an increased understanding of these mechanisms may help in improved control of helminths in endemic regions, for example in designing effective vaccines.

The pig helminth *Ascaris suum*, closely related and morphologically indistinguishable to the human *A. lumbricoides*, is one of the most prevalent helminths in pigs and compromise growth and productivity (Thamsborg et al., 2013). In 2010, 819 million people worldwide were estimated to be infected with *A. lumbricoides*, mainly in East Asia, China, sub-Saharan Africa, and South and Central America (Pullan et al., 2014). Whilst *Ascaris* infections cause substantial morbidity in endemic regions, molecules derived from *A. suum* have shown some promise in treating inflammatory disorders in mouse models. For example, treatment of mice with body fluid derived from adult worms can down-regulate inflammatory responses during conditions such as asthma and allergic conjunctivitis (Lima et al., 2002; McConchie et al.,

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2006; Schopf et al., 2005).

Induction of innate inflammatory responses and immune function is controlled to a large extent by antigen presenting cells such as dendritic cells (DCs) and macrophages (Mϕs). Depending on the nature of the antigen and pattern recognition receptors that are ligated, these cells secrete specific subsets of cytokines that play a crucial role in the subsequent activity of antigen-specific T-cells. Lipopolysaccharide (LPS) is a structural component of the outer membrane of Gram-negative bacteria and induces a strong release of pro-inflammatory cytokines such as TNFα, IL-6 and IL-12 via stimulation of Toll-like receptor 4 (TLR4) (Lu et al., 2008). In addition to being of central importance in the host defense against invading pathogens, Mϕs are equally responsible for maintaining immunological homeostasis. Therefore, dysregulation of their functionality has been implicated in the pathogenesis of various autoimmune and inflammatory disorders (Murray and Wynn, 2011). Similar to the polarization of Th1 and Th2, Mϕs can be divided into different phenotypes depending on their function and location in the body. On the extremes these subsets have been classified as classically (M1) or alternatively (M2) activated, with uncommitted or naïve Mϕs termed as intermediate or M0. M1 Mϕs are generally pro-inflammatory Mϕs that can be induced by intracellular pathogens and bacterial cell wall components such as LPS. M2 Mϕs are regulatory Mϕs involved in tissue repair and reducing inflammation which can be induced by the cytokines IL-10 or IL-4 or, in some cases, helminth products (Mantovani et al., 2004). Indeed, the induction of M2 Mϕs is thought to be a central component of development of Th2-type immunity during helminth infection.

A number of previous in vitro studies have suggested that human Mϕ activity can be modulated by helminth products. Hoeksema et al. (2016) observed that products from the porcine helminth *Trichuris suis* reduced expression and production of IL-6 and TNF-α, as well as chitinase activity in M1 Mϕs and an upregulation of IL-10 and M2 surface markers expression in both uncommitted (M0) and M2 Mϕs. When added early during monocyte-to-macrophage differentiation, *T. suis* products imprinted phenotypic changes on pro-inflammatory cytokine genes in subsequent M1 Mϕs. Similarly, Ottow et al. (2014) showed that *T. suis* products reduced mRNA expression of many pro-inflammatory mediators typical of a LPS-induced profile, including a reduced secretion of the pro-inflammatory cytokines IL-6 and TNF-α, whilst Zawistowska-Deniziak et al. (2017) demonstrated a broad range of cytokines were suppressed after treatment with products from the tapeworm *Hymenolepis diminuta*. Thus, it is likely that the induction of regulatory and anti-inflammatory activity in Mϕs may be a key mechanism in the prolonged states of immune-anergy observed during chronic helminth infection, and which may potentially be harnessed as a novel therapy for autoimmune disorders.

