CD47 regulates parasite burden and promotes pathogenesis in murine malaria models

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Abstract

CD47 is an anti-phagocytic ("don't eat me") signal that inhibits programmed cell self-removal; loss of this molecule by aging erythrocytes is associated with increased likelihood of macrophage phagocytosis. We have investigated the role of CD47 in malaria immunity and pathogenesis in murine malaria models. Previously, we demonstrated that absence of CD47 confers resistance to infection with Plasmodium yoelii 17XNL, a murine malaria that exhibits an aged-based preference for young erythrocytes. Next, we established that CD47 blockade with an anti-CD47 monoclonal antibody promotes survival and reduces the pathologic features of experimental cerebral malaria (ECM) during *Plasmodium berghei* ANKA (Pb-A) infection in C57BL/6 mice, a murine model of ECM. To delineate the immunological mechanism of CD47 regulation of ECM pathogenesis, we present studies comparing *Pb-A* infection in wildtype (WT) versus CD47 KO C57BL/6 mice. In CD47 KO mice, absence of CD47 resulted in partial but highly significant (p<0.001, log-rank) resistance to ECM; following infection with Pb-A parasites, 22/23 (95.6%) WT mice developed ECM by day 10 post-infection. In contrast, only 13/23 (56.5%) of CD47 KO mice succumbed to malaria during the cerebral phase of infection. Through flow cytometric analysis of brain sequestered and splenic immune cell subsets and cytokine profiling of serum, we show that absence of CD47 during Pb-A malaria is associated with a significant reduction in brain sequestered CD8+ T cells which are pathogenic during ECM, an increase in splenic CD107a$^+$ NK cells, and alteration of a subset of cytokines. In addition, comparative analysis of WT versus CD47 KO brain tissue by immunohistology demarcates clear differences in pathologic features such as hypertrophied endothelial cells, presence of parasite hemozoin, macrophage infiltration, vasculopathy, and ring hemorrhages. A further understanding of the mechanism of anti-CD47 antibody-mediated protection from ECM may open avenues for novel immunologic-based treatment options against cerebral malaria in African children.

Introduction

In 2018, there were approximately 218 million cases and 405,000 deaths due to malaria. The majority of deaths due to malaria occur in Africa children and are a consequence of *P. falciparum* infection. Human cerebral malaria is a principal cause of malaria mortality and adjunctive therapies are urgently needed to reduce the high mortality rate (approximately 20%) of cerebral malaria. We present studies examining the role of CD47 in the pathogenesis of cerebral malaria to determine whether it could be a promising target for therapeutic treatment of cerebral malaria.

Materials and Methods

Infection of mice: WT and CD47 KO mice were infected with 10^6 *Pb–A* parasites and susceptibility to ECM and parasite burden was determined. Susceptibility to ECM was measured by evaluating mice twice per day during the cerebral phase (days 6–10 post-infection) for symptoms that resemble ECM. Parasite burden was determined every by preparing and enumerating giemsa–stained thin blood smears every other day.

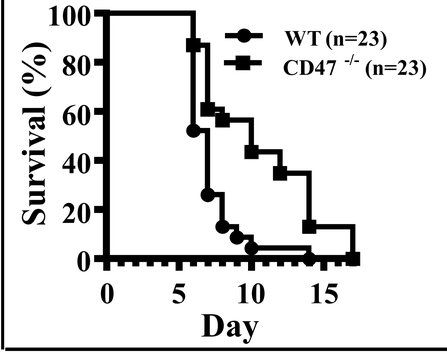
Detection of serum cytokines: Serum cytokine profiles were assessed using the Bio-Plex Pro Mouse Cytokine 23-plex assay (Bio-Rad) specific for IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17A, eotaxin, G-CSF, GM-CSF, IFN-γ, KC, MCP-1, MIP-1α, MIP-1β, RANTES, and TNF–α.

Flow Cytometry: Flow cytometric analysis was performed on innate (neutrophils, macrophages, NK cells) and adaptive (T cells) immune cell subsets in brain sequestered leukocyte and splenocyte populations. Single cell suspensions were prepared from spleen and perfused brain tissue. Cells were then stained with viability dye to distinguish live from dead cells, blocked with anti-CD16/CD32, and then stained with a panel antibodies specific for cells of interest. Cells were acquired on an LSR II or Cytek Flow Cytometer and analyzed using Flojo (Tree Star, Ashland, OR).

