GERMINAL CENTER REACTIONS

Metabolic constraints on the B cell response to malaria

Early plasmablasts responding to *Plasmodium* infection may outcompete germinal center B cells for L-glutamine and delay pathogen control.

Lauren B. Rodda and Marion Pepper

When confronted with a new pathogen, the humoral immune system works to clear the pathogen and prevent a future infection. How pathogen clearance and memory formation are balanced when *Plasmodium yoelii* infects mice, a model of human malaria, is unclear. In this issue of *Nature Immunology*, Vijay et al. suggest that plasmablasts may act as a competitive sink for L-glutamine, thereby constraining the germinal center (GC) response[1]. Early treatment with L-glutamine or plasmablast reduction increased the GC response and improved parasite control (Fig. 1). The authors propose an intriguing model whereby competition for metabolites might regulate the transition from the early plasmablast response to the late GC response.

Plasmodium parasites first infect the liver, where they multiply until their numbers reach the tens of thousands. Next, they burst into the blood and begin infecting red blood cells[2]. Studies, including the current study by Vijay et al., model this by infecting mice intravenously with 1×10^6 *Plasmodium*-infected red blood cells[1,3]. In response to infection, *Plasmodium*-specific B cells rapidly differentiate into plasmablasts, cells that pump out high amounts of *Plasmodium*-specific antibody[3]. *Plasmodium*-specific antibodies bind parasites and support macrophages and T cells as they control the infection[4,5]. However, plasmablasts are short lived, and the population wanes after approximately 12 days.

After a week of infection, GCs begin to form, which constitutes the second wave of the B cell response. GCs are clusters of pathogen-specific B cells and follicular helper T (T_{FH}) cells that work to diversify the B cell receptor repertoire and select B cells that can secrete antibodies with enhanced affinity for the pathogen. These B cells then become long-lived plasma and memory B cells. Plasma cells sustain *Plasmodium*-specific antibody

