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Commentary

Getting oriented with antibodies

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Neisseria meningitidis is a Gram-negative bacterium capable of causing deadly invasive disease. Two recently developed vaccines against *N. meningitidis* serogroup B include recombinant factor H binding protein (fHbp), a surface protein that meningococci use to evade the host immune system. Many anti-fHbp monoclonal antibodies (mAbs) produced against fHbp fail to trigger complement-mediated bacteriolysis when used alone *in vitro*, but are highly synergistic and bactericidal when used in combination. This opened the door to defining the structural basis by which mAbs activate complement synergistically when binding to different epitopes on the same antigen, a story that is told by Malito et al. in a recent issue of the *Biochemical Journal.* Using two separate crystal structures of fHbp bound to Fabs from synergistic mAbs, they were able to model the structure of both full length antibodies bound simultaneously to fHbp. This revealed that the bound antibodies orient their Fc domains 115–130 Å apart, a distance that is compatible with multivalent C1q binding. The need for a precise orientation of Fc domains in order to efficiently activate effector functions is an emerging theme across multiple fields, and its implications could have broad impacts on vaccinology and immunotherapy.

Neisseria meningitidis is a Gram-negative bacterium that benignly colonizes the nasopharynx of many young adults. However, in rare cases, it becomes invasive, gaining access to the bloodstream and cerebrospinal fluid, where it is capable of causing sepsis and meningitis that can be rapidly fatal [1]. A critical part of host defense against meningococci relies on the complement system and the formation of membrane attack complexes (MACs) that lyse the bacteria. Adaptively, *N. meningitidis* has evolved many ways to protect itself from the complement system. While not all details have been elucidated, mechanisms involve the production of a capsule, lipooligosaccharide, and surface proteins that bind soluble complement regulators [2]. One of these surface proteins is known as factor H binding protein (fHbp). As its name implies, fHbp binds to human factor H, a protein that circulates in the plasma, and prevents tissue damage by down-regulating the complement cascade. While fHbp protects meningococci against host innate immunity, it also serves as an attractive target for vaccine development because it is surface-expressed and elicits a broad bactericidal response. It is a particularly important target in *N. meningitidis* serogroup B, where vaccines against the capsular polysaccharide have failed [3]. While capsular polysaccharide vaccines have been developed successfully against most *N. meningitidis* serogroups, they were poorly immunogenic in serogroup B, likely due to the structural similarities between the sialic acid in the capsule and on host cells. New serogroup B vaccines include recombinant fHbp protein, which is highly immunogenic. However, many anti-fHbp monoclonal antibodies (mAbs) failed to trigger complement-mediated bacteriolysis when used alone *in vitro*, but were highly synergistic and bactericidal when used in combination [4]. This opened the door to defining the structural basis by which mAbs activate complement synergistically when binding to different epitopes on the same antigen, a story that is told by Malito et al. in a recent issue of the *Biochemical Journal* [5].

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Classically, IgM and some IgG antibodies activate complement by binding antigens with their Fab domains and presenting their Fc domains to the protein complex C1, which is formed by C1q and a heterotetramer of serine proteases C1r and C1s. C1q acts as the recognition subunit, with six globular

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heads, each capable of binding an Fc homodimer. When binding occurs, a conformational change in C1q causes activation of C1r, which then activates C1s, setting off the complement cascade [6]. To prevent non-specific complement activation, C1q has very low affinity for IgG Fc [7] and only activates C1r efficiently when multiple Fc domains bind. However, antigen-driven antibody clustering allows C1q to simultaneously engage multiple Fc domains, and this highly avid complex formation leads to activation of the complement cascade, and eventually generation of opsonins, anaphylatoxins, chemotactic agents, and MACs [8]. While the importance of Fc binding by C1q has long been known, it was not until recently that evidence began to emerge that a precise orientation of Fc domains is critical in order for C1q to bind productively to IgG and induce complement-dependent cytotoxicity (CDC). IgG has been shown to assemble into hexameric oligomers at the cell surface, which presents an ideal binding platform for the six globular heads of C1q; promoting or disrupting IgG hexamer formation enhances or inhibits complement activation, respectively [9].

