Immunobiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

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

MRP8/14 does not contribute to dissemination or inflammation in a murine model of Lyme borreliosis

Lauren M.K. Mason[a,*], Jeroen Coumou[a], Jasmin I. Ersöz[a], Anneke Oei[b], Joris J.T.H Roelofs[c], Thomas Vogl[d], Tom van der Poll[a,e], Joppe W.R. Hovius[a,e]

[a] Center for Experimental and Molecular Medicine, Academic Medical Center, Amsterdam, The Netherlands
[b] Department of Medical Microbiology, Academic Medical Center, Amsterdam, The Netherlands
[c] Department of Pathology, Academic Medical Center, Amsterdam, The Netherlands
[d] Institute of Immunology, University of Muenster, Muenster, Germany
[e] Division of Infectious Diseases, Academic Medical Center, Amsterdam, The Netherlands

ARTICLE INFO

Keywords:
Borrelia
Lyme
MRP8/14
Calprotectin
S100A8
S100A9

ABSTRACT

Myeloid-related protein (MRP)8 and MRP14 form a complex (MRP8/14) that is released by activated neutrophils and monocytes during infection. MRP8/14 has been shown to have bacteriostatic activity *in vitro* against *Borrelia burgdorferi*, the spirochete that causes Lyme borreliosis. Furthermore, levels of MRP8/14 have been shown to be elevated in the joints of patients with Lyme arthritis. We hypothesized that MRP8/14 has a protective effect during *B. burgdorferi* infection. To determine the role of MRP8/14 in the immune response to *B. burgdorferi*, we studied the course of *B. burgdorferi* infection in wildtype (*wt)* and *mrp14*$^{-/-}$ mice. In addition, we studied the response of leukocytes from mice lacking MRP8/14 to *B. burgdorferi ex vivo*. We demonstrated similar levels of *B. burgdorferi* dissemination, cytokine and immunoglobulin production in infected *wt* and *mrp14*$^{-/-}$ mice after 21 days. Neutrophils and monocytes lacking MRP8/14 were undiminished in their ability to become activated or phagocytose *B. burgdorferi.* In conclusion, we did not find a central role of MRP8/14 in the immune response against *B. burgdorferi.* As the levels of MRP8/14 in the serum of infected mice were low, we speculate that MRP8/14 is not released in levels great enough to influence the course of *B. burgdorferi* infection.

1. Introduction

Lyme borreliosis is caused by *Borrelia burgdorferi* sensu lato and transmitted by *Ixodes* ticks. In disseminated disease, the bacteria can affect the joints, heart, central nervous system and the skin (Steere, 2001). It has been demonstrated that both innate and adaptive immune mechanisms are deployed to combat the bacteria, however the complex immunopathogenesis of the disease and the host factors that affect it are not entirely understood (Mason et al., 2014).

In the early stages of infection, *B. burgdorferi* is recognised by skin immune cells, which instigates an immune response against the invading pathogen. Monocytes and neutrophils are recruited to the tick-bite site where they phagocytose *B. burgdorferi* and produce cytokines, which modulate the immune response (Mason et al., 2015) together with other local inflammatory mediators such as damage-associated molecular patterns (DAMPs).

The DAMPs myeloid-related protein (MRP)8 and MRP14 form a heterodimer (calprotectin), secreted by activated neutrophils and monocytes during infection which amplifies immune responses to pathogens, is involved in cytoskeleton modulation during phagocytosis and has antimicrobial properties (Ehrchen et al., 2009). Indeed, MRP8/14 from human neutrophils was found to have bacteriostatic activity *in vitro* by chelating zinc essential for growth of *B. burgdorferi* (Lusitani et al., 2003). Furthermore, elevated levels of MRP8/14 have been measured in the joints of Lyme arthritis patients, suggesting that it may contribute to or prolong anti-*Borrelia* immunity locally (Montgomery et al., 2006).

We hypothesised that the bacteriostatic effect of MRP8/14 *in vitro* may confer protection against dissemination during infection and that MRP8/14 may augment the response of immune cells against *B. burgdorferi*. To investigate this, we infected wildtype (*wt*) and *mrp14*$^{-/-}$ mice and studied the dissemination of *B. burgdorferi* and the immune response. In addition, we studied the ability of phagocytes *ex vivo* from *mrp14*$^{-/-}$ mouse whole blood to phagocytose and produce cytokines in response to *B. burgdorferi.*

* Corresponding author at: Academic Medical Center, Postbus 22660, 1100 DD, Amsterdam, The Netherlands.
E-mail address: l.m.mason@amc.uva.nl (L.M.K. Mason).

https://doi.org/10.1016/j.imbio.2018.07.017
Received 5 March 2018; Received in revised form 6 July 2018; Accepted 14 July 2018

