Bartell, J.A., Sommer, L.M., Haagensen, J.A.J., Loch, A., Espinosa, R., Molin, S., and Johansen, H.K. (2019). Evolutionary highways to persistent bacterial infection. Nat. Commun. 10, 629.

Basler, M., Ho, B.T., and Mekalanos, J.J. (2013). Tit-for-tat: type VI secretion system counterattack during bacterial cell-cell interactions. Cell 152, 884–894.

Bingle, L.E., Bailey, C.M., and Pallen, M.J. (2008). Type VI secretion: a beginner's guide. Curr. Opin. Microbiol. 11, 3–8.

LeRoux, M., Kirkpatrick, R.L., Montauti, E.I., Tran, B.Q., Peterson, S.B., Harding, B.N., Whitney, J.C., Russell, A.B., Traxler, B., Goo, Y.A., et al.

(2015). Kin cell lysis is a danger signal that activates antibacterial pathways of Pseudomonas aeruginosa. eLife 4, 4.

Lipuma, J.J. (2010). The changing microbial epidemiology in cystic fibrosis. Clin. Microbiol. Rev. 23, 299–323.

Marvig, R.L., Sommer, L.M., Molin, S., and Johansen, H.K. (2015). Convergent evolution and adaptation of Pseudomonas aeruginosa within patients with cystic fibrosis. Nat. Genet. 47, 57–64.

Perault, A.I., Chandler, C.E., Rasko, D.A., Ernst, R.K., Wolfgang, M.C., and Cotter, P.A. (2020). Host Adaptation Predisposes Pseudomonas aeru-

ginosa to Type VI Secretion System-Mediated Predation by the Burkholderia cepacia Complex. Cell Host Microbe 28, this issue, 534–547.

Rosales-Reyes, R., Skeldon, A.M., Aubert, D.F., and Valvano, M.A. (2012). The Type VI secretion system of Burkholderia cenocepacia affects multiple Rho family GTPases disrupting the actin cytoskeleton and the assembly of NADPH oxidase complex in macrophages. Cell. Microbiol. 14, 255–273.

Stubbendieck, R.M., and Straight, P.D. (2016). Multifaceted Interfaces of Bacterial Competition. J. Bacteriol. 198, 2145–2155.

Unwanted Feedback: Malaria Antibodies Hinder Vaccine Boosting

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Whole-organism vaccination is a promising approach to prevent malaria. In this issue of *Cell Host & Microbe*, McNamara and colleagues identify epitope masking as a hindrance to antibody boosting after repeated administration of attenuated *Plasmodium falciparum* sporozoite vaccine.

While the malaria scourge continues across sub-Saharan Africa, the distribution of preventative tools has been disrupted by COVID-19, which could reverse 20 years of hard-earned gains. Vaccines that confer durable protection are needed more than ever.

After decades of research, malaria vaccine candidates have achieved unprecedented levels of protection. Attenuated *P. falciparum* (Pf) whole-sporozoite (SPZ) vaccine candidates have conferred sterilizing immunity against homologous challenge (Mordmüller et al., 2017). Although CD8 T cells could play the major role in protection, antibodies correlate to protection, and neutralizing immunoglobulins have been isolated from PfSPZ vaccinees. Problematically, however, antibody responses to circumsporozoite protein (CSP)—the immunodominant B cell target of PfSPZ vaccines—can be poor after repeated doses. The most advanced malaria vaccine RTS,S, a subunit candidate based on CSP, also

boosts poorly after repeated doses (Regules et al., 2016).

In this issue of *Cell Host & Microbe*, Haley McNamara and colleagues investigated this phenomenon by profiling B cell and antibody responses to SPZ vaccines in humans and mice (McNamara et al., 2020). In malaria-naive humans, CSP-specific antibodies peaked at increasing titers after the first and second dose of the Sanaria radiation-attenuated PfSPZ vaccine candidate, but plateaued thereafter; the expansion of CSP-specific B cells was more modest (Figure 1), and the proportion of CSP-specific plasmablasts was lower after the third dose in comparison with the second dose. Notably, poor responses were most evident among subjects with high antibody titers at the time of the booster dose.