Building on this theme, Malito et al. sought to characterize the structural basis by which two mAbs synergistically activate complement when binding to the same antigen. They used JAR5 and 12C1, two murine IgG2b anti-fHbp monoclonal antibodies that arose due to interest in *N. meningitidis* serogroup B vaccines. 12C1 was a natural choice because a crystal structure had already been solved between its Fab and fHbp. They chose JAR5 because it was known to act synergistically with other anti-fHbp mAbs [4]. As the 12C1 and JAR5 epitopes had been mapped to nonoverlapping surfaces of fHbp, it was likely that they could both bind simultaneously; indeed, Malito et al. confirmed this to be the case. After establishing that JAR5 and 12C1 are ineffective at activating human complement individually, but do so efficiently in combination, they sought to define this molecular mechanism of biological synergy by determining the high-resolution X-ray crystal structure of fHbp in complex with the Fab of JAR5. Now, with crystal structures of both 12C1 and JAR5 Fabs bound separately to fHbp, they were able to model the full length antibodies bound simultaneously to fHbp. They discovered that the antibodies are capable of assembling in a way that separates their Fc domains by 115–130 Å (range here is due to the flexibility of the hinge regions that link Fab and Fc antibodies). Crucially, this distance between Fc domains of the JAR5 and 12C1 antibodies is similar to that observed when IgG forms hexamers during crystallization [10]. These hexamers resemble those that form on a eukaryotic cell surface, which are compatible with multivalent C1q binding [9]. Using anti-fHbp antibodies in combination has two potential benefits. First, they bury more fHbp surface area collectively, hindering the ability of meningococci to bind factor H and evade the immune system; and second, together they facilitate multivalent C1q binding and complement activation. Having antibodies that work synergistically is especially important in the context of antigenic scarcity, where the opportunities for antibody binding are otherwise too limited to effectively engage C1q binding and activate CDC [11].

Not all antibodies function equally. Some are capable of inducing effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) or CDC, while others are not. Proper orientation of the Fc domain upon antigen binding could explain why. The structural evidence presented by Malito et al. echoes discoveries that have been made recently in other fields regarding the requirements for Fc domains to be precisely positioned upon antigen binding in order to activate effector functions. For example, antibodies that induce ADCC against HIV-infected cells were recently found to rely on a precise binding mechanism that cross-links antigens while positioning the CH2 domain for effective Fc receptor interactions [12].

More broadly, the ability of a vaccine to induce non-neutralizing antibodies that offer protection through ADCC and/or CDC is a viable alternative to the traditional goal of inducing broadly neutralizing antibodies, as illustrated by research on HIV-1, HSV-2, and Influenza A [12–14]. This is particularly attractive in cases where antigenic variability makes it difficult to create neutralizing antibodies, as non-neutralizing antibodies could elicit effector functions by binding to more conserved regions. However, it has been difficult to predict which epitopes will produce antibodies that successfully elicit effector functions. Now, one might consider structural constraints when designing such vaccines. For example, by mapping two epitopes a certain distance apart on the same antigen, it might be possible to rationally design multivalent vaccines that elicit CDC. However, one important caveat is that even if two epitopes exist on one antigen, antibodies must still bind with the appropriate orientation for their Fc domains to simultaneously engage C1q or FcR — something that remains a challenge to predict.

Malito et al. advance our understanding of how antibodies orient their Fc domains to work synergistically. In doing so, they help to unravel a story which is applicable to fields outside of vaccinology. Notably, their work has the potential to influence immunotherapy, a field in which antibodies have revolutionized the treatment of cancer and autoimmune disorders. For example, rituximab is an anti-CD20 IgG mAb that has been

approved for use in the treatment of leukemia, lymphoma, and rheumatoid arthritis. It works, in part, by inducing CDC that lyses B cells [15]. In light of recent discoveries concerning the orientation of IgG Fc domains and its relation to inducing ADCC and CDC effector functions, it might be possible to develop novel antibodies that bind CD20 and work synergistically with rituximab. But this is not all — following the discovery that some monovalent antibodies are more effective than their bivalent counterparts for reasons related to Fc orientations upon binding [9], further work could determine if any existing monoclonal antibody therapies would benefit from structural alterations. Whether this would involve creating bispecific antibodies with one functionless arm, or alterations that modulate flexibility of the hinge region, one thing is certain: mind the Fc!

Abbreviations

ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; Fabs, fragments antigen binding; FcR, Fc receptor; fHbp, factor H binding protein; HIV-1, human immunodeficiency virus type 1; HSV-2, herpes simplex virus type 2; mAb, monoclonal antibody; MAC, membrane attack complex.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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