International Journal for Parasitology 43 (2013) 531–539

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

The effects of *Fasciola hepatica* tegumental antigens on mast cell function

Krisztina V. Vukman [a], Paul N. Adams [a], David Dowling [a], Martin Metz [b], Marcus Maurer [b], Sandra M. O'Neill [a,*]

[a] *Parasite Immune Modulation Group, School of Biotechnology, Faculty of Science and Health, Dublin City University, Glasnevin, Dublin 9, Ireland*
[b] *Department of Dermatology and Allergy, Charité – Universitätsmedizin Berlin, Germany*

ARTICLE INFO

Article history:
Received 25 November 2012
Received in revised form 4 January 2013
Accepted 22 January 2013
Available online 25 March 2013

Keywords:
Helminth
Fasciola hepatica
Mast cell
STAT6
Migration
Proliferation
Chemokines
Tegument

ABSTRACT

Fasciola hepatica infection is associated with T helper 2/T regulatory immune responses and increased mast cell numbers. The aim of this study was to examine the interaction between *F. hepatica* tegumental coat antigen and mast cells in vivo and in vitro. Firstly, BALB/C, C57BL/6 or STAT6$^{-/-}$ mice were infected with *F. hepatica* metacercarie or mice were treated with *F. hepatica* tegumental coat antigen and then mast cells numbers in the peritoneal cavity and/or the liver were quantified. Also, the proliferation, chemotaxis, degranulation and cytokine secretion of mast cells from bone marrow or from peritoneal exudate cells stimulated with *F. hepatica* tegumental coat antigen were measured. Finally, we tested whether *F. hepatica* tegumental coat antigen inhibits degranulation of mast cells in vivo in a passive cutaneous and systemic anaphylaxis mouse model. Mast cell numbers increased in the peritoneal cavity and liver of *F. hepatica* infected mice, and this was mimicked by injection of *F. hepatica* tegumental coat antigen in a STAT6$^{-/-}$ independent manner. The increase in mast cell number was not the result of *F. hepatica* tegumental coat antigen-induced proliferation; rather *F. hepatica* tegumental coat antigen indirectly induces mast cell migration by dendritic cell-derived chemokines. *Fasciola hepatica* tegumental coat antigen interactions with mast cells do not drive T helper 2 or T regulatory immune responses. These studies on mast cell and *F. hepatica* tegumental coat antigen interaction may help us to understand the function of mast cells in immunity against *F. hepatica* and the immunomodulatory effect of *F. hepatica* tegumental coat antigen on these cells.

1. Introduction

Helminth infections are associated with increased mast cell numbers and the redistribution of these cells to the site of infection (McCole et al., 1998; Maizels et al., 2004; Weller et al., 2011). Mast cells are associated with helminth-induced T helper (Th)2 immune responses (Dawicki and Marshall, 2007) where Th2 cytokines secreted by CD4$^+$ T cells direct B cells to produce helminth-specific IgE (Anthony et al., 2007; Erb, 2007). Mast cells express the high affinity FcεRI receptor, which in the presence of helminth antigens and the specific IgE antibody induce mast cell degranulation and the release of inflammatory mediators such as histamine, cytokines or chemokines. These mediators can promote Th2 immune responses (Melendez et al., 2007; Pearce, 2007) as *Heligmosomoides polygyrus* infection activates mast cells in mice to induce tissue-derived IL-25, IL-33 and TSLP cytokines (Hepworth et al., 2012a), which drive Th2 cell priming by dendritic cells (DCs) (Hepworth et al., 2012b).

Mast cells contribute to the clearance of helminth infection with gut parasites (Anthony et al., 2007; Klementowicz et al., 2012). For example, studies in mouse and rat models demonstrated that the clearance of *Trichinella spiralis*, *Nippostrogylus brasiliensis* or *Strongyloides ratti* is mast cell-dependent (Abe and Nawa, 1988; McDermott et al., 2003; Marshall, 2004). Nematode fecundity is higher in mast cell-deficient mice when infected by *H. polygyrus* compared with normal or mast cell-reconstituted mice (Hashimoto et al., 2009) and these cells contribute to the development of Th2 responses associated with this gastrointestinal helminth by activating other cells involved in Th2 immunity, such as epithelial cells or DCs (Hepworth et al., 2012b). While mast cells are crucial in the clearance of gut helminths, they do not appear to a have key role in the clearance of other tissue dwelling helminths such as *Schistosoma*, although they are present in the early stages of infection (Gerken et al., 1990).

