Gateway to deLiver: How malaria sporozoites cross the sinusoidal barrier

Insight from David Sacks

After their inoculation by mosquitos and entry into the bloodstream, malaria sporozoites must cross the liver sinusoidal barrier to gain access to hepatocytes, where they can multiply as erythrocyte-infecting forms. In this issue, Cha et al. provide further evidence that Kupffer cells (KCs) offer a gateway for entry of sporozoites into the liver parenchyma and identify the receptor on the KCs that directs the passage of the parasite through this portal.

A remarkably fruitful phage display strategy led to the identification of a peptide, P39, that bound to KCs. P39 strongly inhibited sporozoite entry into KCs in vitro and reduced liver infection in vivo. It was also shown to bind specifically to CD68, a heavily glycosylated transmembrane protein similar to LAMPs in its cellular distribution, sequence, and structure. Furthermore, the expression of CD68, at least in the liver, is confined to KCs. The function of CD68 as a receptor for sporozoite entry into KCs in vitro was supported by silencing, overexpression, and ectopic expression of the CD68 gene and by antibody inhibition. More critically, parasite liver burden in CD68 knockout mice was reduced by 71%, substantiating CD68 as a key receptor for sporozoite interaction with KCs in vivo, and KCs as the paramount gateway cells. Finally, CD68 knockout mice treated with clodronate to deplete KCs showed liver parasite burdens that were comparable to clodronate-treated, wild-type mice, which in turn had comparable infections to untreated wild-type mice. The authors conclude that CD68 serves as such an efficient gateway that sporozoite transit to the liver parenchyma proceeds as if the sinusoidal barrier did not exist.

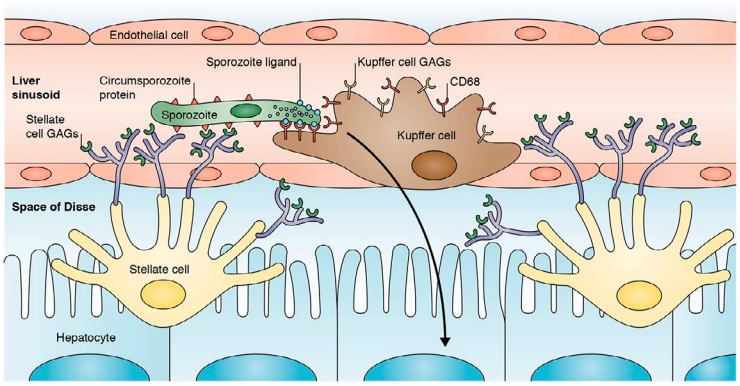

FIGURE COURTESY OF CHA ET AL.

Model for receptor–ligand interactions for sporozoite traversal of Kupffer cells: Interactions between circumsporozoite protein (CSP) and glycosaminoglycans (GAGs) are required for initial sporozoite (SPZ) attachment and sporozoite arrest in the liver sinusoid. The sporozoite then glides until it encounters a Kupffer cell. Recognition of CD68 as the receptor on Kupffer cells mediates sporozoite traversal.

These findings still need to be reconciled with prior quantitative imaging studies that attribute a greater role for endothelial cells in sinusoid traversal and that reveal a negative impact of KCs on sporozoite survival. Beyond its function as a receptor for sporozoite entry, the nature and consequences of CD68 engagement need to be further explored. Does CD68-mediated sporozoite entry into KCs involve active penetration or phagocytosis? Is there damage to the host cell? Does CD68 engagement inhibit antigen presentation function and/or trigger release of antiinflammatory cytokines that contribute to the state of relative immune tolerance in the liver? And, crucially, what is the parasite ligand for CD68? Assuming its accessibility for antibody targeting in its native state, it would seem an ideal component of a pre-erythrocytic vaccine.

Cha, S.-J., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20110575

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Skin-resident T cells keep parasites on a Leish

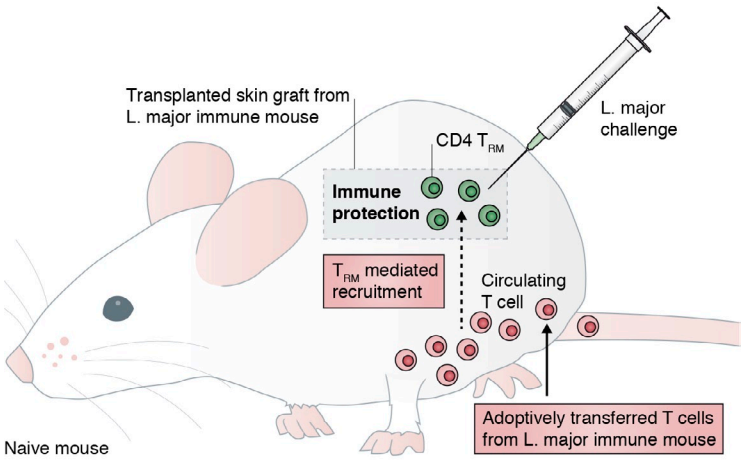
Insight from Laura Mackay (left) and Francis Carbone (right)

