# Deficiency in Mannose-Binding Lectin-Associated Serine Protease-2 Does Not Increase Susceptibility to *Trypanosoma cruzi* Infection

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*Abstract.* *Trypanosoma cruzi* is the causative agent of Chagas' disease, a chronic illness affecting 10 million people around the world. The complement system plays an important role in fighting microbial infections. The recognition molecules of the lectin pathway of complement activation, mannose-binding lectin (MBL), ficolins, and CL-11, bind to specific carbohydrates on pathogens, triggering complement activation through MBL-associated serine protease-2 (MASP-2). Previous *in vitro* work showed that human MBL and ficolins contribute to *T. cruzi* lysis. However, MBL-deficient mice are only moderately compromised in their defense against the parasite, as they may still activate the lectin pathway through ficolins and CL-11. Here, we assessed MASP-2-deficient mice, the only presently available mouse line with total lectin pathway deficiency, for a phenotype in *T. cruzi* infection. Total absence of lectin pathway functional activity did not confer higher susceptibility to *T. cruzi* infection, suggesting that it plays a minor role in the immune response against this parasite.

Chagas' disease (American Trypanosomiasis) is an endemic and chronic illness[1] that affects around 10 million people in Latin America[2] and ~400,000 people in North America, Europe, and Asia.[3] Its causative agent, the flagellated protozoan *Trypanosoma cruzi*, is transmitted through blood-feeding triatomine bugs, oral and transplacental routes, organ transplants, and blood transfusions.[4] Infection with the parasite results in acute parasitemia, followed by a long asymptomatic indeterminate phase. Decades after the initial infection, ~30% of the individuals progress to a chronic phase, characterized by severe cardiomyopathy or pathological enlargement of the digestive tract.[5] Even though immunity plays a fundamental role in the parasite/mammalian host equilibrium,[6] *T. cruzi* has developed effective strategies to escape the host immune response, including the immune defense mediated by the complement system.[7–10]

Three distinct pathways initiate the complement cascade: the classical, the alternative, and the lectin pathways.[11] Lectin pathway activation permits an instant innate immune response to pathogens in naive hosts.[12] Its activation is initiated through the recognition of pathogen-associated carbohydrate or acetylation patterns, present on microbial surfaces, by either the lectin domains of mannose-binding lectin (MBL) or collectin-11 (CL-11), or the fibrinogen-like domains of ficolins, which, upon binding to their respective pathogen-associated patterns, may initiate complement activation through conversion of the three different MBL-associated serine proteases (MASP) described, MASP-1, -2, and -3, into their enzymatically active form.[13–17] Several studies suggest that MASP-2 alone is sufficient to trigger lectin complement activation, because mice deficient in MASP-1 and -3 maintain residual lectin pathway activity,[18–21] which is completely absent in mice deficient of MASP-2.[22]

The role of the lectin pathway during the mammalian host immune defense against several pathogenic agents has been well established in both humans and mice, as shown by the association between increased infection susceptibility to parasitic diseases and genetic deficiencies in key components of the lectin pathway, such as MBL and ficolins.[23–27] In addition, compromised MASP-2 functional activity caused by a polymorphism in the coding sequence of the human *masp2* gene, leading to the loss of a critical calcium binding site, has been associated with autoimmune manifestations and predispositions to frequent infections.[28,29]

Previous *in vitro* reports have shown that human serum MBL and ficolins can bind to glycosylated molecules on the surface of *T. cruzi* metacyclic trypomastigotes, resulting in MASP-2-dependent complement activation.[30] Conversely, a significant decrease in the deposition of complement activation products C3b and C4b on the parasite surface, and compromised complement-mediated parasite lysis, have been described in either MBL, L-ficolin, or H-ficolin-depleted human sera,[30,31] suggesting that lectin pathway recognition molecules not only bind to the parasite surface, but also promote complement activation by the lectin pathway.