Fasciola hepatica causes a chronic liver infection (fascioliasis) in mammals, including cattle and sheep (Collins et al., 2004) and is an important zoonotic disease in humans with approximately 2.4 million people infected worldwide (McManus and Dalton, 2006). The life cycle of the parasite is complex with an intermediate snail host and definitive mammalian host. The definitive host is infected

* Corresponding author. Tel.: +353 1 700 5455; fax: +353 1 700 5412.
E-mail address: Sandra.ONeill@dcu.ie (S.M. O'Neill).

0020-7519/$36.00
http://dx.doi.org/10.1016/j.ijpara.2013.01.011

following ingestion of metacercariae shed from snails. These metacercariae excyst in the duodenum to form newly excysted juvenile flukes that penetrate the intestinal wall. They migrate through the peritoneal cavity and liver for several weeks and then finally the adult worm resides in the bile ducts, releasing eggs for up to 30 years.

Fasciola promotes strong Th2/T regulatory (Treg)-mediated immune responses characterised by the production of IL-4, IL-5 and IL-10 (O'Neill et al., 2000; Miller et al., 2009). Previous studies have shown mast cell numbers are increased in the liver of cattle and sheep infected with *F. hepatica* (Rahko, 1969; Ferreras et al., 2000) and in the Peyer's patches (O'Brien et al., 2008) and the mucosa of the small intestine (Charbon et al., 1991; Van Milligen et al., 1998) in *F. hepatica*-infected rats. We know that injection of *F. hepatica* excretion-secretory antigens (FhES), mimics the Th2 immune response observed during *Fasciola* infection; however, it does not enhance mast cell numbers in rats (Trudgett et al., 2003). Other studies have shown that *F. hepatica*-derived peptides from the tegumental coat induce degranulation of rat peritoneal mast cells and histamine release (Trudgett et al., 2003).

The *Fasciola* tegumental coat is shed from the parasite every 2–3 h and is in constant contact with host immune cells (Lammas and Duffus, 1983). Previously we isolated tegumental antigens from *F. hepatica* (FhTeg) and examined their interactions with innate immune cells. We found that FhTeg inhibits DC maturation and function by inhibiting IL-6, TNF-α, IL-10 and IL-12 production and CD80, CD86 and CD40 cell surface marker expression in toll-like receptor (TLR)4-stimulated DCs. FhTeg also impairs DC function by inhibiting its phagocytic capacity and its ability to prime T cells (Hamilton et al., 2009; Hacariz et al., 2010). In this study we examined the interaction between FhTeg and mast cells in order to help us understand the function of mast cells in immunity against *F. hepatica*.

2. Material and methods

2.1. Animals and antigens

C57BL/6, BALB/C and STAT6$^{-/-}$ mice, 6–8 weeks old, were purchased from Charles River (Carrentrilla, Ireland). Mice were kept under specific pathogen-free conditions at the Bioresource Unit, Faculty of Health and Science, Dublin City University (DCU), Ireland. All mice were maintained according to the guidelines of the Irish Department of Children and Health. Ethical approval for mouse experiments was obtained from the DCU Ethics Committee and the Irish Department of Children and Health.

FhES and FhTeg were prepared as previously published (Hamilton et al., 2009). Endotoxin levels were determined using the Pyrogene endotoxin detection system (Cambrex, Germany). FhES and FhTeg showed endotoxin levels similar to background levels and were less than the lower limit of detection in this assay (<0.01 EU/ml). Heat-inactivated *Bordetella pertussis* (BP) was a kind gift from Professor Bernie Mahon, National University of Ireland Maynooth (NUIM), Maynooth, Ireland (Mahon et al., 1996). All protein concentrations were determined using a bicinchonic acid (BCA) protein assay kit (Pierce, Fischer Scientific, Dublin, Ireland).