To interrogate the mechanisms, the authors generated so-called Igh[92A10] immunoglobulin-knockin mice, whose B cells express the heavy chain of murine mono-

clonal antibody 2A10 that reacts to the PfCSP central repeat region sequence (NANP$_n$). When Igh[92A10] B cells were transferred to congenic mice, NANP-specific plasmablasts, memory B cells, and germinal center cells expanded after the second but not the third SPZ vaccine dose, similar to responses seen in humans, as did bone marrow plasma cells. PfCSP antibody titers also failed to boost after the third dose.

Why does boosting fail? Igh[92A10] memory B cells could be boosted, because they responded appropriately to vaccination after being transferred to naive congenic mice. However, suppression resumed when immune sera were transferred with memory B cells, or when memory cells were transferred to SPZ-immune congenic mice. In addition, Igh[92A10] memory B cells boosted appropriately when transferred to SPZ-immune MD4 mice whose own B cells are unable to mount CSP antibody responses. The evidence pointed to CSP-specific antibodies as

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B cell responses to CSP during vaccination with whole sporozoites
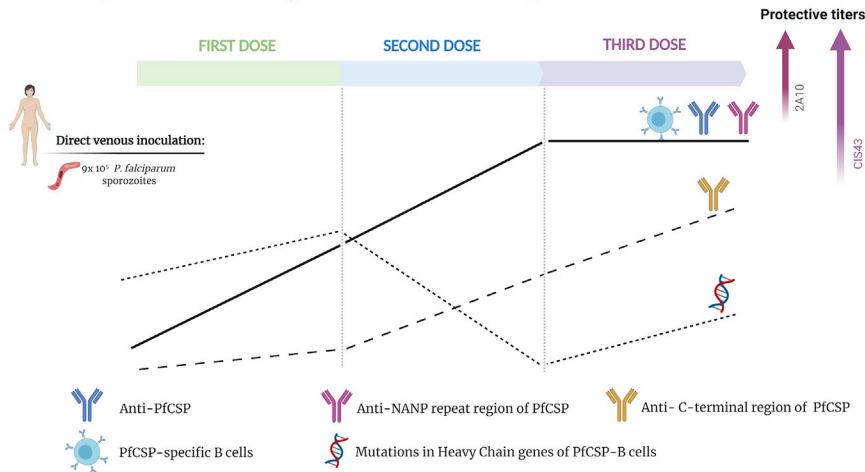
Figure 1. Profile of B Cell and Antibody Responses to *Plasmodium falciparum* CSP Protein over Successive PfSPZ Vaccine Doses in Humans

McNamara et al. (2020) determined that, as seen with the CSP subunit vaccine RTS,S, CSP repeat-specific antibody titers and B cell responses are suppressed after repeated PfSPZ vaccine doses. In mice, sterile immunity required higher titers of mouse mAb 2A10 than of human mAb CIS43, implying that B cell suppression could limit some antibody responses below the level needed for protection. Figure created with BioRender.com.

the culprit suppressing memory B cell responses.

Suppression was recapitulated when mAb 2A10 (or the human PfCSP mAb CIS43 that also binds the repeat region) was transferred together with Igh^{g2A10} memory B cells to congenic mice that were then vaccinated. Mutations in the Fc region that limit binding to inhibitory Fc receptors did not alleviate mAb 2A10 suppression. Nor did delaying mAb 2A10 transfer for 4 h after SPZ vaccination, to exclude the possibility that mAb simply cleared the parasite before B cell activation.

After excluding these other possible mechanisms, the observations suggest that vaccine-induced antibodies likely suppress by binding epitopes, thereby masking them from B cell recognition. Epitope masking has been explored with seasonal and epidemic influenza vaccines (Zarnitsyna et al., 2016) and with the lifesaving immunoglobin therapy that prevents an Rh− mother from developing antibodies that could attack her Rh+ fetus; this study extends the phenomenon to malaria vaccines.

