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Trichostrongylus colubriformis induces IgE-independent CD13, CD164 and CD203c mediated activation of basophils in an in vitro intestinal epithelial cell co-culture model

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ABSTRACT

Gastrointestinal nematodes pose a major risk to the farming of small ruminants worldwide. Infections are typically controlled by anthelmintics, however as resistance to anthelmintics increases, it is necessary that the mechanism of host responses are understood in order to develop alternative control options. It is hypothesised that basophils are involved in the initiation of an anti-parasite immune response, independent of IgE. In this study, the in vitro activation states of $CD203c^+$ basophil-like KU812 cells were determined in the presence of *Trichostrongylus colubriformis* parasitised HT29 epithelial cells with or without mucin. Cell surface expression of CD164, CD107a and CD13 antigens on gated $CD203^+$ cells were determined and qRT-PCR was used to examine gene expression changes of IL33 (a Th2 cytokine) and the high affinity IgE receptor (FcεRIα) within the co-culture. When KU812 basophils encountered *T. colubriformis* and/or mucin in a parasitised epithelium, the basophils increased cell surface expression of CD13 and CD164 antigens, independent of IgE. *T. colubriformis* also increased the number of $CD203c^+$ KU812 cells that expressed CD13 and CD164 antigens. These data support the in vivo observations of *T. colubriformis* primary infections in guinea pigs and sheep.

1. Introduction

Gastrointestinal nematode (GIN) parasites pose a major health and productivity problem to the livestock sector globally (Miller and Horohov, 2006; Sacket et al., 2006). Typically, GIN parasite infections are controlled by grazing management and anthelmintic treatment, however widespread emergence of anthelmintic resistance in nematode parasites (Kaplan, 2004) has increased the need for the development of protective anti-GIN vaccines (LeJambre et al., 2008). By deliberately mimicking the host–parasite interactions that initiate an immune response to GIN, such as damage to intestinal epithelial cells, a vaccine might be designed which is effective against GIN (Andronicos et al., 2012). Knowledge of the activation states and types of innate immune cells that initially respond to the danger signals (Matzinger, 1994) induced by exposure to GIN is expected to be critical for the development of such an effective anti-GIN vaccine.

To successfully combat helminth parasites such as *Trichostrongylus colubriformis* (*T. colubriformis*), a predominantly Th2 response is required. Although research supports the idea that there are several mechanisms for a protective anti-parasite response may exist for example

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passive immunization of anti-CarLA antibodies (Harrison et al., 2008). However, a Th2 type activity predominantly facilitates a protective host response in *T. colubriformis* immunized sheep. Th2 responses induced by GIN are generally characterized by increases in inflammatory cells such as mucosal mast cells/globular leukocytes (Cardia et al., 2011), basophil infiltration and activation (Rothwell and Love, 1975); (Huxtable and Rothwell, 1975), eosinophilia (Amarante et al., 2007), production of type 2 (IgG1, IgE and IgA) anti-parasite antibodies (Andronicos et al., 2010) and the release of bioactive substances such as histamine and leukotrienes (Bendixsen et al., 1995; Jones et al., 1990) at the site of infection. Although immune responses to helminth parasites follow this general trend, the response to different nematodes varies considerably (Gause et al., 2003) and responses from related host species towards the same parasite is also variable (Harris and Gause, 2011). Recently, it has been postulated that the cytokines IL25, IL33 and TSLP may provide a link between the innate and adaptive immune responses to GIN (Coffman, 2010). In sheep genetically resistant to GINs it appears that initiation of Th2 type cytokine production responses are mediated, at least in part, by the release of IL33 from parasite damaged cells (Andronicos et al., 2012).

