4. Ferguson, H.M. and Read, A.F. (2002) Why is the effect of malaria parasites on mosquito survival still unresolved? *Trends Parasitol.* 18, 256–261
5. Werling, K. *et al.* (2019) Steroid hormone function controls non-competitive *Plasmodium* development in *Anopheles*. *Cell* 177, 315–325
6. Atella, G.C. *et al.* (2009) The major insect lipoprotein is a lipid source to mosquito stages of malaria parasite. *Acta Trop.* 109, 159–162
7. Costa, G. *et al.* (2018) Non-competitive resource exploitation within mosquito shapes within-host malaria infectivity and virulence. *Nat. Commun.* 29, 3474
8. Hammond, A. *et al.* (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nat. Biotechnol.* 34, 78–83
9. Mancio-Silva, L. *et al.* (2017) Nutrient sensing modulates malaria parasite virulence. *Nature* 547, 213–216

Spotlight

Initiating the T Cell Response to Liver-Stage Malaria

Komi Gbedande[1] and Robin Stephens[1,2,*]

Kurup *et al.* (*Cell Host Microbe* 2019;25:565–577.e6) define the liver-based antigen-presenting cell driving CD8 T cell responses to mosquito transmission of *Plasmodium* spp., and show direct interaction of CD11c$^+$ cells with infected hepatocytes. We discuss this work in context, highlighting gaps and new approaches suggested by the work to target liver-stage vaccine antigens.

Malaria remains an important parasitic infection in the world, with 217 million cases and 435 000 deaths in 2018. During the blood meal, infected *Anopheles* mosquitos introduce *Plasmodium* spp. sporozoites into the host dermis, which quickly transit to the liver of the host. The liver stage of *Plasmodium* is a potential target to induce immunity by vaccination. The mechanisms of immunity to the very effective, radiation-attenuated sporozoite (RAS) vaccination have been well characterized [1]. Here, we briefly review the host response to the liver stage of *Plasmodium* spp., and discuss the recent findings of Kurup *et al.* [2] on the role of hepatic inflammatory dendritic cells (DCs) in priming CD8 T cells against the liver stage of *Plasmodium yoelii* infection in this context.

Liver-Stage Immunity to *Plasmodium* spp.

Liver-stage infection with *Plasmodium* spp. induces both innate and adaptive immune responses. The live parasite is now known to trigger interferon (IFN)-inducible genes in infected hepatocytes, recruiting CD8α$^+$ DCs and T cells to the liver more strongly than RAS [3,4]. Liver-localized DCs have been shown to take up both RAS and sporozoites after infection, and drive generation of T cell responses [1,4,5]. In humans, immune responses to liver-stage parasites have been measured. Antibodies to liver-stage antigens, such as LSA-3, are generated from the earliest exposures. The specificity for the antigens LSA-1 and STARP was present in most children of 8–10 years of age during a malaria-free rainy season, but fewer showed this specificity in the group with clinical disease [6]. These antibody responses likely require CD4 T cell help. However, most T cell epitopes tested to date have elicited responses in only a small fraction of infected people, though more are detected when epitopes are human leukocyte antigen (HLA)-matched [7]. Interactions between liver macrophages, or Kupffer cells, and the *Plasmodium* sporozoites are necessary for hepatocyte invasion. While this interaction induces cytokines [8], mechanisms of antigen presentation are unclear in the case of liver infection.

CD11c$^+$ Cells Find Infected Hepatocytes and Prime CD8 T Cell Immunity

Although pathways of antigen presentation in the liver, including RAS vaccination, leading to *Plasmodium* immunity are beginning to be well understood, induction of immunity to infectious sporozoites has not been well explored [1,5]. The recent study by Kurup *et al.* identified a subset of hepatic CD11c$^+$ cells that present antigens upon mosquito transmission of *P. yoelii* and *P. berghei* infection [2]. While the frequencies of Kupffer cells (CD45$^+$ CD11c$^-$ MHC-II$^+$ F4/80$^+$ CD11b^{int} CX3CR1$^{lo\text{-}int}$), macrophages (CD45$^+$ CD11c$^-$ F4/80$^+$ CD11b^{hi} CX3CR1^{hi}), and monocytes (CD45$^+$ MHC-II^{int} CD11c$^-$ F4/80$^-$ Ly6C$^+$) remain unchanged in the livers of infected mice, the numbers of CD11c$^+$ cells increased, suggesting a possible role for CD11c$^+$ DCs in antigen presentation [2,4]. The authors showed that CD11c$^+$ cells from the liver primed a CD8 T cell response to liver-stage-restricted antigens using CD11c-reporter and T cell receptor (TCR) retrogenic mice, and fluorescent parasites. While DCs in the liver have been observed with blood-stage parasites or RAS inside, tissue-localized antigen-presenting cells had not been previously observed in the less inflammatory liver stage. It had been hypothesized that hepatocyte apoptosis led to priming of liver-stage responses. However, it is now clear that Mda5 can initiate the intracellular response to *Plasmodium* infection, suggesting that the infection is not silent [3]. The authors carefully demonstrated that T cell priming requires hepatocyte invasion, ruling out an exclusive role for dead parasites as antigen for priming in the local lymph node. In addition, live video microscopy shows CD11c$^+$ cells taking up merosomes. Intriguingly, the cytokine, chemokine, and integrin signals used by DCs to find infected hepatocytes may also be used by T cells that could then find and kill them. These signals, which are not yet understood, could reduce the need for an extremely large number of CD8 T cells to be retained after vaccination to allow identification and elimination of the parasite. Though generally thought to inhibit liver-stage immunity, the inflammation caused in the liver from the blood stage of infection