CD4$^+$ T cells have long been known to play an important role in the immune response to skin infection with *Leishmania major*. However, the role of permanently skin-resident cells in immune protection has not been explored in any detail, with the majority of studies implicitly assuming that all the memory cells recirculate back to the blood. In this issue, Glennie et al. report that skin-resident CD4$^+$ T cells are critical for optimal immune control of *Leishmania* due to their ability to recruit circulating cells to the site of infection.

Tissue-resident memory T cells (T_{RM}) are a nonrecirculating subset of T cells that provide local immune protection from reinfection. Although the majority of T_{RM} studies have focused on CD8$^+$ T cells, their CD4$^+$ counterparts have received less attention, and the role

of T_{RM} in protection against parasitic infection has not been explored. After *L. major* infection, Glennie et al. identify a population of $CD4^+$ T cells that remains in the skin, including sites distal to primary inoculation, for up to a year after infection. At least a proportion of these cells were long-term residents in the tissue, as they persisted in the skin after transplantation onto naive animals. Importantly, *L. major*–specific $CD4^+$ T_{RM} in these grafts provided enhanced protection against secondary infection. These cells were insufficient to mediate protection alone, instead recruiting circulating memory T cells to the site of infection in a chemokine-dependent fashion.

The Glennie et al. study allows a key commonality to be drawn between T_{RM} subsets, as $CD8^+$ T_{RM} in the skin and mucosa have been shown to provide immune protection in a similar fashion, involving the enhanced recruitment of immune cells into the infected tissue. Diseases caused by *Leishmania* represent a significant global health problem, and there is no available vaccine against this parasite. This study has major implications for the design of an effective *Leishmania* vaccine, highlighting that control of this pathogen depends on both resident and circulating memory T cell populations. A major advantage of a vaccine designed to generate T_{RM} is that these cells might also directly provide rapid protection at the site of infection, which would allow for immune control before pathogen dissemination and spread.



Glennie et al. grafted skin containing $CD4^+$ T_{RM} cells from *L. major*–immune mice onto the flank of naive animals that also received *L. major*–immune splenocytes (i.v.) and then challenged the transplanted grafts with *L. major*. The combination of graft-resident *L. major*–specific T_{RM} and circulating T cells resulted in enhanced immune protection compared with mice that received immune skin grafts or circulating cells alone.

Glennie, N.D., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20142101

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No "Kindlin," it's all about HSC balance

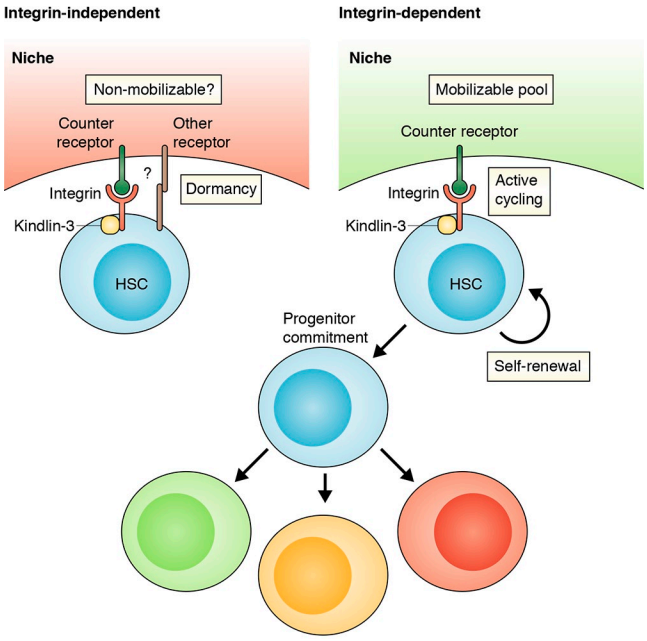
Insight from Paul Frenette

Integrins are known to promote the quiescence of hematopoietic stem cells (HSCs), but the precise mechanism has been unclear. In this issue, Ruppert et al. use mice deficient in Kindlin-3 (K3), an intracellular protein that regulates bidirectional integrin signaling, to show that integrin function differentially regulates quiescent and activated HSCs in the bone marrow (BM).