Additionally, an *in vivo* study using a mouse model of experimental trypanosomiasis compared the severity of the parasitic disease between MBL-null mice, which are deficient in both of the murine MBL genes, MBL-A and MBL-C,[32] with that of their matched wild-type (WT) controls.[33] The results revealed that MBL-null mice presented with a moderately increased parasitemia load and cardiac pathology,[33] indicating that MBL regulates host resistance and cardiac inflammation following *T. cruzi* infection. Nevertheless, the MBL-null mouse strain fails to provide a reliable model for total lectin pathway deficiency, because they can still activate the lectin pathway through ficolin A, ficolin B, and/or CL-11.[34,35] In addition, the MBL-null phenotype also includes the loss of MBL-mediated biological activities, which are independent of complement activation.[32]

In this study, we report the assessment of a MASP-2-deficient mouse line as the only presently available mouse model of total lectin pathway deficiency (which retains fully functional

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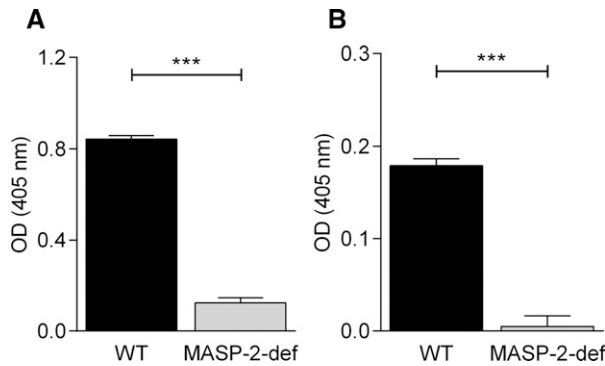† These authors contributed equally to this article.

activity of both the classical and the alternative complement pathways)[22] in experimental *T. cruzi* infection. The phenotype of MASP-2-deficient mice exclusively defines the loss of MASP-2-mediated complement activation, whereas all complement independent functions of the serum resident lectin pathway recognition components are retained.[22]

We evaluated the phenotype of MASP-2-deficient mice bred on a C57BL/6 background,[22] a mouse strain with a genetic background that is highly susceptible to *T. cruzi* infection.[36] All experimental procedures involving animals, which were maintained under internationally accepted guidelines, were approved by our Institutional Bioethics Committee (Faculty of Medicine, University of Chile). Genotyping using heteroduplex polymerase chain reaction (PCR) was performed to screen for MASP-2-deficient (MASP-2^{-/-}) and WT alleles (MASP-2^{+/+}) in the progeny of heterozygous intercrosses to generate littermate controls, as described previously[22] (data not shown).

The lack of lectin pathway-dependent complement activation in MASP-2-deficient mice was confirmed *in vitro* using C4 cleavage assays on both, mannan or lipoteichoic acid-coated enzyme-linked immunosorbent assay (ELISA) plates, as described previously.[22] As shown in Figure 1, lectin pathway-dependent C4 turnover was only detectable in WT mice sera, whereas sera of homozygous MASP-2-deficient mice presented with a total absence of C4 cleavage.
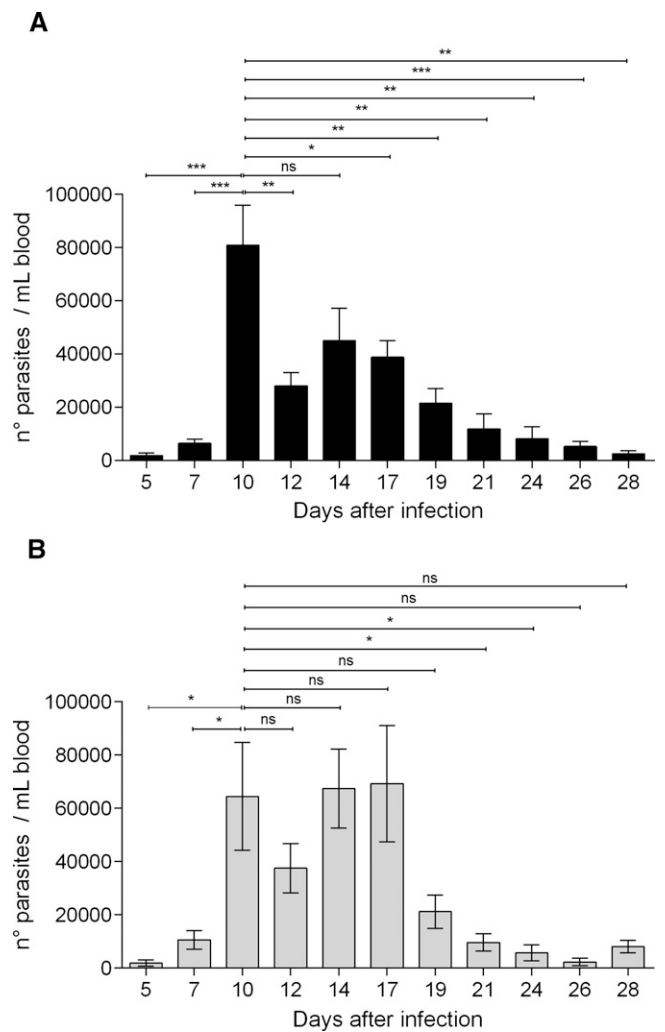
We subsequently challenged 10 female and 5 male WT littermates and 2 female and 4 male MASP-2-deficient mice with an intraperitoneal inoculation of 100 *T. cruzi* trypomastigotes