could also augment migration of T cells into the liver via upregulation of these signals.

Parasites were found in CD11c$^+$ cells, which could be generated from transferred monocytes. CD11c is typically associated with DCs; however, they also expressed the macrophage markers F4/80 and CSF1R, the inflammatory monocyte markers CD11b, CD14, Ly6C, and CCR2, as well as CD103 and CD207, reminiscent of migratory or inflammatory DCs. A previous paper indeed identified a CD8α$^+$ CD11c$^+$ cell type as presenting *P. berghei* antigen to CD8 T cells [4], but many markers used here were not overlapping with previous studies to allow confirmation. Malaria research has uncovered other unique activated subsets of immune cells, including atypical B cells, monocytes, and hybrid effector T cell types [9], several of which upregulate CD11c, an integrin associated with activation, during infection. Kurup *et al.* clearly demonstrate that CD11c$^+$ cells from livers of infected mice stimulated parasite-specific IFN-γ$^+$ tumor necrosis factor (TNF)$^+$ CD8 T cells both *in vivo* and *in vitro*. This study explains how liver-stage T cell responses are generated. While it was known that DCs play a central role in the response to RAS and sporozoites in the skin, it had not been demonstrated that infected hepatocytes attracted antigen-presenting cells in the liver [10], and further investigation of this exciting phenomenon is warranted.

New vaccines might target multistage antigens to the newly identified inflammatory DC, for example using CD207, as DEC205 has been used. In order to manipulate the migration of DCs and T cells for the purposes of vaccines, it would be important to know if CCR2, CX3CR1, or CD103 were critical for their infiltration of the liver or location of infected hepatocytes. Most importantly, it will be critical to follow up by examining the prevalence and mechanisms of *Plasmodium*-specific CD8 T cell immunity in exposed humans. While this is challenging, phenotypes and responses could soon be studied using new HLA tetramers. Given the variation between findings in studies testing for various antibody specificities and T cell epitopes, much more needs to be done to understand the natural generation of *Plasmodium*-specific CD8 T cells, which are known to be beneficial in vaccines that seek to destroy infected hepatocytes.

[1]Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0435, USA
[2]Department of Microbiology and Immunology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555-0435, USA

*Correspondence:
rostephe@utmb.edu (R. Stephens).

https://doi.org/10.1016/j.pt.2019.04.011

References

1. Long, C.A. and Zavala, F. (2016) Malaria vaccines and human immune responses. *Curr. Opin. Microbiol.* 32, 96–102
2. Kurup, S.P. *et al.* (2019) Monocyte-derived CD11c(+) cells acquire *Plasmodium* from hepatocytes to prime CD8 T cell immunity to liver-stage malaria. *Cell Host Microbe* 25, 565–577.e6
3. Liehl, P. *et al.* (2014) Host-cell sensors for *Plasmodium* activate innate immunity against liver-stage infection. *Nat. Med.* 20, 47–53
4. Parmar, R. *et al.* (2018) Infectious sporozoites of *Plasmodium berghei* effectively activate liver CD8alpha(+) dendritic cells. *Front. Immunol.* 9, 192
5. Silvie, O. *et al.* (2017) Tissue-specific cellular immune responses to malaria pre-erythrocytic stages. *Curr. Opin. Microbiol.* 40, 160–167
6. Crompton, P.D. *et al.* (2010) A prospective analysis of the Ab response to *Plasmodium falciparum* before and after a malaria season by protein microarray. *Proc. Natl. Acad. Sci. U. S. A.* 107, 6958–6963
7. Dodoo, D. *et al.* (2011) Measuring naturally acquired immune responses to candidate malaria vaccine antigens in Ghanaian adults. *Malar. J.* 10, 168
8. Tweedell, R.E. *et al.* (2018) Kupffer cells survive *Plasmodium berghei* sporozoite exposure and respond with a rapid cytokine release. *Pathogens* 7, 4
9. Hansen, D.S. *et al.* (2017) Emerging concepts in T follicular helper cell responses to malaria. *Int. J. Parasitol.* 47, 105–110
10. Radtke, A.J. *et al.* (2015) Lymph-node resident CD8alpha$^+$ dendritic cells capture antigens from migratory malaria sporozoites and induce CD8$^+$ T cell responses. *PLoS Pathog.* 11, e1004637