HSC quiescence is a defining behavior associated with preservation of self-renewal. Integrins have been shown to promote HSC homing to and retention in the BM and also to regulate HSC function. For example, deletion of α4 integrin, an adhesion molecule critical for HSC migration and homing, does not alter BM HSC numbers in the steady state but reduces HSC activity in the context of competitive reconstitution. It has been difficult, however, to tease apart the homing deficit from an intrinsic function of the integrin in HSC maintenance.

Ruppert et al. used a series of elegant experimental approaches to show convincingly that integrin function was dispensable for quiescent, long-term repopulating cells but was essential for activated HSCs and progenitors. One of the most convincing pieces of evidence was obtained from mixed chimeric mice harboring both $K3^{+/+}$ and $K3^{-/-}$ HSCs, in which deletion of K3 was induced after stable engraftment in the BM, allowing the authors to bypass the homing step. This approach revealed that $K3^{+/+}$ HSC numbers were sustained, whereas $K3^{-/-}$ progenitors were progressively depleted in the steady-state BM. The increased cycling of $K3^{-/-}$ versus $K3^{+/+}$ progenitors was confirmed by BrdU labeling and 5-fluorouracil, which selectively labels and kills, respectively, cycling HSCs/progenitors. Proliferative HSCs and progenitors were not retained in the BM and were mobilized in blood and spleen, a finding consistent with the notion that integrins contribute to retention in BM. This study strongly supports the idea the K3 (and integrin activation) is dispensable for HSC maintenance under homeostasis but essential for progenitors when the hematopoietic system is under stress.

The differential requirement of quiescent and activated HSCs for integrin-mediated adhesion argues for hitherto unknown regional specifications of niche components and/or extracellular matrices maintaining active and quiescent HSCs.



Although at first glance one can envision that integrins enable HSC migration toward a niche that promotes active cell divisions, the study by Ruppert et al. shows that integrin signaling exerts critical roles for *retention* in the BM, whereas it appears completely dispensable for the migration out of the marrow. Indeed, HSCs/progenitors deficient in activated integrins are readily mobilized, suggesting that integrin signaling, rather than its physical migratory function, may drive the phenotype in the active niche. The question then arises how dormant HSCs are anchored in their quiescent microenvironment. This question remains puzzling because signaling downstream of CXCR4/CXCL12, the sole niche factor–receptor pair thus far shown to promote HSC quiescence, leads to integrin activation. The most intuitive answer, as suggested by the authors, may lie in the overlapping array of adhesion receptors that could compensate for any individual loss. Additionally, other physical forces (e.g., neighboring cells and matrix) may contribute to maintain niche integrity without the specific need of brute adhesion strength.

Ruppert, R., et al. 2015. *J. Exp. Med.* http://dx.doi.org/10.1084/jem.20150269

HSC niches have distinct requirements for Kindlin-3 and integrin signaling where slow cycling (dormant) HSCs do not require the expression of Kindlin-3 and perhaps rely on other receptor pairs (brown sticks). Active HSCs and progenitors, in contrast, are dependent on Kindlin-3 and integrin function for self-renewal and progenitor proliferation. Loss of integrin activation is associated with higher mobilization of HSC in blood and spleen, suggesting that integrins regulate the mobilizable HSC/progenitor pool.

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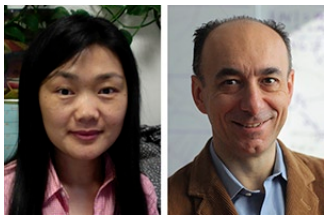
Inborn errors underlying herpes simplex encephalitis: From TLR3 to IRF3



Insight from Shen-Ying Zhang (left) and Jean-Laurent Casanova (right)