of the virulent Tulahuén strain.[36] All mice were 12 weeks of age when challenged with parasites. For each mice group (MASP-2-deficient and WT controls), optical microscopy was carried out every 2 (or 3) days during 28 days after parasite inoculation to determine the number of circulating parasites in fresh blood obtained from tip cuts of mice tails, following standard protocols.[37] The survival of infected mice was monitored on a daily basis. Mice that did not succumb to infection were euthanized 82 days after parasite inoculation, following internationally accepted procedures.

The WT mice reached the highest parasitemia load at Day 10 post-infection (p.i.) (Figure 2A). In these mice, the circulating parasite burden decreased significantly 2 days later (i.e., Day 12 p.i.), followed by a constant and steady decrease until

FIGURE 1. The lectin pathway is not functional in MBL-associated serine protease (MASP)-2-deficient mice. Enzyme-linked immunosorbent assay (ELISA) plates were coated with (**A**) 1 µg/well of mannan from *S. cerevisiae* (Sigma-Aldrich, USA) or (**B**) 5 µg/well of lipoteichoic acid (LTA) (Sigma-Aldrich) in 100 µL carbonate buffer. Control wells received buffer alone. Residual protein binding sites were blocked using 1% bovine serum albumin (BSA) in Tris-buffered saline (TBS), followed by several washes. Sera from 15 wild-type (WT) and 6 MASP-2-deficient (MASP-2-def) mice, diluted in high salt binding buffer (20 mM Tris-HCl, 10 mM CaCl$_2$, 1 M NaCl, pH 7.4), were added to plates coated with mannan (serum dilution 1/160) or LTA (serum dilution 1/20). C4 (Complement Technology Inc., USA) (0.05 µg/well in 50 µL barbital buffered saline) was added, and C4b deposition was detected with goat anti-human C4 IgG (Calbiochem), followed by incubation with horseradish peroxidase-conjugated rabbit anti-goat IgG (Calbiochem). Enzyme activities were assessed, at 405 nm, after addition of 2,2′-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt (ABTS) with hydrogen peroxide. Background values of unspecific reactivity with BSA were subtracted from OD values obtained upon reactivity with carbohydrates. Results are from three independent experiments in triplicate wells. Differences between groups were determined by the Student's *t* test using GraphPad Prism Software v6.01 (USA). Mean values ± SE are shown (***$P < 0.001$).

FIGURE 2. MBL-associated serine protease (MASP)-2-deficiency promotes sustained high circulating parasite load. Fifteen wild-type (WT) (**A**) and 6 MASP-2-def (**B**) 12-week-old mice received an intraperitoneal inoculation of 100 bloodstream *T. cruzi* trypomastigotes (Tulahuén strain). The number of parasites per mL of blood was estimated every 2 or 3 days, by microscopical counting, during 28 days after parasite inoculation. Differences between parasitemia values within each mice group were determined by the Student's *t* test. The results represent the mean ± SE values. Ns = non-significant; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$ in relation to parasitemia load on Day 10 p.i. for both mice groups. All data were analyzed with GraphPad Prism software.
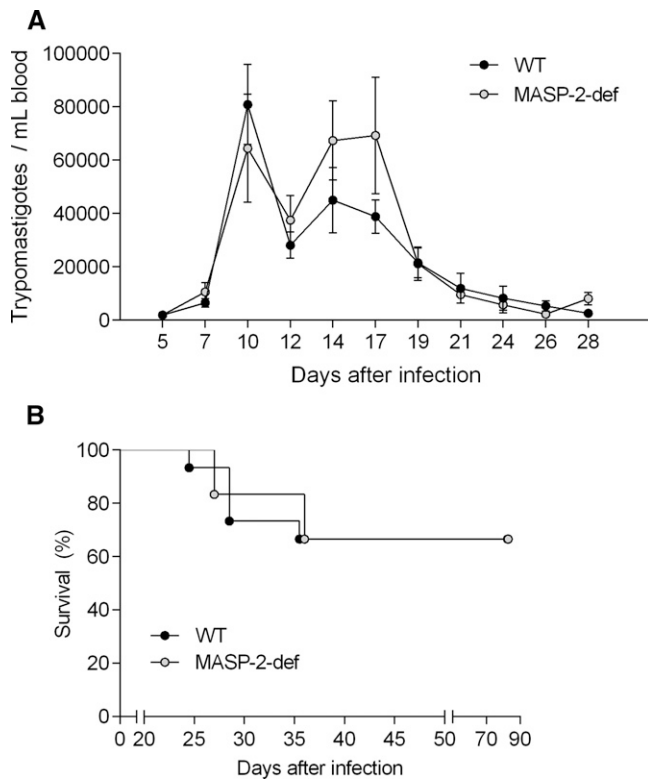
Figure 3.   MBL-associated serine protease (MASP)-2-deficiency does not increase susceptibility to *T. cruzi* infection. (**A**) Parasitemia curves of 15 wild-type (WT) and 6 MASP-2-def mice infected with *T. cruzi* trypomastigotes (Figure 2) were compared between mice groups by two-way analysis of variance followed by Sidak's analysis for multiple comparisons. The results represent the mean ± SE values. (**B**) Kaplan-Meier curves for survival rates of trypomastigote-infected WT and MASP-2-deficient mice. Log-rank test was used to compare survival curves. All data were analyzed with GraphPad Prism software.