Importantly, suppression can occur when antibody levels are still below the level needed to protect mice—raising concern that full protection might never be achieved with some antigens. In the PfSPZ vaccine trial in humans, antibody

feedback targeted the immunodominant central repeat region of CSP; C-terminal region antibodies continued to increase after repeated doses, although titers were relatively low (Figure 1). Low titers against the C-terminal region might account for the lack of suppression, or perhaps intrinsic features of the PfCSP repeat region or its corresponding antibodies could make it distinctly prone to suppression. McNamara and colleagues suggest the latter: passive transfer of human mAb targeting the C-terminal region of CSP had only a modest effect on the B cell response to SPZ vaccines in mice.

CSP is common to *Plasmodia*, but each species encodes a distinct central repeat region. Subjects that received *P. vivax* CSP vaccine similarly showed evidence of limited boosting to its ANGAGNQPG or DRADGQPAG repeats (Bennett et al., 2016). Thus, the CSP repeat region could represent a general strategy for *Plasmodia* to limit antibody responses against this key surface protein integral to sporozoite motility and invasion and therefore to successful infection.

Somatic hypermutation of plasmablast heavy-chain genes specific to CSP, unlike other B cell responses, was greater after the third dose than the second dose (Figure 1). Mutation rates may differ by antibody specificity; for example, post-dose-three antibodies could dispropor-

tionately include those to the C terminus whereas those after dose two could be to the repeat region. Human antibodies to the CSP repeat region often undergo limited mutation (Murugan et al., 2018). The results could be relevant to RTS,S, where booster doses can also fail to increase titers against the repeat region and full protein but nevertheless increase somatic hypermutation, antibody avidity, and vaccine efficacy (Regules et al., 2016). It will be interesting to determine the specificity of the resulting serum antibodies for different CSP regions, because C-terminal seroreactivity and avidity have also been associated with RTS,S protection (Dobaño et al., 2019). Similarly, B cell suppression after repeated PfSPZ vaccine administration does not necessarily imply ineffective antibody, as confirmed by the isolation of the potent CIS43 antibody two weeks after the third dose.

At the molecular level, mutations in heavy-chain genes revealed interesting features: 68% of CSP-specific B cell antibodies using the IGHV3-33 gene, a germline previously associated with NANP-specific B cells (Imkeller et al., 2018), had mutations that introduced Asparagine (Asn) in CDR1 and Isoleucine (Ile) in CDR2. Asn and the isobaric residues Ile and Leucine (Leu) are common substitutions in antibody CDRs, and structural studies show their importance for the affinity and specificity of antigen-antibody interactions (Yokota et al., 2010). McNamara and colleagues show that the two substitutions in IGHV3-33 increase affinity for CSP 35-fold.

This study brings new impetus to identify epitopes and corresponding antibodies involved in masking, B cell suppression, and impaired vaccine responses. How frequently are such antibodies elicited in response to different malaria vaccines? Does natural infection also lead to epitope masking of NANP repeats? Because malaria-experienced Kenyan adults developed lower antibody titers than malaria-naive American adults receiving the same RTS,S regimen (Polhemus et al., 2009, Regules et al., 2016), antibody feedback-related limitations should continue to be assessed whenever RTS,S or PfSPZ are delivered to Africa.

Ultimately, vaccine developers will want to know how to avert antibody feedback and B cell suppression. McNamara

and colleagues report that simply delaying a third dose until vaccine antibody levels wane can alleviate B cell suppression. RTS,S trials are already examining delayed doses for their effects on antibody responses and efficacy (Regules et al., 2016). The report in this issue of *Cell Host & Microbe* suggests RTS,S developers are on the right track.

REFERENCES

Bennett, J.W., Yadava, A., Tosh, D., Sattabongkot, J., Komisar, J., Ware, L.A., McCarthy, W.F., Cowden, J.J., Regules, J., Spring, M.D., et al. (2016). Phase 1/2a Trial of Plasmodium vivax Malaria Vaccine Candidate VMP001/AS01B in Malaria-Naive Adults: Safety, Immunogenicity, and Efficacy. PLoS Negl. Trop. Dis. 10, e0004423.

Dobaño, C., Sanz, H., Sorgho, H., Dosoo, D., Mpina, M., Ubillos, I., Aguilar, R., Ford, T., Díez-Padrisa, N., Williams, N.A., et al. (2019). Concentration and avidity of antibodies to different circumsporozoite epitopes correlate with RTS,S/AS01E malaria vaccine efficacy. Nat. Commun. 10, 2174.