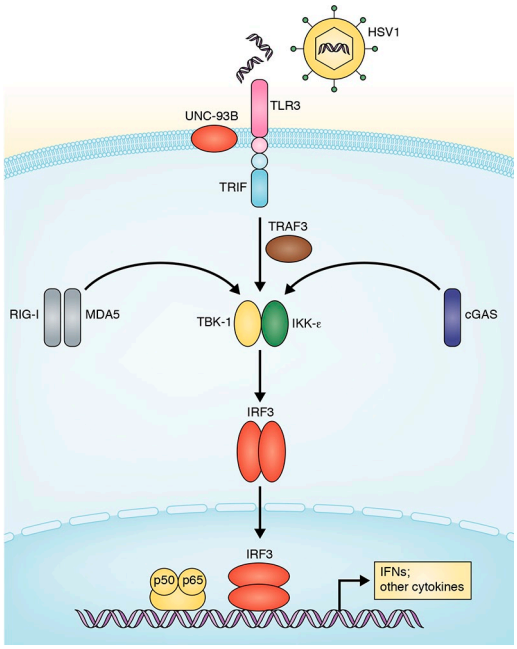
Most humans experience herpes simplex virus 1 (HSV-1) infection at some point in their lives, with no ill effects. Only a very small number develop HSV-1 encephalitis (HSE), which has an estimated prevalence of about 1/10,000. HSE is more common in childhood, affecting previously healthy individuals during primary infection. In the course of HSE, only the brain is affected. HSE is the most common form of sporadic, as opposed to epidemic, viral encephalitis in the Western world. Although sporadic, rather than familial, HSE can be caused by inborn errors of single genes. Mutations of five genes of the Toll-like receptor 3 (TLR3) signaling pathway have been identified in children with HSE: *TLR3*, *UNC93B1*, *TRIF*, and, more surprisingly, *TRAF3* and *TBK1*, which encode downstream, nonspecific components of the pathway. These mutations impair central nervous system (CNS)–intrinsic interferon (IFN)-α/β production in response to HSV-1. In this issue, autosomal dominant (AD) interferon regulatory factor 3 (IRF3) deficiency is reported by Andersen et al. as a new genetic etiology of HSE.

The transcription factor IRF3 controls multiple IFN-α/β–inducing pathways, including that of TLR3, which can be triggered by dsRNA, and those of other RNA and DNA sensors. IRF3 is normally activated by the kinases TBK1 and IKKε. The new IRF3 missense mutant, found in an adolescent with HSE, cannot undergo serine phosphorylation or dimerization and thus cannot activate transcription. In the heterozygous leukocytes of this patient, haploinsufficiency for IRF3 impairs the induction of antiviral IFN-α/β in response to various stimuli. Moreover, IFN-β induction in heterozygous fibroblasts stimulated by poly(I:C), which mimics dsRNA and for which recognition in fibroblasts depends on TLR3, is also impaired. IFN-β induction by HSV-1 is also impaired in these fibroblasts. Overall, the AD IRF3 deficiency is consistent with a key

role for IRF3 in IFN-α/β induction, with a broad cellular phenotype, including impaired TLR3 and HSV-1 responses, reminiscent of that seen in AD TRAF3 and TBK1 deficiencies.

Despite the broad cellular impact of AD IRF3 deficiency, affecting multiple signaling pathways and cell types, its clinical phenotype in the single patient studied is surprisingly narrow, restricted to HSE. Moreover, clinical penetrance may be incomplete, as a heterozygous relative of unknown HSV-1 infection status is healthy. This neatly illustrates that not all cellular phenotypes translate into clinical phenotypes and that this translation varies from individual to individual. The molecular basis underlying these intriguing observations is not understood and will require further study. Incomplete clinical penetrance has been observed for most known genetic etiologies of HSE, consistent with the general view of HSE as sporadic. Overall, the study reported in this issue shows that human IRF3 is a key component of TLR3-dependent, IFN-α/β-mediated, CNS-intrinsic immunity to HSV-1. It adds weight to the emerging notion that impaired TLR3–IFN-α/β intrinsic immunity can underlie childhood HSE, suggesting that IFN-α treatment may be beneficial to patients.

Andersen, L.L., et al., 2015. *J. Exp. Med*. http://dx.doi.org/10.1084/jem.20142274



Mutations in six genes (*TLR3*, *UNC93B1*, *TRIF*, *TRAF3*, *TBK1*, and *IRF3*), all involved in the TLR3 signaling pathway, have been found in HSE patients. *TLR3*, *UNC93B1*, and *TRIF* are specifically involved in TLR3 signaling. *TRAF3*, *TBK1*, and *IRF3* are also involved in signaling of other RNA and DNA sensors (RIG-I, MDA5, and cGAS). TLR3 signaling is initiated by the recognition of dsRNA, inducing activation of the IRF3 and NF-κB pathways via TRIF, leading to the production of IFN-α/β and/or IFN-λ. TLR3, UNC-93B, TRIF, TRAF3, TBK1, and IRF3 deficiencies are associated with impaired IFN-α/β and/or IFN-λ production upon stimulation of TLR3 or infection with HSV-1. A mutation in the gene encoding IRF3 is described in the paper by Andersen et al.

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