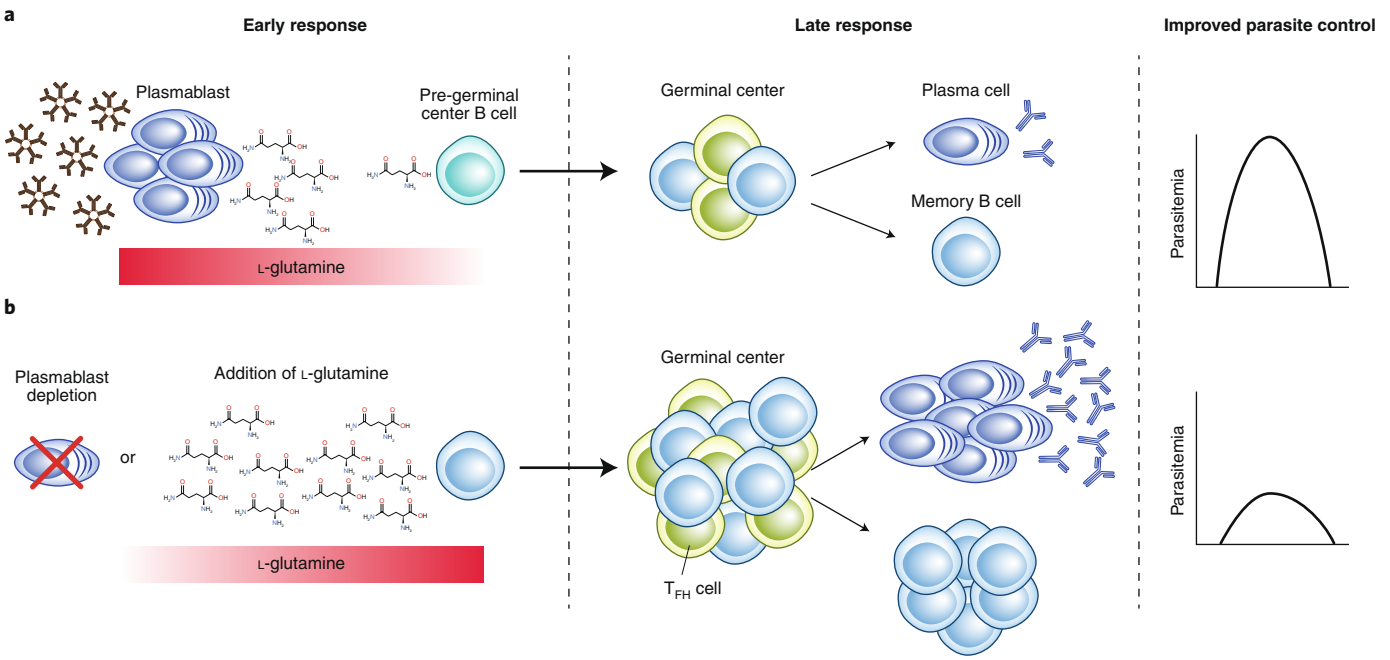

Fig. 1 | Model of the influence of L-glutamine and plasmablasts on the GC response to *Plasmodium* infection. a. Early in the response to blood-stage *Plasmodium* infection in mice, pathogen-specific B cells in the spleen differentiate into plasmablasts to rapidly secrete antibody and, as a result, consume L-glutamine. *Plasmodium*-specific B cells differentiate into GC B cells more slowly, and this process also requires L-glutamine. These GC B cells can become plasma cells that secrete higher affinity antibodies and help control the infection. **b**. Supplementation of drinking water with L-glutamine or genetic depletion of plasmablasts increases the number of GC B cells, the number of T follicular helper cells (T_{FH}), the amount of *Plasmodium*-specific antibody and improves parasite control. Credit: Gretchen Harms Pritchard

production, while memory B cells can rapidly differentiate into plasmablasts to boost antibody production when they detect antigen. Both function to protect the host from a future infection[6,7]. Studies of blood-stage *Plasmodium* infection in mice demonstrated that parasite clearance during the primary infection required both the formation of GCs and the production of antibodies by GC-derived plasma cells or activated memory B cells[8]. Thus, it is important to understand what regulates the formation of GCs and protective antibodies during *Plasmodium* infection.

Both plasmablasts and GC B cells proliferate rapidly. They have a high demand for glutamine, which supports their proliferation and other functions via ATP production, the Krebs cycle and mTORC1 signaling[9]. However, how plasmablasts and GC B cells might compete for this resource has not been previously interrogated. Vijay et al. investigate the possibility of metabolic competition during mouse infection with blood-stage *P. yoelii*. The authors show that supplementation with L-glutamine reduced peak parasitemia and increased the formation of GCs. By contrast, treatment with L-alanine or L-valine did not decrease peak parasitemia. L-glutamine treatment increased the numbers of GC B and T_{FH} cells early in the response and increased the titer of *P. yoelii*–specific antibodies. Additionally, oxygen consumption rates and the basal respiration of activated B cells increased, supporting the idea that L-glutamine can enhance GC B cell proliferation. The authors found L-glutamine was reduced at day 5 of infection in untreated mice, and the effects of L-glutamine addition were only seen when L-glutamine was supplied early after infection. This work suggests that increasing the level of L-glutamine early in the response enables GCs to form earlier, grow bigger, or both.

Interested in whether proliferating plasmablasts may be consuming the L-glutamine that the GC B cells need, Vijay et al. depleted plamablasts in several ways during the response to *P. yoelii* infection. Most convincingly, they treated CD138-DTR (diphtheria toxin receptor) bone-marrow-chimeric mice with diphtheria toxin early during the response. This treatment depleted CD138-expressing plasmablasts while still enabling the formation of GC-derived plasma cells. Similar to L-glutamine supplementation, plasmablast depletion reduced peak parasitemia and increased the quantity of GC B cells, GC T_{FH} cells and *P. yoelii*–specific antibodies.

Vijay et al. suggest that plasmablasts, which express higher levels of glutamine transporters and expand earlier in the response, deplete the available L-glutamine, thereby limiting early GC formation. In support of this model, combining depletion of plasmablasts and L-glutamine supplementation had only a small additive effect. Further study is needed to establish the mechanism of this redundancy and to determine whether the supplemental L-glutamine is directly affecting lymphocytes in the spleen. Identifying which cell type is consuming the L-glutamine will require cell-specific deletion of amino acid transport channels such as Slc7a5, and *Slc7a5*$^{f/f}$ mice have been bred[10]. For example, *P. yoelii*–specific T cells rapidly expand early in response to *Plasmodium* infection and express *Slc7a5* transcript. Interferon-γ-secreting T helper 1 (T_H1) cells and GC-supporting T_{FH} cells can both help control parasitemia. These cells may also be competing for L-glutamine and may therefore benefit from supplementation. In addition, it would be interesting to understand whether the limited GC response is due to constrained mTORC1 signaling, proliferation or another cellular process.