It is still not clear to what extent helminth products can affect the polarization of human Mϕ and/or cytokine production of previously polarized Mϕ. Moreover, the effects of *Ascaris* antigens on human Mϕ activity have not yet been investigated. Given the extremely high prevalence of *Ascaris* infection worldwide, and the putative anti-inflammatory effects of *A. suum* antigens in mice (McConchie et al., 2006), an increased understanding of the immune-modulating effects of *Ascaris* may aid both novel therapeutic options for inflammatory disorders as well as improved control of *Ascaris* infections in endemic regions. Therefore, in the present study, we aimed to investigate the effects of *A. suum* adult body fluid (ABF) on human Mϕ phenotype and functional activity when added at three different points of differentiation − during monocyte-to-macrophage differentiation, and during and after Mϕ polarization. We hypothesized that treatment with ABF would result in a suppression of the pro-inflammatory M1 profile by reducing LPS-induced pro-inflammatory cytokines and typical M1 cell surface markers, and additionally result in an increased M2 phenotype characterized by anti-inflammatory cytokine secretion and typical M2 surface marker expression.

2. Materials and methods

2.1. Preparation of Ascaris suum adult body fluid (ABF)

A. suum ABF was obtained from freshly collected adult worms by decapitation and collection of the pseudocoelomic fluid, followed by centrifugation at 10,000*g* for 15 min. The supernatant was filtered through a 0.2 µm syringe filter and stored at −80 °C before use. The protein content was determined by the BCA assay (Pierce).

2.2. Purification of PBMCs and isolation of monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats collected from different blood donors at Rigshospitalet blood bank (Copenhagen, Denmark) following written, informed consent. The PBMCs were obtained by centrifugation over Histopaque-1077 (Sigma-Aldrich, USA). The PBMC-enriched band was then collected and monocytes were obtained by positive selection by magnetic cell sorting with anti-CD14 beads (MACS, Miltenyi Biotec, Germany). Monocytes were washed, stained with Trypan Blue solution and counted.

2.3. Differentiation and polarization of human macrophages

Monocytes were seeded (10^6/mL) in Dulbeco's Modified Eagle's Medium (DMEM)–high glucose (Sigma-Aldrich, USA) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 µg/mL streptomycin and 100 U/mL penicillin (Complete DMEM). For Mϕ differentiation, 3 different methods were used (Fig. 1–A, B and C). The resulting Mϕ subtypes were defined according to the nomenclature described in Murray et al., whereby each subtype is described by the specific stimulus rather than by the over-generalized M1 and M2 classifications (Murray et al., 2014).

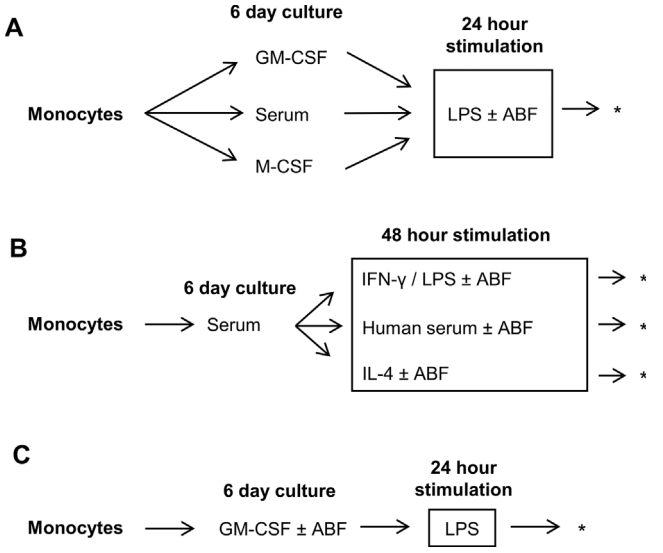

Fig. 1. Experimental design. Human monocytes were differentiated (A) into pro-inflammatory type-1, uncommitted or alternatively activated type-2 macrophages using either granulocyte/macrophage colony-stimulating factor (GM-CSF), human serum (serum) or macrophage colony-stimulating factor (M-CSF) for 6 days, respectively and subsequently stimulated with lipopolysaccharide (LPS) alone, *A. suum* adult body fluid (ABF) alone, or LPS plus ABF for 24 h. Alternatively, monocytes were differentiated first into uncommitted M(−) macrophages (B) and further polarized into type-1, M(−) or type-2 macrophages in the presence or absence of ABF using either IFNγ and LPS, human serum, or IL-4 for 48 h, respectively. Another group of monocytes (C) were differentiated in the presence of GM-CSF with or without ABF and later stimulated with LPS. * Time of media collection and cell harvest, for cytokine quantification by ELISA and phenotypic analysis by flow cytometry, respectively.