Immunobiology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

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

Short communication

Mast cells and histamine alter intestinal permeability during malaria parasite infection

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ARTICLE INFO

Article history:
Received 9 August 2015
Received in revised form
11 November 2015
Accepted 11 November 2015
Available online xxx

Keywords:
Mast cell
Histamine
Co-infection
Bacteremia
Malaria
Plasmodium
Salmonella

ABSTRACT

Co-infections with malaria and non-typhoidal *Salmonella* serotypes (NTS) can present as life-threatening bacteremia, in contrast to self-resolving NTS diarrhea in healthy individuals. In previous work with our mouse model of malaria/NTS co-infection, we showed increased gut mastocytosis and increased ileal and plasma histamine levels that were temporally associated with increased gut permeability and bacterial translocation. Here, we report that gut mastocytosis and elevated plasma histamine are also associated with malaria in an animal model of falciparum malaria, suggesting a broader host distribution of this biology. In support of mast cell function in this phenotype, malaria/NTS co-infection in mast cell-deficient mice was associated with a reduction in gut permeability and bacteremia. Further, antihistamine treatment reduced bacterial translocation and gut permeability in mice with malaria, suggesting a contribution of mast cell-derived histamine to GI pathology and enhanced risk of bacteremia during malaria/NTS co-infection.

1. Introduction

Nearly half of the global population is at risk for malaria, which results in approximately 600,000 deaths annually (WHO, 2014). Most of these deaths are due to *Plasmodium falciparum* infection in sub-Saharan Africa in pediatric patients, a population with a high prevalence of co-infection with non-typhoidal serotypes of *Salmonella* (NTS) (Reddy et al., 2015). In healthy individuals, infections with NTS are associated with gastroenteritis, a localized infection with low mortality. However, co-infected individuals can develop a life-threatening NTS bacteremia (Scott et al., 2015) that is complicated by the increasing prevalence of antibiotic resistance in Africa (Feasey et al., 2015; Kingsley et al., 2009).

In the course of infection, sequestration of parasitized red blood cells (RBCs) causes capillary blockage that is prominent in intestinal villi (Seydel et al., 2006) and associated with increased gastrointestinal (GI) permeability (Molyneux et al., 1989; Wilairatana et al., 1997). Severe malaria has also been associated with high circulating histamine (Enwonwu et al., 2000; Srichaikul et al., 1976), suggesting a connection between allergic inflammation and disease. To identify mechanisms behind increased GI permeability, invasive bacterial disease, and bacteremia during malaria infection, we developed a murine model of co-infection with *Plasmodium yoelii* and the NTS strain *Salmonella enterica* serotype Typhimurium (Roux et al., 2010). In this model, co-infected mice have higher levels of *S.* Typhimurium in their mesenteric lymph nodes, spleens, and livers than do mice infected with *S.* Typhimurium alone (Roux et al., 2010). Further, we observed that rising parasitemia is associated with increased intestinal permeability, increased circulating and ileal histamine levels, and ileal mastocytosis, suggesting that allergic inflammation contributes to GI pathology in our mouse co-infection model (Chau et al., 2013).

Mucosal mast cells are primary regulators of the physical integrity and function of the intestinal epithelial barrier (Gurish and Austen, 2012). In the context of parasitic infections, intestinal mastocytosis is required for expulsion of GI nematodes, via intesti-

Abbreviations: NTS, non-typhoidal *Salmonella* serotypes; GI, gastrointestinal; RBC, red blood cell; H1, histamine receptor 1; H2, histamine receptor 2; i.p., intraperitoneally; CFU, colony forming units; PBS, phosphate-buffered saline; LB, Luria–Bertani medium; TBST, Tris-buffered Saline; IL, interleukin (IL-10,4,6); TNF-α, tumor necrosis factor alpha; IFNγ, interferon gamma; B2 M, beta-2-microglobulin; EcN, *Escherichia coli* Nissle; NO, nitric oxide.

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http://dx.doi.org/10.1016/j.imbio.2015.11.003
0171-2985/

nal permeability that is enhanced by mast cell protease-dependent alterations to intercellular tight junctions and adherens junctions (Grencis et al., 2014). In this context, mast cells are recruited and traffic from the submucosa to the villus tips, returning to the crypts once infection is resolved (Friend et al., 1998). Other mast cell-derived mediators, however, have been associated with secretory diarrhea and intestinal malabsorption, including histamine (Castro et al., 1987; Madden et al., 2002). This is particularly notable in mastocytic enterocolitis, chronic intestinal permeability that is reversible by treatment with H1 and H2 histamine receptor antagonists (Jakate et al., 2006).

Based on our observations of increased ileal mastocytosis and histamine in our mouse malaria model and related facts regarding mastocytosis and other parasitic infections, we asked whether mastocytosis and elevated circulating histamine levels were more broadly evident in malaria using a non-human primate model of falciparum malaria and whether mast cell ablation and antihistamine therapy in in our mouse model could increase the integrity and function of the intestinal epithelial barrier, thereby reducing bacterial translocation during *S.* Typhimurium co-infection.