While these are important first observations, future studies are needed to understand the mechanism of improved parasite control in this system. The authors detected increased anti-*Plasmodium* antibodies after L-glutamine supplementation at day 12 post-infection, and they imply that this antibody is aiding parasite control and depends on the presence of GCs. While GC-derived plasma cells produce, on average, higher affinity antibodies than plasmablasts, the authors only detected increases in plasma cell numbers after day 15. Previous work with protein immunization showed memory B cells are produced from GCs earlier than plasma cells[11]. Since memory B cells can turn into plasmablasts and produce antibodies, perhaps the antibodies derived from memory B cells are contributing to parasite control. Additional studies are needed to determine whether antibodies are directly required for the improved parasite control and what distinguishes these protective antibodies from early plasmablast-derived antibodies.

It will be fascinating to learn whether the plasmablast response constrains the GC response in the context of other infections or whether this is specific to infection with *Plasmodium*. Additional studies using immunization would allow for the investigation of how antigen load, plasmablast expansion and the parasites themselves might contribute to this metabolic balance. Work in mouse models demonstrated that the formation of GCs against the liver-stage of *Plasmodium* infection can be disrupted when the subsequent blood-stage *Plasmodium* parasites emerge[12]. As this coincides with the rapid proliferation of plasmablasts and reduced L-glutamine, it would be interesting to determine whether L-glutamine treatment can rescue the production of liver-stage-specific GCs and antibodies. These antibodies can prevent a second liver-stage infection[13]. These findings may also have important implications for vaccination strategies. While there is no licensed vaccine for malaria, there are several vaccines that rely on repeated immunization currently in trials. As each boost induces a rapid plasmablast response, it will be important to determine whether this disrupts the previous GC and impairs the formation of more protective and long-lived antibody responses to the vaccine.

Humans form antibodies to the blood-stage of *P. falciparum* after one infection and are mostly protected from severe disease[14]. Vijay et al. take the first step in investigating their findings by describing a correlation between the rapid plasmablast response and parasitemia during blood-stage *Plasmodium* infection in humans. This confirms previous work demonstrating rapid plasmablast formation in naturally infected children[15]. Future studies correlating plasmablasts, parasitemia and GC kinetics in humans are needed but will require fine needle aspirate sampling of human lymph nodes.

Finally, we are currently experiencing a SARS-CoV-2 pandemic. Massive plasmablast responses and high-antibody titers have been described in patients with severe COVID-19 disease. Determining how plasmablasts, GC-dependent antibody and memory formation interact will be of the utmost importance to developing treatments and vaccines. ❐

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References

1. Vijay, R. et al. *Nat. Immunol.* https://doi.org/10.1038/s41590-020-0678-5 (2020).
2. Crompton, P. D. et al. *Annu. Rev. Immunol.* **32**, 157–187 (2014).
3. Krishnamurty, A. T. et al. *Immunity* **45**, 402–414 (2016).
4. Langhorne, J., Simon-Haarhaus, B. & Meding, S. J. *Immunol. Lett.* **25**, 101–107 (1990).
5. Fontana, M. F. et al. *PLoS Pathog.* **12**, e1006046 (2016); erratum **13**, e1006192 (2017).

6. Weisel, F. & Shlomchik, M. *Annu. Rev. Immunol.* **35**, 255–284 (2017).
7. Hirunpetcharat, C. et al. *J. Immunol.* **159**, 3400–3411 (1997).
8. Pérez-Mazliah, D. et al. *EBioMedicine* **24**, 216–230 (2017).
9. Boothby, M. & Rickert, R. C. *Immunity* **46**, 743–755 (2017).
10. Sinclair, L. V. et al. *Nat. Immunol.* **14**, 500–508 (2013).
11. Weisel, F. J., Zuccarino-Catania, G. V., Chikina, M. & Shlomchik, M. J. *Immunity* **44**, 116–130 (2016).
12. Keitany, G. J. et al. *Cell Rep.* **17**, 3193–3205 (2016).
13. Tan, J. et al. *Nat. Med.* **24**, 401–407 (2018).
14. Crompton, P. D. et al. *Proc. Natl Acad. Sci. USA* **107**, 6958–6963 (2010).
15. Sullivan, R. T. et al. *Malar. J.* **15**, 139 (2016).

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Competing interests
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ASTHMA & ALLERGY

A soluble allergen sensor sounds the alarm

The identification of the acute phase protein serum amyloid A as a soluble allergen sensor sheds new light on the mechanisms involved in the induction of type II airway inflammation.

