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INSIGHTS

Antibody barriers to going viral

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Antibody neutralization of a virus in vitro is often associated with protection against viral exposure in vivo, but the mechanisms operational in vivo are often unclear. By investigating a large number of antibodies, Earnest et al. (https://doi.org/10.1084/jem.20190736) show the importance of antibody effector function in neutralizing antibody protection against an emerging alphavirus in a mouse model.

Antibodies (Abs) are important in immune defense against a variety of foreign agents, including bacteria, viruses, parasites, and toxins. In a number of instances, e.g., defense against bacteria, Abs act as adaptor molecules linking their recognition (the Fab arms of the Ab molecule) with their elimination by functions such as complement, phagocytosis, and Ab-dependent cellular cytotoxicity (the Fc region). In some cases, notably for viruses and toxins, agent inactivation can occur in the absence of Fc functions in vitro. The classic case is virus neutralization, which is typically assessed in vitro as the ability of an Ab to prevent viral infection of a target cell in the absence of other factors (Klasse, 2014). Indeed, neutralization is such a fundamental attribute that neutralizing Abs (nAbs) are often measured as the best correlates of protection from viral infection. The ability of $F(ab')_2$ fragments or effector function–crippled Abs to protect implies that neutralization can be sufficient in vivo, in at least some instances (Parren and Burton, 2001; Hessell et al., 2007). In one case, direct evidence suggests the critical nature of neutralization in vivo, but with notable differences from in vitro observations (Day et al., 2010). However, overall it is not clear if neutralization per se is universally the sole, or even dominant, mechanism of antiviral activity of neutralizing Abs (nAbs) in vivo, in either protective or therapeutic modes. Neutralization generally requires that an Ab interacts with functional molecules on the viral surface and, as such, can be interpreted as giving a readout of binding to such molecules on virus and/or infected cells, which are subsequently cleared in vivo by other mechanisms. Indeed, animal model experiments going back to the 1980s convincingly showed a critical dependence of protection for a number of viral infections on Ab effector function, particularly interaction with Fc receptor–bearing cells (Parren and Burton, 2001). In recent years, there has been a resurgence of interest in Fc-dependent mechanisms of antiviral activity of nAbs in vivo (Lu et al., 2018), not least because the understanding of these mechanisms may assist in vaccine design (Bournazos and Ravetch, 2017). In this issue of *JEM*, Earnest et al. describe one of the most thorough studies to date with some interesting surprises.

The virus investigated is an emerging mosquito-transmitted alphavirus, Mayaro virus (MAYV), which has caused outbreaks of fever and arthritis in tropical South and Central America. Earnest et al. (2019) generated 18 monoclonal nAbs directed to the envelope glycoproteins E1 and E2 on the viral surface by immunization of mice. The nAbs were highly potent: 17 of 18 nAbs neutralized virus at <100 ng/ml (EC50 values), with 11 of those neutralizing at <10 ng/ml. However, even when given prophylactically to mice at doses that should produce serum Ab at several orders of magnitude higher than the neutralization EC50, only 9 of 18 nAbs protected mice against lethal MAYV challenge, and only two prevented mortality completely. MAYV is not lethal for immunocompetent mice, so the authors developed an anti-IFNAR1 mAb-treated mouse model in which viral infection is uniformly fatal. The mAb blocks type 1 interferon signaling, reduces protection due to innate immunity, and renders infection uniformly fatal in the absence of Ab. For a number of viruses, serum nAb concentrations in the range of 100–1,000× EC_{50}s measured in vitro provide sterilizing immunity from viral challenge (Parren and Burton, 2001), and therefore it was expected that protection might have been more effective than observed here.

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The authors then noted that protection is strongly associated with nAb isotype; all the protective Abs were of the IgG2a subclass, whereas IgG1 Abs of similar potency as the IgG2a Abs failed to protect. The importance of isotype was confirmed for the two most protective nAbs by isotype-switching them from IgG2a to IgG1 when protective activity was notably reduced. This result implies the importance of nAb effector function for protection, since IgG2a generally mediates Fc effector functions well, whereas IgG1 is poor. In agreement, an Fc substitution that greatly reduces effector activity decreased