2. Materials and methods

2.1. Animals

Control uninfected and *Plasmodium fragile*-infected Rhesus macaque (*Macaca mulatta*) tissues were from two previous studies (Mooney et al., 2014; Raffatellu et al., 2008). Six- to 8-week-old female CBA/J mice and WBB6F1/J-Kit^{W}/Kit^{W-v} mice and their wild type littermate controls were purchased from Jackson West. Female CD-1 mice for parasite stock expansion were from Harlan. Macaque and mouse protocols were reviewed and deemed to be in accord with all relevant institutional policies and federal guidelines by the University of California Davis Institutional Animal Care and Use Committee.

2.2. Parasites and bacteria

Mice were inoculated intraperitoneally (i.p.) on day 0 with 0.1 mL of uninfected CD-1 RBCs (uninfected control) or with 10^7 *P. yoelii* 17XNL-infected RBCs. Mice were orally gavaged at 9 days after RBC inoculation with 20 mg of streptomycin to enhance subsequent colonization (at 10 days after RBC inoculation) of *Escherichia coli* Nissle, which is resistant to streptomycin and ampicillin, or *S.* Typhimurium strain IR715 (pHP45 Ω), which is resistant to streptomycin, ampicillin, and nalidixic acid (Supplementary Fig. 1). For both bacterial species, mice were inoculated with 0.1 mL of an overnight culture (37 °C) of 10^8 colony-forming units (CFU) following our previous protocol (Chau et al., 2013).

2.3. Microbial readouts for mouse infection

Parasitemias were recorded beginning 2 days following infection with *P. yoelii* 17XNL (hereafter, *P. yoelii*) as infected RBCs divided by the total number of RBCs in Giemsa-stained thin blood films. To quantify tissue *S.* Typhimurium CFU, liver and spleen were aseptically removed and homogenized in cold phosphate-buffered saline (PBS) using an Ultra Turrax T25 basic mixer (IKA Works, Inc., Wilmington, NC). Serial dilutions were plated on selective media (LB agar plus ampicillin or LB agar plus

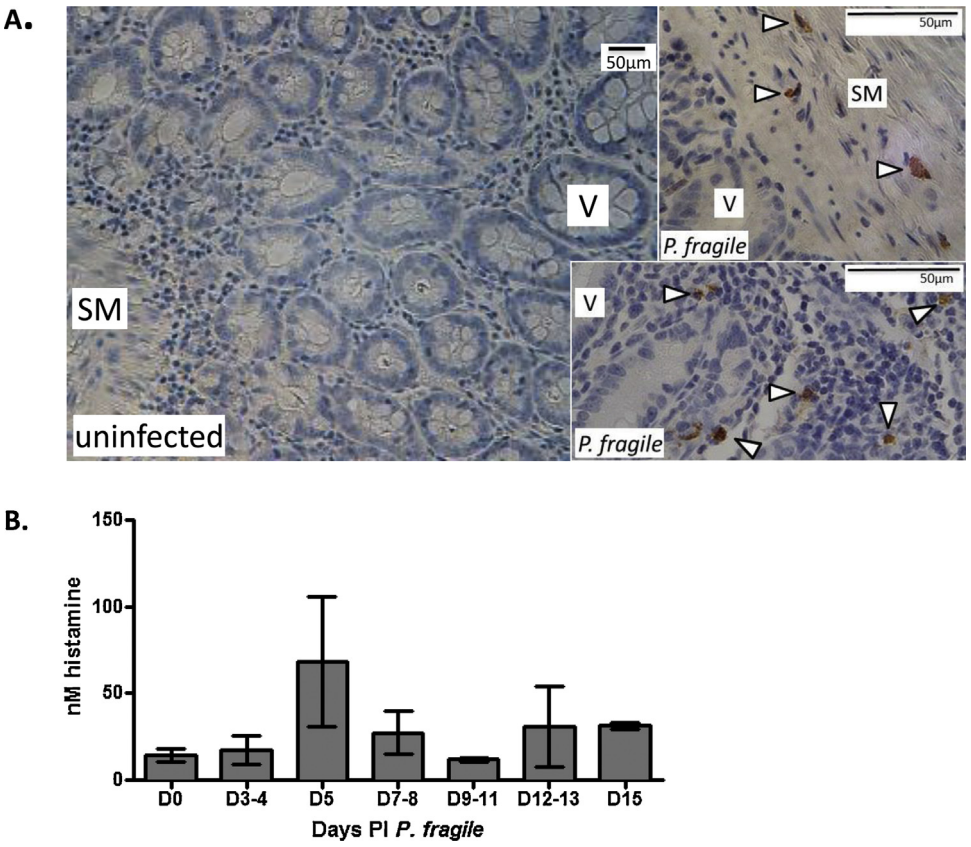

Fig. 1. *Plasmodium fragile*-infected Rhesus macaques exhibit increased ileal mastocytosis and plasma histamine levels. (A) Representative ileum sections from macaques infected with *P. fragile* immunostained with anti-tryptase and counter-stained with hematoxylin. Mast cells were detected in *P. fragile*-infected animals (arrowheads, $n = 4$) but not in uninfected controls ($n = 4$). SM denotes submucosa, V denotes villi. Bars equal 50 μm. (B) Mean histamine levels (+/−SEM) in plasma of *P. fragile*-infected macaques from panel A.

Please cite this article in press as: Potts, R.A., et al., Mast cells and histamine alter intestinal permeability during malaria parasite infection. Immunobiology (2015), http://dx.doi.org/10.1016/j.imbio.2015.11.003