Imkeller, K., Scally, S.W., Bosch, A., Martí, G.P., Costa, G., Triller, G., Murugan, R., Renna, V., Jumaa, H., Kremsner, P.G., et al. (2018). Antihomotypic affinity maturation improves human B cell responses against a repetitive epitope. Science 360, 1358–1362.

McNamara, H.A., Idris, A.H., Sutton, H.J., Vistein, R., Flynn, B.J., Cai, Y., Wiehe, K., Lyke, K.E., Chatterjee, D., Kc, N., et al. (2020). Antibody Feedback Limits the Expansion of B Cell Responses to Malaria Vaccination but Drives Diversification of the Humoral Response. Cell Host Microbe 28, this issue, 572–585.

Mordmüller, B., Surat, G., Lagler, H., Chakravarty, S., Ishizuka, A.S., Lalremruata, A., Gmeiner, M., Campo, J.J., Esen, M., Ruben, A.J., et al. (2017). Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature 542, 445–449.

Murugan, R., Buchauer, L., Triller, G., Kreschel, C., Costa, G., Pidelaserra Martí, G., Imkeller, K., Busse, C.E., Chakravarty, S., Sim, B.K.L., et al. (2018). Clonal selection drives protective memory B cell responses in controlled human malaria infection. Sci. Immunol. 3, 3.

Polhemus, M.E., Remich, S.A., Ogutu, B.R., Waitumbi, J.N., Otieno, L., Apollo, S., Cummings, J.F., Kester, K.E., Ockenhouse, C.F., Stewart, A., et al. (2009). Evaluation of RTS,S/AS02A and RTS,S/AS01B in adults in a high malaria transmission area. PLoS One 4, e6465.

Regules, J.A., Cicatelli, S.B., Bennett, J.W., Paolino, K.M., Twomey, P.S., Moon, J.E., Kathcart, A.K., Hauns, K.D., Komisar, J.L., Qabar, A.N., et al. (2016). Fractional Third and Fourth Dose of RTS,S/AS01 Malaria Candidate Vaccine: A Phase 2a Controlled Human Malaria Parasite Infection and Immunogenicity Study. J. Infect. Dis. 214, 762–771.

Yokota, A., Tsumoto, K., Shiroishi, M., Nakanishi, T., Kondo, H., and Kumagai, I. (2010). Contribution of asparagine residues to the stabilization of a proteinaceous antigen-antibody complex, HyHEL-10-hen egg white lysozyme. J. Biol. Chem. 285, 7686–7696.

Zarnitsyna, V.I., Lavine, J., Ellebedy, A., Ahmed, R., and Antia, R. (2016). Multi-epitope Models Explain How Pre-existing Antibodies Affect the Generation of Broadly Protective Responses to Influenza. PLoS Pathog. 12, e1005692.

Recipe for Zoonosis: How Influenza Virus Leaps into Human Circulation

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The features that permit or prevent a virus from becoming a zoonotic threat is an ongoing area of investigation. In this issue of *Cell Host & Microbe*, Herfst et al. and Henritzi et al. help define the molecular and host determinants of influenza virus spillover from animal to human populations.

Humans are continually threatened by the ongoing emergence and circulation of potentially zoonotic viruses in wild and domestic animal populations. Understanding which, if any, of these viruses pose a threat to human health requires understanding of characteristics of the virus, the host, and the species-specific barriers that must be overcome. In this issue of *Cell Host & Microbe*, two separate groups report key findings that extend our understanding of the molecular and host determinants that could drive influenza virus spillover from animal to human populations (Figure 1).

Zoonotic transmission and establishment of a novel virus in the human population are largely constrained by three features: the opportunity to spill over from the animal host, the capacity to transmit and replicate in the human population, and the ability to escape human immunity. First, spillover from the reservoir or intermediate host must occur through direct or indirect contact between an infected vector and the naive host. Second, the virus must be able to transmit effectively and replicate in a human host. This is constrained by receptor-mediated entry to cells and the replication competence in that new host environment. Third, to successfully establish itself in the human population, successful zoonotic viruses must escape human immunity, including innate