Results and Discussion

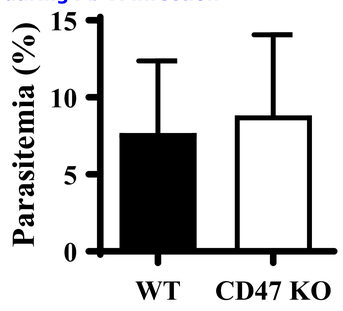
Results 1

- **CD47 KO mice display partial but significant (p<0.001, log-rank) resistance to ECM**



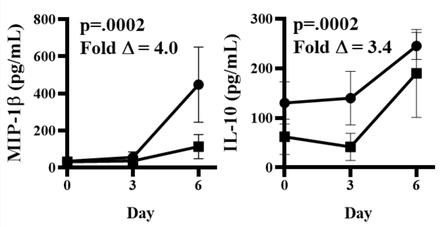
Results 2

- **CD47 does not regulate parasite density during *Pb–A* infection**



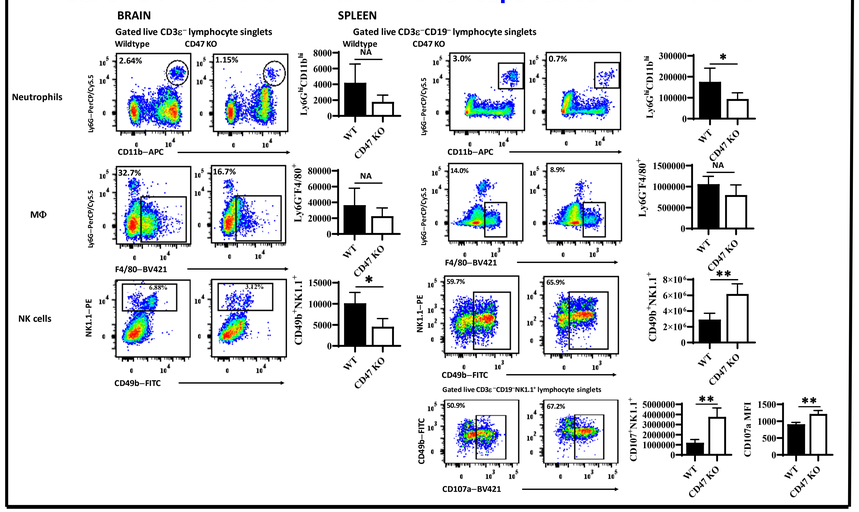
Results 3

Absence of CD47 results in a significant reduction in MIP1β and IL-10



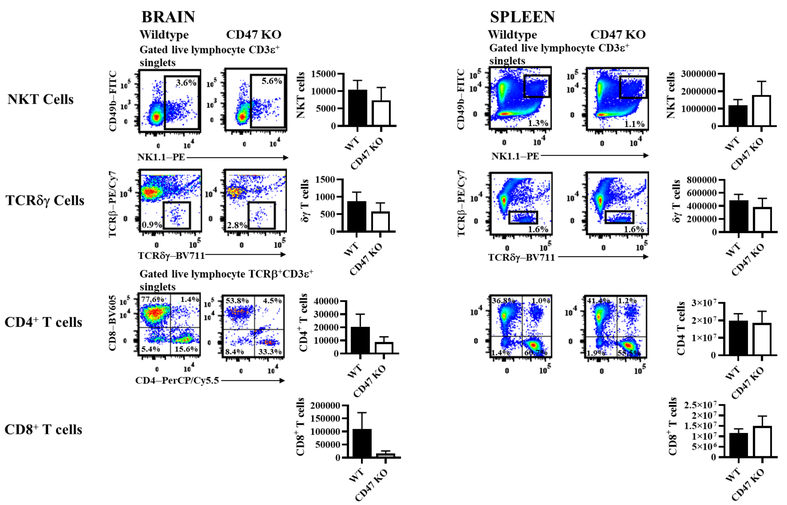
RESULTS 4

- **Effect of CD47 on the innate immune response to *Pb–A* malaria**



RESULTS 5

- **CD47 KO mice have reduced numbers of brain–sequestered CD4$^+$ and CD8$^+$ T cells**



Results 5

Summary of in depth flow cytometric analysis of immune cell subsets during *Pb—A* infection:

- **CD47 does not alter the Th1/Th2 balance in CD4$^+$ T cells**
- **CD47 does not change the pathogenic signature (granzyme, IFN–γ, T–bet, CD69, CXCR3, CCR5) of CD8$^+$ T cells**
- **Brain sequestered NK cells have altered levels of CD11b, CD49b, and CD226 in the absence of CD47**

Conclusion

CD47 displayed partial but significant resistance from ECM. In contrast to previous studies conducted in the *P. yoelii* murine model of malaria, CD47 does not regulate parasite density during a *Pb–A* infection and there is no significant difference in parasite density between CD47 KO mice that are susceptible versus resistant to ECM. CD47 KO mice have reduced numbers of brain–sequestered NK cells, CD4$^+$ T cells, and CD8$^+$ T cells during the cerebral phase of infection. Furthermore, The phenotype of brain–sequestered NK cells is altered in the absence of CD47. Brain–sequestered NK cells have lower levels of CD11b and CD49b and elevated levels of CD226 in the absence of CD47. Lastly, absence of CD47 results in a significant reduction in MIP1β and IL-10 during the cerebral phase of ECM during a *Pb–A* infection.