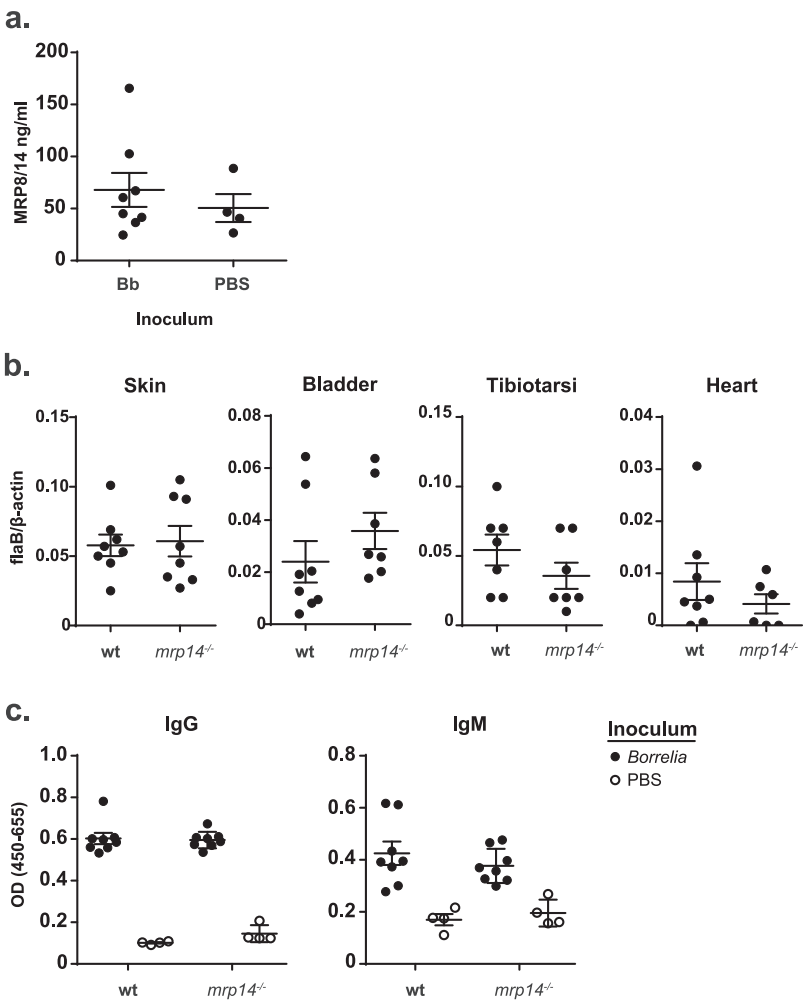

Fig. 1. MRP8/14 deficiency does not affect *B. burgdorferi* loads or immune responses in mice.
Wildtype and *mrp14*$^{-/-}$ mice were syringe inoculated with 10^6 spirochetes and sacrificed after 21 days. a) Dot plots show MRP8/14 levels in serum of *wt* infected and uninfected mice ± SEM. b) Dot plots show *B. burgdorferi* loads measured in the skin, bladders, tibiotarsi and hearts of infected *mrp14*$^{-/-}$ and *wt* mice ± SEM. c) Dot plots show IgG and IgM levels measured in the serum of infected *wt* and *mrp14*$^{-/-}$ mice ± SEM.

2. Materials and methods

2.1. B. burgdorferi

B. burgdorferi sensu stricto strain N40, previously recovered from an experimentally infected mouse was cultured in modified Kelly-Pettenkofer medium (MKP; AMC, Amsterdam, Netherlands). Low passage (< 5) spirochetes were washed and resuspended in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Paisley, UK) supplemented with 10% foetal calf serum (FCS, Lonza, Verviers, Belgium) for *ex vivo* experiments, or phosphate buffered saline (PBS, Fresenius Kabi, Graz, Austria) for mouse inoculation. Organs of infected mice were cultured in MKP with rifampicin (50 µg/ml) and phosphomycin (100 µg/ml) at 33 °C and checked weekly for presence of spirochetes.

2.2. Mice

Female *wt* C57Bl/6 mice were purchased from Charles River Laboratories (Maastricht, the Netherlands). Female mrp14$^{-/-}$mice, backcrossed > 10 times to a C57BL/6 background were generated as described (Manitz et al., 2003) and were bred in the animal facility of the Academic Medical Center (Amsterdam, the Netherlands). Six to eight-week-old mice were infected by subcutaneous inoculation in the midline of the back with 1×10^6spirochetes in 100 µl PBS, or PBS control, as described previously (Hovius et al., 2009). Mice were sacrificed by bleeding from the inferior vena cava. Paraffin-embedded sections of skin, ankle and heart were processed and H&E stained by routine histological techniques. Inflammation was scored on a scale from 0 to 3 by a pathologist who was blinded to the experimental design, as previously described (Hovius et al., 2009).

2.3. Cytokine and immunoglobulin measurements

Serum MRP8/14 was measured by ELISA as previously described (Achouiti et al., 2012). N40 strain-specific IgG and IgM in serum was measured by ELISA as previously described (Hovius et al., 2009). Cytokines were measured in whole blood stimulation supernatant and the serum of infected mice using a mouse inflammation CBA kit (BD, Franklin Lakes, NJ), according to manufacturer's instructions.

2.4. qPCR

DNA from murine tissues was obtained by blood and tissue kit (Qiagen, Venlo, The Netherlands) according to manufacturer's instructions. Quantitative (q)PCR detecting Borrelia flaB and mouse β-actin was performed using the lightcycler480 (Roche, Nutley, NJ) and SYBR green dye (Roche) in triplicate as described previously (Hovius et al., 2009). Results were analyzed using LinregPCR software (Amsterdam, the Netherlands).