2.2. Animal experiments

BALB/C, C57BL/6 or STAT6$^{-/-}$ mice were infected for 2 weeks with a single application of 20 *F. hepatica* metacercariae in 100 μl of water orally using a 200 μl pipette (Baldwin Aquatics Ltd, USA), or injected i.p. three times per week for 3 weeks with PBS (100 μl), FhTeg (10 μg in 100 μl) or FhES (20 μg in 100 μl). Mice were killed by cervical dislocation and peritoneal cells were obtained from the mice by injecting 10 ml of PBS into the peritoneal cavity. Cell numbers and viability were monitored using trypan blue staining. Percentages of mast cells were determined by measuring cell surface expression of c-kit and FcεRI and by Kimura staining (Kimura et al., 1973).

The abdominal cavity was opened and the posterior vena cava was cut. Using a 1 ml syringe with a pipette tip, approximately 1 ml of blood was collected from each mouse. Blood samples were incubated for 1 h at room temperature and centrifuged for 15 min at 1,500*g* at 4 °C. Serum (the top yellow phase) was collected and tested without dilution on bone marrow-derived cultured mast cells (BMMCs) in a β-hexosaminidase assay as described previously (Vukman et al., 2013).

2.3. Histological studies

The livers were gently removed from *F. hepatica*-infected mice after 2 weeks and fixed in 4% formalin. Sections (5 μm thick) were stained with toluidine blue or alcian blue/safranin staining as previously described (Vukman et al., 2012). Mast cells were identified by their metachromatic staining. Mast cell density was expressed as cells/mm^2.

2.4. Passive cutaneous and systemic anaphylaxis model

Briefly, C57BL/6 mice were injected i.p. with FhTeg (10 μg in 100 μl of PBS) or PBS at 24 and 48 h prior to administering dinitrophenol (DNP)-specific IgE and 2.5 h prior to challenge with DNP by i.p. injection (Passive Systemic Anaphylaxis model; PSA) or injection into the ears of mice (Passive Cutaneous Anaphylaxis model; PCA) (Siebenhaar et al., 2007). In the PSA model, rectal temperature was recorded every 10 min for 2 h. In the PCA model ear swelling was measured 1, 2, 4 and 8 h after DNP challenge.

2.5. Isolation, maturation and characterisation of bone marrow and peritoneal cell-derived mast cells

BMMCs were generated from the femoral and tibia bone marrow cells of C57BL/6 mice and maintained in complete Iscove's modified Dulbecco's medium (IMDM) in the presence of 10% heat-inactivated FCS, 100 u/ml of penicillin/streptomycin, L-Glutamine (2 mM) and 30% WEHI-3 conditioned IMDM-medium (ATTC TIB-68) as a source of the murine mast cell growth-factor, IL-3, for 4 weeks. Peritoneal cells were obtained from C57BL/6 mice after i.p. injection of 10 ml of sterile PBS and then cultured in RPMI 1640 medium supplemented with 10% FCS and 100 u/ml of penicillin/streptomycin, 10 ng/ml of recombinant mouse IL-3 (Calbiochem, Merck, Darmstadt, Germany) and 30 ng/ml of recombinant mouse stem cell factor (SCF) at 37 °C as previously described (Vukman et al., 2012). Non-adherent cells were removed 48 h later and replaced by fresh culture medium. Seven days later, >95% of the total cells were identified as mast cells on the basis of c-kit and FceRI cell surface expression or Kimura staining. Cell number and viability were monitored using trypan blue staining. β-Hexosaminidase release from mast cells was measured to test functionality. Degranulation was measured following stimulation of mast cells with various antigens or with DNP (200 ng/ml)/anti-DNP IgE (400 ng/ml) in the presence or absence of FhTeg.

2.6. Stimulation of mast cells for cytokine measurement and flow cytometry

Mast cells were cultured with PMA (Phorbol myristate acetate, 25 ng/ml), A23187 (1 μM), or FhTeg (10 μg/ml) and after 1, 4, 6 or 24 h, levels of TNF-α, IL-6, IL-4, IL-5, IL-10 and IFN-γ were measured in the supernatant by commercial ELISA (BD Biosciences, Oxford, UK). Cells were incubated for 15 min with CD16/CD32 (FcγIII/II, BD Biosciences) to block FcR prior to flow cytometric