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Brief Definitive Report

Endogenous galectin-3 controls experimental malaria in a species-specific manner

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SUMMARY

Galectins are evolutionarily conserved glycan-binding proteins with pleiotropic roles in innate and adaptive immune responses. Galectin-3 has been implicated in several immunological processes as well as in pathogen recognition through specific binding to glycosylated receptors on the surface of host cells or microorganisms. In spite of considerable evidence supporting a role for galectin-3 in host–pathogen interactions, the relevance of this lectin in the regulation of the host defence mechanisms in vivo is poorly understood. In this study, we analysed the impact of galectin-3 deficiency during infection with three distinct species of rodent malaria parasites, Plasmodium yoelii *17XNL,* Plasmodium berghei *ANKA and* Plasmodium chabaudi *AS. We found that galectin-3 deficiency showed a marginal effect on the course of parasitaemia during* P. chabaudi *infection, but did not alter the course of parasitaemia during* P. berghei *infection. However, lack of galectin-3 significantly reduced* P. yoelii *parasitaemia. This reduced parasitaemia in Lgals3*$^{-/-}$ *mice was consistent with higher titres of anti-*P. yoelii *MSP1*$_{19}$ *IgG2b isotype antibodies when compared with their wild-type counterparts. Our results reflect the complexity and singularity of host–pathogen interactions, indicating a species-specific role of endogenous galectin-3 in the control of parasite infections and the modulation of antibody responses.*

Keywords *galectin-3, immunoglobulin G,* Plasmodium spp.

INTRODUCTION

Galectins are evolutionarily conserved glycan-binding proteins with pleiotropic roles in innate and adaptive immune responses (1–3). To date, 15 galectins have been identified in mammals, which share a common structural fold and at least one conserved carbohydrate recognition domain (CRD) of approximately 130 amino acids that mediates carbohydrate binding (1–3). Galectins are classified based on their structural similarities as 'proto-type' galectins (galectin-1, -2, -5, -7, -10, -11, -13, -14 and -15), which have one CRD and exist as monomers or dimers, 'tandem repeat-type' galectins (galectin-4, -6, -8, -9 and -12), which contain two different CRDs separated by a linker of up to 70 amino acids, and the 'chimera-type' galectin-3, which contains a CRD connected to a nonlectin amino-terminal region (1–3).

These endogenous lectins have a wide tissue distribution and are present intracellularly both in the nucleus and in the cytoplasm of different cell types. However, galectins are also secreted through a nonclassical pathway and can be found associated to the cell surface and the extracellular milieu, where they play essential roles in cellular signalling events, including cell survival, differentiation, extracellular matrix remodelling and immune cell homeostasis (2,3). In particular, galectin-3 is involved in monocyte and neutrophil adhesion and chemotaxis (4–6), modulation of cytokine production (7,8), regulation of T-cell viability (9,10) and B-cell differentiation (11,12). In addition, galectin-3 has been implicated in pathogen recognition through specific binding to glycan structures on the surface of

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different microorganisms (1,2). In fact, this lectin recognizes the lipophosphoglycan structure on the surface of *Leishmania* parasites, the LacdiNAc (GalNAcβ1, 4GlcNAc) structures on *Schistosoma mansoni* and the β-1,2-linked mannans on certain *Candida* species (13–15), leading to activation of innate immunity and microbial killing (2). However, in spite of considerable progress, more studies are needed to understand the relevance of this immunoregulatory lectin in the regulation of host defence mechanisms.

Malaria is a disease, produced by protozoan parasites of the genus *Plasmodium*, which causes an estimated of 250 million cases and approximately 1 million deaths every year (http://www.who.int/features/factfiles/malaria/en/index.html) (16,17). *Plasmodium falciparum* is the species with greater impact in human health; the clinical symptoms associated with this infection are elicited mainly by the destruction of red blood cells and occlusion of capillaries by infected erythrocytes, especially in the brain. Although murine malaria models do not recapitulate all the features of human malaria, experimental infection of mice with various species and strains of rodent malaria parasites has facilitated the understanding of the immunological and pathological mechanisms involved in parasite clearance and disease (16,18). In addition, the use of genetically modified mice has proven to be useful to better identify the mechanisms and pathways involved in host responses to pathogens. In this study, we have analysed the impact of galectin-3 deficiency during infection with rodent malaria parasites (*Plasmodium chabaudi*, *Plasmodium yoelii*, *Plasmodium berghei*). Interestingly, we found that the clinical outcome in galectin-3-null mutant mice (*Lgals3*$^{-/-}$) depends on the species of the infecting organism. Our results demonstrate that galectin-3 is particularly important in the control of *P. yoelii* infection, indicating a possible role for this lectin in the modulation of parasitaemia and antibody-mediated responses.

