Previous *Plasmodium* Infection Reprograms Neutrophil PD-L1 Responses to a Secondary Inflammatory Challenge

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Malaria is a disease caused by protozoan parasites of the *Plasmodium* genus, responsible for thousands of deaths annually. While the innate immune system limits parasite growth, an unbalanced inflammatory response contributes to severe manifestations, such as cerebral malaria (CM). Neutrophils, key players in the host defense, produce inflammatory mediators that amplify inflammation and shape adaptive immunity. Recent studies have revealed phenotypic and functional diversity among neutrophil. Moreover, innate immune cells are now understood to exhibit a form of memory, whereby previous exposures to infections or vaccines can modulate their responses, upon encountering subsequent stimuli. This study aimed to assess whether *P. berghei* ANKA infection in C57BL/6 mice, a model for CM, induces immune memory in neutrophils. Mice were infected and treated with chloroquine before CM signs emerged. After recovery, mice were challenged with lipopolysaccharide (LPS) to evaluate whether activation or suppression markers persisted in neutrophils, potentially indicating the establishment of innate immune memory. Our data revealed that, during active infection, the expression of PD-L1, a co-inhibitory molecule associated with immune regulation, increased significantly in splenic and bone marrow-derived neutrophils. Following treatment, at a later stage, neutrophils from previously infected animals exhibited a considerable reduction in PD-L1 expression. However, upon secondary LPS challenge, PD-L1 levels increased again but remained significantly lower than those in LPS-challenged, non-infected controls. This modulation was observed in both spleen and bone marrow compartments. These findings suggest that prior plasmodial infection altered the capacity of neutrophils to respond to inflammatory stimuli. While reduced PD-L1 expression may reflect altered regulatory potential, further investigation is needed to determine whether this represents the development of innate immune memory.

Keywords: Malaria; *P. berghei* ANKA; Neutrophils; Innate immune memory; Trained immunity.