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The airway epithelium forms a tight barrier to the outside world and plays a central role in the pathogenesis of allergic asthma and rhinitis[1]. A growing body of evidence indicates that many allergens can directly engage epithelial pattern recognition receptors (PRRs). These PRRs should be able to discriminate between harmful and innocuous substances but, in susceptible individuals, sensing of inhaled allergens by epithelial cells is thought to be dysregulated. As a result, PRR engagement by allergens evokes the epithelial secretion of chemokines, innate cytokines and alarmins, culminating in a robust type II adaptive immune response. However, the molecular mechanisms by which allergens stimulate PRRs and thereby induce the recruitment and activation of critical players in type II inflammation remain largely undefined. In this issue of *Nature Immunology*, Smole and colleagues[2] demonstrate that the acute phase protein serum amyloid A (SAA) acts as a soluble innate environmental sensor that promotes pulmonary type II inflammation. The authors discovered that the interaction between SAA and allergens of the fatty acid binding protein (FABP) family, which includes the house dust mite (HDM) allergen Der p 13, drives the active release of interleukin (IL)-33 by epithelial cells. Their findings reveal a surprising and previously unrecognized role for SAA, identifying a new mechanism of allergen sensing that provides insight into how the expression of IL-33, a crucial innate cytokine in allergic inflammation, is induced.

Allergen sensitization is a critical step in the development of allergic asthma. Allergens are derived from complex living organisms and comprise a diverse group of proteins with unique molecular structures and biological functions. It is therefore not surprising that many different mechanisms exist for the recognition of these allergens. Remarkably, all of these distinct pathways converge to induce eosinophilic airway inflammation. Allergens derived from the HDM species *Dermatophagoides pteronyssinus* are a major cause of asthma worldwide. HDM fecal particles contain a composite mixture of more than twenty mite-derived immunostimulatory protein groups, as well as endotoxin and proteases[3]. HDM allergens are strong activators of airway epithelial cells and innate immune cells because they contain specific lipid and carbohydrate ligands that can be recognized by a variety of PRRs. HDM-derived serine proteases, which interact with protease-activated receptor PAR-2, and the cysteine protease Der p 1 induce epithelial cytokine production and facilitate transepithelial delivery of allergens by disrupting tight junctions (Fig. 1). Another major HDM allergen, Der p 2, promotes airway inflammation via Toll-like receptor (TLR) 4, resulting in epithelial production of IL-1α, which then triggers granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-33 release in an autocrine fashion[4] (Fig. 1). Together, these proinflammatory signals trigger the activation of group 2 innate lymphoid cells (ILC2s) and dendritic cells (DCs), driving a T helper 2 (T_H2)-mediated response. Airway epithelial cells do not only express membrane-bound or cytoplasmic PRRs but are also a major source of soluble PRRs, such as SAA. Interestingly, SAA is known to bind to the bacterial outer membrane protein A (OmpA), which shares structural homology with the FABP Der p 13, a minor HDM allergen. This, together with the published finding that SAA is significantly increased in the blood and sputum of patients with asthma, prompted Smole and colleagues to explore the role of SAA in allergic airway disease.

SAA, originally identified as a protein deposited in amyloid A–type amyloidosis in the early 1970s, is a major acute-phase protein that is present in the circulation bound to high-density lipoprotein. Its production in the liver is upregulated in response to trauma and systemic infection, resulting in an up to 1,000-fold increase in its serum concentration. SAA serves as an innate immune opsonin for Gram-negative bacteria and blocks virus entry into cells. Locally produced lipid-free SAA has been implicated in the recruitment of immune cells and in epithelial repair and has cytokine-inducing properties[5].

Using mouse models, the authors provide convincing evidence that the interaction of SAA with allergens of the FABP family is required for the induction of HDM-mediated allergic airway inflammation. They show that targeted deletion of the *Saa* gene, which results in the loss of expression of SAA1 and its less abundant isoform SAA2, or SAA neutralization with specific antibodies diminishes the hallmarks of the HDM-driven allergic inflammatory phenotype. Compared with HDM-exposed wild-type mice, $Saa^{-/-}$ mice displayed significantly reduced numbers of DCs, IL-13$^+$ T cells and IL-13$^+$ ILC2s in the lung. Bronchoalveolar lavage eosinophilia, goblet cell hyperplasia, total serum immunoglobulin E (IgE) and bronchial hyperresponsiveness were also diminished. Furthermore, Smole and colleagues established that SAA can interact with FABPs of arthropod group 13 allergens and that depletion of Der p 13 impaired the capacity of HDM extract to induce IL-33 release in bronchoalveolar lavage fluid