MATERIALS AND METHODS

Mice

Galectin-3-deficient mice (*Lgals3*$^{-/-}$; C57BL/6) (5) and wild-type mice (WT; C57BL/6) were housed and bred at the animal facility of the London School of Hygiene and Tropical Medicine (London, UK). All experiments were carried out in compliance with the British Home Office Animals (Scientific Procedures Act) 1986.

Malaria infections

Cryopreserved *P. berghei* ANKA, *P. chabaudi* AS and *P. yoelii* 17XNL parasites were passaged in C57BL/6 mice, before use in experiments. Six- to 8-week-old female *Lgals3*$^{-/-}$ and WT mice were infected intraperitoneally with 10^4 parasite-infected erythrocytes in 200 μL of PBS. Control mice received an equal number of uninfected erythrocytes. Parasitaemia was monitored by Giemsa-stained thin blood smears obtained from tail bleeds. Serum obtained from clotted blood was stored at −20°C for further use in antibody enzyme-linked immunosorbent assays (ELISA).

Haematological parameters

Percentages of lymphocytes, monocytes and polymorphonuclear cells were determined in Giemsa-stained thin blood smears obtained from tail bleeds. Reticulocytes were visualized by staining with new methylene blue, and results were expressed as the percentage of red blood cells that stained positive for RNA. Packed cell volume (PCV) was measured by centrifugation of sealed capillary tubes filled with heparinized blood. PCV was expressed as the height of the resulting column of red cells calculated as a fraction of the height of the entire column of blood.

Antibody detection by ELISA

IgG and IgG subclass antibodies were measured by direct ELISA as described previously (19). Microtiter plates were coated with recombinant GST-PyMSP1$_{19}$ (1 μg/mL), washed, blocked and serum samples (serially diluted in phosphate-buffered saline–0·05% Tween 20 with 3% nonfat milk) were added to duplicate wells. After incubation overnight at 4°C, the plates were washed, incubated with horseradish peroxidase-conjugated antibodies to murine IgG or IgG subclass (Southern Biotech, Birmingham, AL, USA) and developed with *o*-phenylenediamine (Sigma, St. Louis, MO, USA) and hydrogen peroxide. The reaction was stopped after 10 min with 2 M H_2SO_4, and the optical density was measured at 492 nm.

Statistical analysis

Comparison of two groups was made using the Student's *t*-test for unpaired data when appropriate using Prism software (GraphPad, San Diego, CA, USA). *P* values of 0·05 or less were considered significant.

RESULTS

To evaluate the impact of galectin-3 deficiency during the infection with rodent malaria parasites, *Lgals3*$^{-/-}$ and WT mice were infected with *P. yoelii* 17XNL, *P. berghei* ANKA and *P. chabaudi* AS (Figure 1a–c). Interestingly, *Lgals3*$^{-/-}$ mice infected with *P. yoelii* showed significant

