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When is a malaria immune complex not an immune complex?

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SUMMARY

Immune complexes (ICs) are believed to play an important role in malaria pathology, and an interesting article by Mibei et al. recently published by *Parasite Immunology* suggests that IgG4 and IgE are particularly important. However, researchers should be aware of potential pitfalls to current assays aimed at measuring plasma ICs and correlating these to deposition in tissues.

Keywords

Fc-receptors, IgG, immune complex, malaria, Plasmodium

Antibody-antigen immune complexes (ICs) are common in normal healthy individuals, where they can be found at levels up to 15 µg/mL, indicating a physiological role in maintaining immune homeostasis (1). ICs are vital in clearing and/or neutralizing infectious agents and are crucial in presenting antigen via Fc and/or complement receptors to professional antigen presenting cells (APCs) during vaccination. IC binding to Fc-receptors (FcRs) can trigger activatory and/or inhibitory signalling pathways that culminate in a well-balanced immune response (1). However ICs are also associated with ill health in many diseases, including malaria, where they are believed to cause pathology through pro-inflammatory cytokine induction and/or by depositing and interfering with the function of important organs, e.g. the kidney with *Plasmodium malariae*, or the brain with *P. falciparum*. Studies in FcR or complement deficient mice argue for a prominent role for FcR engagement rather than complement in IC mediated pathology in a wide variety of tissue sites (2).

It is somewhat surprising therefore, that so little work has been done on investigating the biochemical nature of these ICs in malaria. A search of Pubmed with the terms, 'plasmodium', 'immune' and 'complex' in the title revealed a paltry seven publications, of which only one dealt with human malaria! It is therefore refreshing that *Parasite Immunology* recently published a paper by Mibei *et al.* where the authors investigated the contribution of antibody subclasses within circulating ICs from children with cerebral malaria (CM) and severe malarial anaemia (SMA) (3). Assuming that circulating ICs correlate with IC deposition in the tissues, and that their detecting reagents are specific for each subclass of IgG, the authors went on to show that total IgG (in particular IgG4) and IgE circulating ICs were significantly elevated in CM and SMA patients.

Unfortunately, there are a number of technical considerations that put these findings in conflict with the field in general, of which the most worrying is the ability to detect IgG4 in C1q binding ELISAs, given that IgG4 is generally believed not to bind C1q (4–6). There are

two possible interpretations of their data: (i) IgG4 ICs do bind C1q, or (ii) malaria ICs are not as homogeneous with respect to their subclass composition as the authors assume, in other words they contain C1q binding subclasses such as IgG1, as well as non-C1q binding types such as IgG4. If the latter hypothesis is correct then any subsequent conclusions made about the role of individual subclasses to IC mediated CM or SMA must be questionable. Furthermore IgM ICs may also be present in these samples, and given IgM's very high affinity for C1q, this antibody class would presumably block the binding of the ICs being detected. Therefore evaluating circulating ICs, especially of subclasses like IgG4 that are found at very low concentration in plasma, requires even more care than the interpretation of tissue complexes that have been detected in brain capillaries of CM fatalities (7). Presumably, circulating ICs would already be occupied with C1q, in which case, levels seen in the ELISA by Mibei *et al.* would be an under representation? ELISA assays based on binding to C1q do not take account of the varying affinities that ICs of differing subclasses have for C1q; nor of the known ability of monomeric IgG and IgM to bind C1q attached to a plate (4–6). The possibility that monomeric antibodies or IgM containing ICs block binding of IgG ICs were not accounted for in these assays.