the end of the observation time (Day 28 p.i.) (Figure 2A). However, MASP-2-deficient mice presented a high parasitemia load on Days 10, 14, and 17 p.i. (Figure 2B). Such high and sustained parasitemia burden was observed until Day 19 p.i., which decreased significantly only 2 days later (Day 21 p.i.) (Figure 2B).

Surprisingly, even though WT mice were able to begin peripheral parasite clearing earlier than MASP-2-deficient mice (Figure 2), no significant difference in parasite load could be detected between infected MASP-2-deficient and WT mice at any time point of the acute experimental infection (Figure 3A).

Accordingly, both WT and MASP-2-deficient mice survived the acute/high parasitemia phase, with the first cases of mortality presenting only at the later stage of acute trypanosomiasis (i.e. Days 24 and 26 p.i. for WT controls and MASP-2-deficient mice, respectively) (Figure 3B). After 28 days of infection, the percentage of surviving WT and MASP-2-deficient mice was 73% and 83%, respectively. There was no difference whatsoever in the overall long-term survival of both mice groups, with 67% of WT and MASP-2-deficient mice being alive at the end of the experiment (i.e., 82 days p.i.) (Figure 3B).

Our findings described here for the MASP-2-deficient mouse line may not reflect the phenotype of the MBL-null mouse strain previously reported by Rothfuchs and others,[33] because complete deficiency in the lectin pathway activity neither resulted in increased parasitemia levels nor higher mortality rates during *T. cruzi* experimental infection (Figure 3). We therefore conclude that complement activation by the lectin pathway plays a more or less redundant role in the immune clearance of this parasite. Undoubtedly, other mechanisms of mammalian host protection, which does not rely upon downstream activation of complement lectin pathway, may be crucial to prevent host death caused by the high parasitemia burden that characterizes the acute phase of the *T. cruzi* infection. These may include macrophage-mediated killing of intracellular parasites,[38] circulating parasite destruction mediated by the alternative pathway of complement,[30] and the generation of lytic antibodies and subsequent activation of complement classical pathway, after a specific antibody response is mounted.[39,40]

It is worth mentioning that our short-term experiment does not challenge the disease modifying polymorphisms reported in clinical studies on the severity of human trypanosomiasis. The possibility remains that, in long-term disease manifestations, *T. cruzi*-mediated dilated cardiomyopathy and/or enlargement of the digestive tract, or inherited polymorphic or acquired variations in lectin pathway functional activity, may present as disease modifying determinants in the pathophysiology of human trypanosomiasis. For instance, in a recent large cohort study with patients affected by Chagas' disease, an association between low MASP-2 serum levels and higher risk of chagasic cardiomyopathy was found, suggesting that deficiency in this serine protease could favor disease progression.[41] Whether this response can also be observed in the mouse model of complete lectin pathway deficiency remains to be established.

It is also noteworthy that, apart from the *T. cruzi* Tulahuén strain used in this study, which frequently leads to a lethal acute disease in C57BL/6 mice,[36] mammalian infection by genetically distinct parasite strains has been well described,[42] which may induce differentially extended complement activation. Therefore, further characterization of the protective activity of the lectin pathway under infection with other virulent *T. cruzi* strains may be important to understand the relevance of this arm of the immune response against this parasite.

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