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INSIGHTS

Why it might be bad for brain cells to eat malaria parasites

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In this issue, Adams et al. (2021. *J. Exp. Med*. https://doi.org/10.1084/jem.20201266) show that red blood cells infected with strains of *Plasmodium falciparum*, which are commonly found in cerebral malaria patients, are specifically internalized by brain endothelial cells, perhaps contributing to the symptoms of the disease.

Malaria becomes very dangerous when it affects the brain. Cerebral malaria is caused when red blood cells, infected by the parasite *Plasmodium falciparum*, accumulate within tiny brain blood vessels, blocking blood flow (White et al., 2013). This can increase blood pressure and induce brain swelling, crushing precious parts of the brain against the skull (Newton et al., 1991; Seydel et al., 2015). As a result, patients may suffer from seizures and coma and, if they survive, experience permanent brain damage. But why do some malaria episodes cause cerebral symptoms while the majority do not?

The surfaces of *Plasmodium falciparum*–infected erythrocytes display a small number of parasite-derived molecules, organized into families (Wahlgren et al., 2017). One of these, the PfEMP1 proteins, mediates binding of infected erythrocytes to tissue and blood vessel surfaces, preventing their destruction by the spleen (Jensen et al., 2020). Each parasite genome contains many PfEMP1 proteins that form a highly diverse protein family. Parasites switch which of these they produce to avoid antibody-mediated detection. But this also varies the binding properties of infected erythrocytes as different PfEMP1 bind to different endothelial receptors.

Two of the major human receptors recognized by PfEMP1 are endothelial protein C receptor (EPCR) and intercellular adhesion molecule-1 (ICAM-1; Jensen et al., 2020). PfEMP1 that bind to these receptors are associated with parasites that cause different malaria symptoms. Parasites expressing PfEMP1 that bind EPCR are more commonly found in children suffering from severe malaria episodes (Turner et al., 2013), while those that can simultaneously bind to both EPCR and ICAM-1 are more commonly associated with episodes of cerebral malaria (Lennartz et al., 2017). But why is this? How does binding to particular receptors lead to the symptoms of cerebral disease? The study in this issue by Adams et al. presents a novel discovery that leads the authors to propose a novel hypothesis.

They started by taking a panel of parasite strains that produce different PfEMP1, selecting some that bind to just ICAM-1, some that bind to just EPCR, and some of the dual EPCR and ICAM-1 binders. They incubated human brain endothelial cells with erythrocytes infected with these parasites and observed what happened to the ICAM-1. When, and only when, the erythrocytes used in this study expressed dual binding PfEMP1, they observed an interesting phenomenon. ICAM-1 became enriched on endothelial cell surfaces, forming unusual rings and protrusions, changing in both surface density and in distribution.

Looking more closely, they spotted something very unexpected. As well as observing infected erythrocytes attached to the surfaces of brain endothelial cells, they found infected erythrocytes inside these cells. This occurred in three independent experiments using three different brain endothelial cell lines. In each case, only infected erythrocytes that can bind both ICAM-1 and EPCR were internalized. The same effect was also seen using a spheroid model. These spheroids are clusters of cells that assemble to mimic the blood–brain barrier. Once again, only dual ICAM-1– and EPCR-binding infected erythrocytes were internalized by the endothelial cells of the spheroids. What was going on? Brain endothelial cells have previously been shown to internalize damaged erythrocytes, perhaps to clear them from the blood. Could they be doing the same with parasite-infected erythrocytes?