Given that every publication examining the relationship between malarial infection and IgG ICs have depended on this single C1q solid-phase assay, whether ICs even exist in malaria infected individual's needs to be reappraised in light of these concerns. However, the work raises a number of important questions concerning the nature of ICs in malaria. For example, do ICs found in CM or SMA even contain *Plasmodium* antigens, and if they do which ones? Given the ease of purifying ICs by size exclusion chromatography and technological advances in high throughput protein analysis, addressing what is actually in an IC from a malaria patient; especially in the context of parasite antigens, immunoglobulin subclasses, and differing disease states, would be a very worthwhile exercise. Neither did their assays take account of allotypic variation within populations for IgG1 (4 allotypes), IgG2 (5 allotypes) and IgG3 (13 allotypes); that may affect C1q binding in these experiments. In truth, although much is known concerning affinities of monomeric IgGs for FcγRs and complement (4–6), very little is known about the relative affinities of multimeric ICs for the same targets, let alone ICs bound to a variety of antigens that may also contain more than one subclass. The ability to generate recombinant human ICs for each subclass, free of contamination with other subclasses, and specific for soluble malaria antigens, for example, the MSP1 or RhopH complexes, would allow their role to be investigated for both CM and SMA in FcR knockout or transgenic models of malaria (8).

Notwithstanding these concerns and problems involved in interpreting correlative analyses, the authors were faced with another dilemma; how could they explain the increased risk from CM and SMA in patients with elevated IgG4 ICs? IgG4 is a poor activator of FcγR mediated pro-inflammatory cytokines, and an even poorer activator of complement (4–6). However, IgG4 is commonly found on the surface of red cells from patients with malaria or autoimmune haemolytic anaemia (9), and a number of studies have found associations between elevated total and parasite-specific IgG4 and increased susceptibility to clinical malaria attacks or severe malaria (10,11). How can these results be explained for IgG4 in the light of such poor effector function? Recent work has shown that IgG4 can undergo dynamic Fab arm exchange *in vivo*, a post-translational modification that leads to anti-inflammatory activity (12). This exchange of Fab arms means that IgG4 molecules lose their ability to cross-link antigen and to form ICs under most conditions, making these results for malaria questionable at best. In this important work, non-cross-linking IgG4 Abs provided protection against pathogenic IgGs in experimental autoimmune myasthenia gravis by interfering with IC formation by other Ab classes. Although these hybrid IgG4s can only bind monovalently, even to repeating antigen, they are capable of cross-linking two different antigens. Thus

patients predisposed to making high IgG4 may simply have worse malaria because the IgG4s are interfering with the more protective cytophilic subclasses, like IgG1 and IgG3.

Polyethylene glycol (PEG) precipitation was used to purify IgE and IgA containing immune-complexes. Unfortunately this method is likely to be even less specific for ICs than the C1q assay. Regrettably, studies have shown that the types of ICs precipitated either by PEG or bound by C1q are very different with respect to their antibody composition and frequently demonstrate inverse correlations between levels of ICs obtained by either assay, despite the fact that both are widely used clinically (13).

Nonetheless, the finding that IgE containing ICs also correlate with CM is on the face of it easier to explain, since cross-linking of the low affinity FcεRII (CD23) can lead to the production of tumour-necrosis factor (TNF) and other proinflammatory cytokines (such as IL-1 and IL-6) from monocytes (14,15). However, once again caution is required in correlating plasma IgE concentrations with pathology, since most IgE *in vivo* is sequestered away in tissues by nature of its high affinity for FcεRI found on tissue tropic cells including mast cells and basophils. Much of the published work in malaria suggests that elevated plasma IgE levels correlate with protection (16–19), although the finding that IgE deposits in brain capillaries of CM fatalities does support a role of IgE in the pathogenesis of CM (4). Recombinant human IgE molecules directed to infected erythrocytes or soluble plasma antigens may resolve some of these controversies using mouse models transgenic for human FcεRs, as have been used recently with recombinant human IgG1 to demonstrate the importance of human FcγRI in controlling malaria (7).

If ICs do make an important contribution to pathology in malaria, then agents that block the interaction of ICs with cell surface FcRs may represent a novel approach for treating CM and SMA, as has recently been demonstrated using mouse models of arthritis with soluble FcR based reagents (20,21).

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