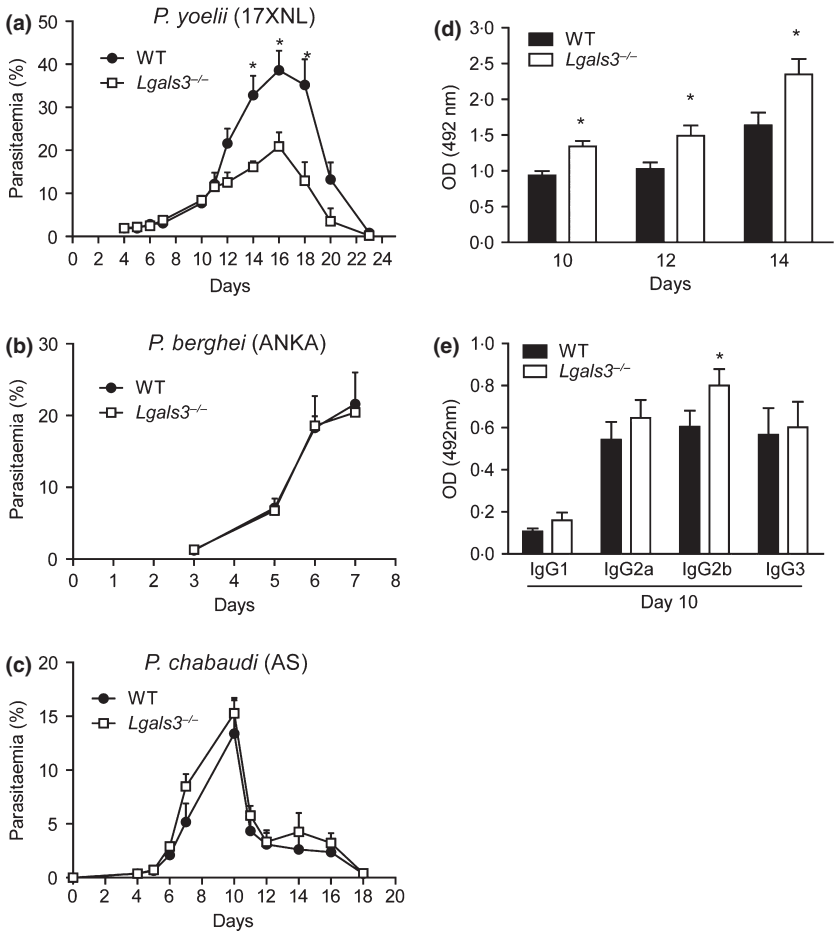

Figure 1 Galectin-3 deficiency selectively alters the course of infection with rodent malaria parasites. (a–c) *Lgals3*$^{-/-}$ and wild-type (WT) mice were infected with *Plasmodium yoelii* 17XNL (a), *Plasmodium berghei* ANKA (b) or *Plasmodium chabaudi* AS (c) through intraperitoneal injection of 10^4 parasitized red blood cells. Parasitaemia was monitored by Giemsa-stained thin blood smears obtained from tail bleeds. (d,e) Serum levels of IgG (d) and IgG subclass (e) antibodies specific for $PyMSP1_{19}$ in *P. yoelii*-infected *Lgals3*$^{-/-}$ and WT mice by ELISA (1 : 800 dilution). *$P < 0{\cdot}05$. Results shown are representative of two independent experiments (5–6 mice per group) for each infection.

reductions in parasite load compared with WT mice (Figure 1a). However, we found no differences in parasitaemia between *Lgals3*$^{-/-}$ and WT mice during *P. berghei* or *P. chabaudi* infection (Figure 1b,c). These results suggest that galectin-3 is involved in biological processes that specifically affect *P. yoelii* replication or infectivity.

We then examined *Lgals3*$^{-/-}$ and WT mice for differences in haematological parameters that may affect parasite load and found no significant differences in the percentage of lymphocytes, monocytes and polymorphonuclear cells in peripheral blood of these mice (Table 1). In addition, we found no differences in the percentage of reticulocytes or red blood cells PCV (Table 1), suggesting that the differences in the ability to control parasitaemia between *Lgals3*$^{-/-}$ and WT mice were not related to intrinsic alterations in the hematopoietic or lymphopoietic systems.

Effective immunity against blood stage malaria parasites involves both cellular- and antibody-mediated immune responses (17,19). To study the immune response against *P. yoelii* in *Lgals3*$^{-/-}$ and WT mice, we measured IgG antibody titres against the *P. yoelii* merozoite surface protein 1_{19} ($PyMSP1_{19}$), a widely recognized vaccine candidate antigen (19,20). We found that *Lgals3*$^{-/-}$ mice had higher titres of anti-$MSP1_{19}$ IgG (Figure 1d) when compared with WT mice, and this difference could be assigned to an increase in the titres of the IgG2b isotype (Figure 1e).

These results indicate that galectin-3 is involved in biological processes that affect the replication of *P. yoelii*, but not other *Plasmodium* species and that this difference may be associated to the ability of *Lgals3*$^{-/-}$ mice to produce higher levels of IgG, particularly the cytophilic IgG2b isotype.

Table 1 Haematological parameters in uninfected *Lgals3*$^{-/-}$ and wild-type mice

	Lgals3$^{-/-}$	Wild type
Packed cell volume	55·0 ± 4·4	51·8 ± 2·9
Reticulocytes (%)	1·37 ± 0·52	1·72 ± 0·65
Lymphocytes (%)	69·8 ± 2·4	65·0 ± 2·8
Monocytes (%)	7·7 ± 0·9	6·7 ± 0·9
Polymorphonuclear cells (%)	22·4 ± 2·0	28·1 ± 2·6

DISCUSSION

Understanding host defence mechanisms is a challenging goal that relies on the careful study of numerous interrelated parameters involved in host response to pathogens, including susceptibility of host strains, virulence of different pathogen species and the genetic, epigenetic and environmental factors influencing these unique interactions (21). Here, we analysed the impact of galectin-3 deficiency during rodent malaria infections and found that this lectin is particularly relevant for the control of *P. yoelii* infection, but not for other *Plasmodium* spp. such as *P. chabaudi* or *P. berghei*. Moreover, we found that *Lgals3*$^{-/-}$ mice infected with *P. yoelii* had increased levels of IgG2b antibodies specific for the merozoite surface antigen, PyMSP1$_{19}$, compared with their WT counterparts. These observations are consistent with previous studies showing that enhancement of IgG antibody responses to *P. yoelii* is associated with higher levels of protective immunity (22).