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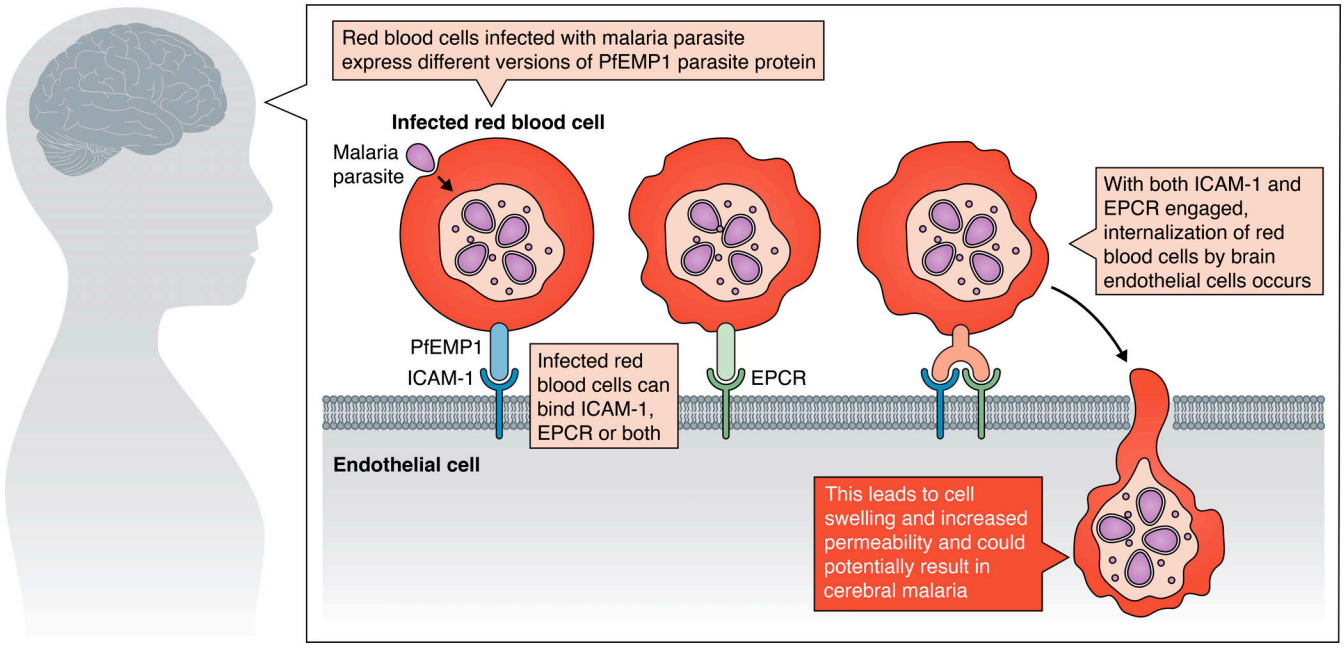

Human erythrocytes infected with the malaria parasite, *Plasmodium falciparum*, produce adhesive PfEMP1 proteins that allow them to interact with human endothelial receptors. Some of the these PfEMP1 bind to ICAM-1 and some bind to EPCR, while some can simultaneously bind to both ICAM-1 and EPCR. Adams et al. (2021) show that brain endothelial cells can specifically internalize infected erythrocytes that bind to both ICAM-1 and EPCR. This leads to endothelial cell swelling and increased permeability, perhaps contributing to the severe symptoms of cerebral malaria.

The next set of experiments suggested that internalization of infected erythrocytes is not a good move. Two distinct and potentially deleterious effects were observed. First, Adams et al. (2021) studied the uptake of a large fluorescent dye, which cannot enter healthy cells. Spheroids containing internalized dual ICAM-1– and EPCR-binding infected erythrocytes took up more of this dye. Could this indicate that internalization of infected erythrocytes causes damage to brain endothelial cells, increasing their permeability? Second, the authors noticed that the spheroids increased in volume when incubated with dual ICAM-1– and EPCR-binding infected erythrocytes. Could this swelling of endothelial cells play a role in brain swelling in cerebral disease? In both cases, internalization of infected erythrocytes seems likely to spell trouble for the brain endothelium.

This fascinating discovery raises a number of questions. The first relates to the specificity of internalization. It appears as though only infected erythrocytes producing dual ICAM-1– and EPCR-binding PfEMP1 are internalized. As these are the same PfEMP1 whose expression in patients is associated with development of cerebral symptoms (Lennartz et al., 2017), this is tantalizing. But how does this selectivity arise, and what is the mechanism of internalization?

