Second, building disease classifiers based on the correlation, over time, of modular signature components with clinical traits (rather than with the mere presence of a given molecular trait at a specific point in time), could allow hard-wiring of resulting molecular classifications, with the development and selection of treatment modalities. Third, this implies, specifically in SLE, that in future IFN-blocking trials, the selection of patients should not be based on the presence or absence of a signature, but rather on whether this signature correlates with changes in disease activity. Fourth, therapeutic modalities targeting other components of the SLE signature should be developed to provide adequate treatment coverage for other molecular disease subtypes identified by this study. Finally, the reported findings suggest that genetic association studies would greatly benefit from using molecular classifications derived from this type of sequential molecular phenotyping approach.

The work from Virginia Pascual's group offers new avenues for stratification of patients, in terms of both designing a new generation of trials and developing near-term personalized medical treatments for patients with SLE. Importantly, while such studies combine information from costly high-throughput methods, the use of less expensive and more focused transcriptional profiling approaches (e.g, multiplex PCR) that target only some representative genes (e.g., within the modules of interest), could constitute a practical stratification and monitoring strategy in future studies and trials [8]. The work also proposes that genetic variants may also be used as adjunct or surrogate markers in the identification of molecular classes of patients with SLE. Lastly, with these conceptual and methodological advances in place, it may be possible to develop serial molecular repertoires and clinical monitoring-based classifications for the development of drug selection frameworks in other disease settings.

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References

1. Banchereau, R. *et al.* (2016) Personalized immunomonitoring uncovers molecular networks that stratify lupus patients. *Cell* Published online March 30, 2016. http://dx.doi.org/10.1016/j.cell.2016.03.008
2. Bennett, L. *et al.* (2003) Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. *J. Exp. Med.* 197, 711–723
3. Baechler, E.C. *et al.* (2003) Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc. Natl. Acad. Sci. U.S.A.* 100, 2610–2615
4. Chiche, L. *et al.* (2014) Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol.* 66, 1583–1595
5. Khamashta, M. *et al.* (2016) Sifalimumab, an anti-interferon-α monoclonal antibody, in moderate to severe systemic lupus erythematosus: a randomised, double-blind, placebo-controlled study. *Ann. Rheum. Dis.* Published online March 23, 2016. http://dx.doi.org/10.1136/annrheumdis-2015-208562
6. Kalunian, K.C. *et al.* (2016) A Phase II study of the efficacy and safety of rontalizumab (rhuMAb interferon-α) in patients with systemic lupus erythematosus (ROSE). *Ann. Rheum. Dis.* 75, 196–202
7. Chaussabel, D. *et al.* (2008) A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. *Immunity* 29, 150–164
8. Chaussabel, D. and Baldwin, N. (2014) Democratizing systems immunology with modular transcriptional repertoire analyses. *Nat. Rev. Immunol.* 14, 271–280

Forum

Endothelial Glycocalyx: Shedding Light on Malaria Pathogenesis

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Malaria is estimated to kill 438 000 people annually, mostly due to severe malaria, which is closely associated with microcirculatory vasculopathy, although its pathogenesis remains incompletely understood. Here, we propose that the largely ignored glycocalyx of the vascular endothelium plays an important role in facilitating the pathogenesis of severe malaria.

Malaria and Severe Malaria Pathogenesis

Malaria is caused by intracellular protozoan parasites of the genus *Plasmodium.* When the parasites enter the intra-erythrocytic stages of their life cycle, patients exhibit symptoms that include spiking fever and rupture of infected erythrocytes, resulting in the release of hemozoin, which in turn stimulates endogenous pyrogens, such as tumor necrosis factor (TNF) [1]. If untreated, the infection leads to severe malaria (SM), which represents a range of conditions encompassing cerebral malaria (CM), multiorgan failure (including kidney and liver), severe anemia, and metabolic derangement, as well as pregnancy-associated malaria (leading to fetal growth retardation) [1].

It is widely accepted that the parasites cause severe pathology through interactions with the microcirculation [1] (Box 1). Different lines of study have focused on the effects of parasite binding on endothelial cells (EC). Notably, *Plasmodium falciparum* infections seem to disrupt the blood–brain barrier, which appears to be a crucial step in the development of CM [2]. Some authors have ascribed this to localized inflammation and dysregulated coagulation, associated with binding to specific endothelial receptors, such as EPCR and ICAM-1 [3], whereas others have focused on reduced nitric oxide (NO)-levels as the key event [1].