A major question arising from our study is why *Lgals3*$^{-/-}$ mice are protected from *P. yoelii* 17XNL, but not from *P. chabaudi* AS or *P. berghei* ANKA infection. As *P. berghei* infection results in a fatal neuropathological syndrome within 7 days in C57BL/6 mice, it is possible to argue that this experimental setting does not allow a proper time frame to detect differences between *Lgals3*$^{-/-}$ and WT mice and that galectin-3 could be involved in biological processes that require longer time periods, such as those observed during *P. yoelii* infection. However, in contrast to our findings, Oakley *et al.* (23) found that deletion of the galectin-3 gene resulted in a minor increase in parasitaemia after infection with *P. berghei* ANKA. A possible explanation for this difference might be related to the number of parasitized red blood cells used to induce infection (10^6 vs. 10^4) providing clues about the importance of the initial parasitic load in addition to environmental and genetic factors involved in the clinical outcome of the disease. It is interesting to note that Oakley *et al.* (23) also observed a partial protection from cerebral malaria (CM) in *Lgals3*$^{-/-}$ mice and that the role of galectin-3 in the pathogenesis of CM was not mediated by recognition and binding of galectin-3 to *P. berghei* ANKA parasites. This finding indicates that galectin-3 is not acting as a pathogen recognition receptor for *P. berghei* ANKA glyco-antigens. In this regard, whether galectin-3 recognizes specific glycan structures on *P. yoelii* or *P. chabaudi* parasites remains to be elucidated.

Although *P. yoelii* 17XNL and *P. chabaudi* AS exhibit a nonlethal clinical course, a phylogenetic analysis of rodent malaria parasites showed that *P. berghei* and *P. yoelii* seemed to be more evolutionarily related to each other, while *P. chabaudi* appears to differ considerably (24). These observations are consistent with older studies showing that *P. berghei* and *P. yoelii* share a higher degree of serological cross-reactivity than either species shares with *P. chabaudi* (25), indicating that despite the similar clinical course of the infection, these species have different antigenic properties.

Recent studies on the role of galectin-3 during infection showed that this lectin can modulate activation of innate immune cells and their ability to polarize adaptive immune responses (3). Interestingly, the effect of galectin-3 deficiency depends on the nature of the invading pathogen. In particular, it has been described that galectin-3 deficiency promotes an increased Th2 response when challenged with the fungus *Paracoccidioides brasiliensis* (7), while induces a Th1-biased response when infected with the intracellular bacteria *Rhodococcus equi* (8) or with the helminth parasite *S. mansoni* (26). Taken together, results arising from these experimental models, rather than pointing out to a single direction, indicate that galectin-3 may exert a selective modulatory role during pathogen-evoked innate and adaptive immune responses. Nonetheless, a consistent finding in the literature relates to the ability of this endogenous lectin to modulate fate decision within the B-cell compartment. In accordance with our findings showing an increased IgG level in *Lgals3*$^{-/-}$ mice infected with *P. yoelii*, previous work has demonstrated that galectin-3 interferes with plasma cell differentiation. In particular, blockade of galectin-3 expression via antisense oligonucleotides led to an increase in the number of plasma cells in the course of *Trypanosoma cruzi* infection (11). Moreover, Oliveira *et al.* (27) showed that galectin-3 is required for memory B-cell differentiation in a model of *S. mansoni* infection and that *Lgals3*$^{-/-}$ mice exhibit increased number of plasma cells and higher levels of IgG and IgE. Interestingly, this effect also occurs within the B1 cell compartment where galectin-3 prevents the differentiation of B1 cells into IgM-secreting plasma cells (12). Of note, whether the lack of differences between *Lgals3*$^{-/-}$ and WT mice during *P. chabaudi* infection is reflected in equal numbers of plasma cells and IgG titres remains to be further elucidated. Altogether, the evidence presented here further substantiates the key role of galectin-3 in modulating antibody-mediated responses and highlights the importance of critically analysing species-specific differences

when assigning immunomodulatory roles to emerging endogenous mediators.

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