Adams et al. (2021) show clearly that the interaction between PfEMP1 and ICAM-1 is critical for internalization, with antibodies targeting either ICAM-1 or the ICAM-1–binding domain of the PfEMP1, preventing the effect. However, infected erythrocytes that express a PfEMP1 protein that binds to ICAM-1 alone are not internalized. While it is true that PfEMP1 that bind to ICAM-1, but not EPCR, bind ICAM-1 with a subtly different binding mode than those that bind both ICAM-1 and EPCR (Lennartz et al., 2019), it seems unlikely that this is the cause of a major difference in internalization efficiency. It is much more likely that EPCR binding is also involved in uptake into the brain endothelium. In the highlighted study, the authors added an antibody that targets EPCR and found that this led to a nonsignificant reduction in internalization (Adams et al., 2021). It will be interesting to probe the role of the EPCR–binding domain of these PfEMP1 with further experiments—for example, assessing internalization in the presence of soluble EPCR or antibodies against the EPCR-binding domain—to see if this reduction is real.

If both ICAM-1 and EPCR binding prove to be involved in internalization, how do they do it? Previous studies have shown that dual EPCR- and ICAM-1–binding PfEMP1 are able to simultaneously bind to both receptors, with dual binding tethering infected erythrocytes more tightly to endothelial cells than when either receptor is used for adhesion alone (Avril et al., 2016; Lennartz et al., 2017). Could this tighter binding provide more time for internalization? Alternatively, and perhaps more likely, EPCR-binding PfEMP1 have been shown to block the natural ligand of EPCR, protein C, from mediating signaling (Lau et al., 2015; Turner et al., 2013). One consequence of this could be up-regulation of ICAM-1 expression on the endothelium (Moxon et al., 2013; Turner et al., 2013). Could EPCR-mediated signaling lead to the up-regulation of ICAM-1 seen in the brain endothelial cells and, together with tighter binding due to simultaneous dual receptor attachment, trigger internalization? More studies are needed to dissect these mechanisms and to understand their specificity.

The second question is the degree to which internalization of infected erythrocytes contributes to cerebral disease and,

if it does, how. The authors speculate that swelling of spheroids on infected erythrocyte internalization could contribute to brain swelling seen in cerebral disease (Seydel et al., 2015). Indeed, upon observing a brain section taken postmortem from a victim of cerebral malaria, they observe internalized infected erythrocytes, showing that internalization does happen during natural infection as well as in culture conditions. But, do infected erythrocytes need to enter into endothelial cells in order to cause brain swelling? Will infected erythrocytes that tightly adhere to the surface of the endothelium not have just the same effect in increasing the volume of cellular material in the brain? Estimates suggest that as much as 50 ml of parasite biomass might be sequestered in the brain of a cerebral malaria sufferer (White et al., 2013). It will be fascinating to see how much of this parasite material is inside cells and how much outside, and whether it matters for the brain swelling.

Another way in which internalization of infected erythrocytes could cause harm is through damage to the brain endothelium. The increased permeability of spheroids after infected erythrocyte internalization could contribute to disruption of the integrity of the endothelium. This could combine with an effect already observed for EPCR-binding PfEMP1. Signaling through EPCR-mediated pathways has been shown to protect the endothelium from thrombin-mediated disruption, and PfEMP1 binding to EPCR interferes with this protective effect (Bernabeu et al., 2016; Kessler et al., 2017). Could the increased permeability of the endothelium resulting from infected erythrocyte internalization combine with this effect from infected erythrocytes attached to cell surfaces, spelling trouble for endothelial integrity?

Whatever the answers to these questions, the discovery that brain endothelium can internalize infected erythrocytes, and that this internalization is specific to parasite variants found more commonly in sufferers of cerebral malaria, is fascinating and tantalizing. It opens up a series of questions, the answers to which will require complex and challenging in vivo experiments. But one thing appears clear. Those brain cells need to be careful what they eat. Gobbling up parasite-infected erythrocytes might not be good for them.

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