New insights have recently emerged supporting a role for basophils in the initiation stages of the immune response (Gibbs, 2005). Several studies in mice have demonstrated that basophils are a source of Th2 cytokines, driving the production of Th2 cells (Sullivan and Locksley, 2009). Basophils can also act as antigen presenting cells (APCs) driving Th2 and IgE responses against protease, protein allergens and parasites (Nakanishi, 2010). Both mucosal mast cells and basophils can activate B cells directly to produce IgE, IL-4 or IL-13 (Henz et al., 2001). Indeed during a primary *T. colubriformis* infection in guinea pigs the number of basophil/mast cells increases in the jejunum (Rothwell, 1975). Moreover, impaired basophil activity in juvenile mice is associated with a delayed Th2 immune response (i.e. decreased Th2 cytokine production, IgE responses, mouse mast cell protease 1 levels, and goblet cell proliferation) during primary infection with *Nippostrongylus brasiliensis* (Nel et al., 2011). Basophils have been shown to secrete IL4 and IL13 early in the immune response when stimulated with IL18 or IL33, promoting Th2 differentiation independently of IgE (Kroeger et al., 2009).

The aim of this study was to determine if basophils are activated in an IgE-independent manner by *T. colubriformis* larvae in a damaged mucosal epithelial environment. The parasite-human epithelial cell co-culture system established by Andronicos et al. (2012) was modified and used to examine the activation of a human basophil-like cell line by *T. colubriformis* larvae. These parasites have been shown to establish patent infections in humans (Sato et al., 2011).

2. Materials and methods

2.1. Cell lines

All cell culture reagents were obtained from Invitrogen, USA and all fluorochrome-conjugated antibodies were from Biolegend, USA unless otherwise stated. The human colonic epithelial cell line HT29 and the human basophil cell line, KU812 were routinely passaged according to EDAAC guidelines using McCoys 5a media supplemented with 10% heat-inactivated FCS and 1× penicillin-streptamycin or in RPMI supplemented with 10% FCS and 1× penicillin-streptamycin respectively. Cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere.

2.2. Protein preparations

Post exsheathment antigen (PEA) from infective L3 McMaster *T. colubriformis* larvae and whole cell lysate (WCL) from HT29 cells were produced as described previously (Andronicos et al., 2012). Preparations were aliquoted and stored at −80 °C until required. Porcine mucin (40 mg/ml; Sigma, USA) was dissolved in sterile complete RPMI medium (20 ml) overnight at 4 °C. The solution was then aliquoted and frozen at −20 °C until required. All protein concentrations were determined using BCA protein assay using BSA as a standard.

2.3. Air/liquid interface intestinal epithelial cell-parasite co-culture system

The epithelial cell-parasite co-culture model described by Andronicos et al. (2012) was used with the exception that KU812 cells were co-cultured with the HT29 cells for testing (where indicated). Briefly, after culturing HT29 cells in six-well plates using an air-liquid interface architecture for 24 h in a humidified incubator, plates were initially prepared by adding 1×10^6 KU812 cells to the well inserts and the plate incubated for a further 1–2 h. To establish the remainder of the time course experiments, 1×10^6 KU812 cells in 250 μl of complete RPMI media were added to each of the inserts. Complete RPMI media (250 μl) was added to control inserts and previously exsheathed *T. colubriformis* L3 larvae in 250 μl complete RPMI media with or without 2 μM ivermectin (Sigma, USA) were added to the insert. In some treatments exsheathed larvae were also resuspended with or without 2.5 mg/ml porcine mucin in 250 μl complete RPMI media. When PEA was included as a treatment it was added at 1 mM in 250 μl of complete media to the insert containing cells. All plates were incubated at 37 °C in a 5% CO_2 humidified atmosphere for up to 7 days.

2.4. Parasite motion assays

Exsheathed *T. colubriformis* L3 larvae were co-cultured with either HT29 cells or HT29 and KU812 cells with or without 2 μM ivermectin or 2.5 mg/ml mucin and their motion recorded using ProgRes C10 camera (Jenoptik, Germany) attached to a TE300 inverted microscope (Nikon, Germany). To assess the motility changes in the parasites under the different conditions, the video microscopy was recorded using the live image option of the ProgRes CapturePro 2.1 software (Jenoptik, Germany) and captured from the screen using ZD Soft Screen Recorder 5.4. Parasite motion under different co-culture conditions was