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Dissection of Protective Innate Immune Signatures in a Murine Malaria Model

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Malaria, caused by the apicomplexan parasite *Plasmodium*, is one of the most important public health problems worldwide. Severe forms of malaria, including cerebral malaria, mostly affect children and naïve individuals, who have not acquired partial immunity to the parasite. In these patients non-adaptive immune mechanisms are crucial to control the infection, but the host's immune response is also involved in severe immunopathology.

This work systematically assessed the capacity of distinct TLR2, -3, -4, and -9 signalling pathways to alter a subsequent *Plasmodium* infection in the *P. berghei* ANKA / C57BL/6 murine infection model, which develops experimental cerebral malaria (ECM), arguably the best model of human cerebral malaria available. This work demonstrates for the first time, that stimulation of TLR4 or -9 prior to a lethal challenge with *Plasmodium* sporozoites or infective mosquito bites significantly reduces the occurrence of ECM. In contrast, pre-stimulation of the TLR2 and -3 pathways had no clinical benefit. Partial protection against ECM is associated with prevention of immunopathology around the time of the onset of neurologic symptoms, which is characterized by high levels of pro-inflammatory cytokines/chemokines in untreated mice. Mice, which had received a TLR4 or -9 ligand, did not show the characteristic peak of serum IFN-γ, TNF-α, MCP-1 and counterregulatory IL-10 on day 5 post-infection. Earlier in the course of infection these mice showed a strong serum cytokine response followed by regressive kinetics and a condition refractory to the dysregulation associated with ECM.

In addition to the modulation of the clinical outcome of blood-stage malaria, activation of TLR9 also shared an anti-parasitic effect against *P. berghei* liver-stages. This work ruled out possible direct physicochemical inhibition of pre-erythrocytic stages and cell-autonomous growth inhibition in the infected hepatocyte. In contrast, reduction of the hepatic parasite load depended on MyD88 signalling. Inhibition of *P. berghei* liver stages stages was associated with high serum levels of IL-12, IFN-γ and MCP-1 at the time of infection and beyond, local expression of IL-12, IFN-γ, and IFN-β in the liver, and activation of T cells, NK cells and NKT cells. The inhibitory capacity of TLR9 activation was reduced without T and B cells, but not abolished. In contrast, intact IFN-γ signalling was indispensible. Depletion of $F4/80^{+}/CD11b^{+}$ macrophages with clodronate-liposomes completely reversed the TLR9 triggered anti-parasitic effect. Specific activation of the TRL9 pathway provides a previously unrecognized opportunity to target liver-stages, a bottle neck of the *Plasmodium* life cycle, through an IL-12 and IFN-γ mediated activation of macrophages.

Together, systematic comparative immune profiling in a mouse malaria model revealed a critical role of activated macrophages to limit the first parasite replication cycle in malaria naïve hosts