The glycocalyx is a vasculoprotective, carbohydrate-rich matrix that has a crucial role in maintaining the integrity of the endothelial lining of blood vessels (Box 2) [4]. However, the effect of parasite binding

Box 1. Host Microcirculation and Malaria

General inflammatory responses cannot fully explain the pathogenesis of SM [1]. Thus, attention has gathered around two phenomena: one is the adhesion of infected erythrocytes to ECs lining postcapillary venules, so-called 'sequestration'; the other is the aggregation of uninfected erythrocytes around a single infected erythrocyte, so-called 'rosetting'. Both mechanisms impair blood flow, resulting in hypoxia and local inflammatory processes. They also provide parasites with a survival advantage, because infected erythrocytes avoid systemic circulation and splenic clearance. The binding is mediated by parasite antigens inserted into the infected erythrocyte membrane. The responsible variant surface antigen (VSA) classes include proteins encoded by *rif* and *stevor* genes and, in the case of *Plasmodium falciparum*, also *var* genes. The binding to EC is mediated by a range of receptors, including CD36, intercellular adhesion molecule 1, and endothelial protein C receptor, all of which are glycoproteins [3]. Glycosaminoglycans and polysaccharides also assist in EC binding [7,8,15]. With the aim of identifying vaccine and drug targets, research has mostly dealt with identifying parasite VSAs responsible for sequestration and rosetting as well as their cognate binding receptors [3]. However, the downstream effects that binding might have on the glycocalyx have not yet been studied.

to this endothelial structure and its contribution to malaria pathogenesis has been largely neglected by current SM investigations. Here, we propose that this effect may have key implications for our understanding of severe disease and the development of new adjunct therapies.

Endothelial Glycocalyx and its Possible Role in Malaria Pathogenesis

All cell membranes are covered with a carbohydrate-rich matrix, termed the glycocalyx. Several findings suggest that a breakdown of the glycocalyx is a key event in the pathogenesis of severe malaria. *Plasmodium falciparum* and *Plasmodium vivax* both cause increased local inflammation, ischemia, and activation of procoagulant signaling and fibrinolysis in the microcirculation (Box 1) [1,5,6]. These events lead to endothelial activation and remodeling of the endothelial surface [1,6]. Due to shielding of the endothelial surface, the initial contact between the parasite-infected erythrocytes and the vascular lining occurs via the glycocalyx. Both heparan sulfate and chondroitin sulfate are well-established binding partners for malaria-infected erythrocytes [7,8] and binding to other components of the glycocalyx may also take place. Furthermore, SM is associated with shedding of several selectins and cellular adhesion molecules that are glycoproteins [6]. In terms of permeability, a lost or diminished glycocalyx may account for part of the brain swelling that has convincingly been associated with mortality in CM [2].

Glycocalyx loss has been studied in murine models of CM. The endothelial glycocalyx could be imaged by light microscopy using fluorescently labeled lectins recognizing specific carbohydrates and by transmission electron microscopy using cationic dyes binding negatively charged glycosaminoglycans (Box 2, Figure I) [9]. By using these protocols, a significant loss of glycocalyx was found in brain tissue from mice with experimental CM. In uncomplicated malaria, there was no significant loss of glycocalyx in the brain, but since plasma levels of glycocalyx components increased, shedding from other organs might be occurring [9].

Loss of endothelial glycocalyx as a consequence of malaria has so far not been studied in humans. Plasma levels of hyaluronic acid and sulfated glycosaminoglycans can be measured as surrogate markers for glycocalyx loss [9], but other cells in the circulation also bear a glycocalyx and can shed it in response to stressful stimuli [10]. Unfortunately, endothelial shedding cannot be appropriately addressed by routine investigation of human tissue samples, because the endothelial glycocalyx can be lost during processing *post mortem*. In pilot studies, increased plasma levels of hyaluronic acid and sulfated glycosaminoglycans have been found in human and nonhuman primate (NHP) malaria, but these results remain to be validated. Several observations make it reasonable to speculate that the integrity of the endothelial glycocalyx may be damaged as a consequence of malaria. Malaria-induced endothelial remodeling may ensue as a result of excessive localized inflammation and lead to shedding of cellular adhesion molecules and other glycocalyx components, as recently reported in human sepsis cases [11]. This, in turn, may improve access of infected erythrocytes to smaller, well-established endothelial binding partners, such as CD36 and ICAM-1 [1,3].

Exploring the Etiology behind Glycocalyx Loss in Experimental Malaria Models

In some diseases with glycocalyx loss, sheddases that cleave various components of the glycocalyx have been identified [10,12]. Sheddases include members of the matrix metalloprotease family [10] that have been previously implicated in experimental malaria, and are known to be increased in the plasma of patients with malaria [13]. Enzymes associated with coagulation and fibrinolysis, including thrombin and plasmin, are also capable of inducing glycocalyx shedding [12]. In malaria, thrombin is activated via the contact activation pathway and the procoagulant state is compensated by plasmin, removing excessive fibrin clots [5]. Thus, both enzymes may contribute to glycocalyx loss.

A different possibility is that the parasites themselves produce substances with sheddase-like activities. Further studies are clearly needed to understand the role of the endothelial glycocalyx in malaria. *In vitro* models have been widely used in malaria research since they enable the use of human material [8], but they are of limited value in establishing the connection between damaged glycocalyx and infection, due to the fact that cultured cells present an immature glycocalyx [4]. Well-defined *in vivo* models of malaria exist and, consequently, these may allow the glycocalyx to be properly studied.