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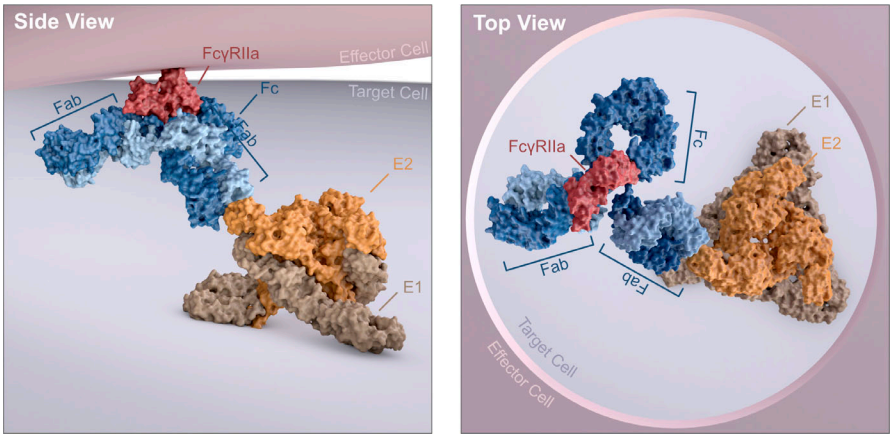

Ab linking infected cell and effector cell: interactions in a tight space. Earnest et al. (2019) show that nAb protection against MAYV requires Ab interaction with an effector function, most likely an Fc receptor–bearing cell. The type of interaction probably involved is modeled here using available structures and known interaction sites. The alphavirus Chikungunya E1 (pale orange) and E2 (orange) spike proteins (PDB 6NK5), full length human IgG (IgG b12, PDB 1HZH, bright and dark blue), and Fc-bound human FcγRIIa (PDB 3RY6, red) structures are modeled together to reveal the tight space in which the three-way interaction occurs. To avoid structural clashes, the immunoglobulin Fab arms are bent away from the bound Fcγ receptor, and the Fc portion is lain flat on the surface of the Fc-bearing cell (pink) in order to allow for insertion of the C-terminal end of the FcγR into the cell membrane. The side view shows the three molecules involved, but the Fc is not well seen; this is clearer in the top view. Modeling and 3D rendering by Christina Corbaci and Lars Hangartner.

protective efficacy of the two nAbs. Furthermore, if two nonprotective mouse IgG1 nAbs were isotype-switched to human IgG1, which does mediate effector functions here across species, then there were significant gains in terms of protective activity.

What is the effector function involved? Earnest et al. (2019) showed that several of the protective nAbs promoted neutrophil and monocyte-dependent phagocytosis of E2-coated beads, as might be expected. Of note, the protective activities of IgG2a nAbs with similar neutralizing titers varied widely, suggesting a potentially rich area for further research to understand the underlying mechanisms involved.

Although nAbs are most often considered in the context of protection, i.e., Abs given or induced before infection, recent studies have shown that highly potent Abs ("super-Abs"; Walker and Burton, 2018) can have dramatic therapeutic effects, i.e., Abs given after infection. Cases that spring to mind include Abs to Ebola virus that prevent mortality in monkeys due to Ebola virus infection after symptoms have appeared (Saphire et al., 2018) and Abs to HIV in monkeys and man that promote drug-free virus control when given during chronic infection (Caskey et al., 2019). In a post-exposure (nAb given 1 d after infection) treatment modality, Earnest et al. (2019) found that only the two Abs that were described above as providing complete protection from challenge were effective. A combination of these two Abs was most effective. For nAbs given only 1 d before death was expected, the combination was able to save half the animals. The ability of nAbs given before infection to limit MAYV-induced musculoskeletal disease in immunocompetent mice was also studied. All the IgG2a-protective nAbs had some beneficial effects with a complex pattern of responses observed.

For such a comprehensive and detailed study as this, one would like to draw as many general conclusions as possible with regard to nAbs and viruses. In particular, what can the study teach us about desirable features in passive nAbs and nAbs induced through vaccination in humans—first, against MAYV; second, against alphaviruses as a class; and third, more broadly against viruses in general? The clear suggestion from the mouse study is that nAb Fc effector function is important in protection against MAYV. As with any animal model, there are important caveats. The mouse model is a highly artificial one, and it is possible that protection from MAYV in humans may be less demanding and that Fc effector function would be less critical. In terms of alphaviruses generally, it is intriguing that nonneutralizing Abs have been shown to protect against Sindbis and Semliki Forest virus in mouse models (Schmaljohn et al., 1982; Wust et al., 1987), which clearly suggests the importance of Fc effector function in protection in those cases. It may be that Fc effector function has a particularly strong role to play in defense from alphaviruses, although it is not immediately apparent why this would be the case. In terms of viruses generally, as above, there is ample evidence in many cases of the significance of Fc effector function for protection in animal models, albeit that many of these model studies may offer a more stringent challenge than typical human exposure and therefore the conclusions should be treated with caution. It is also worth emphasizing that, although we strive to derive general rules for Ab behavior, it is likely that every Ab–virus combination has, to some degree, its own characteristics. Thus, for example, two Abs, nominally to the same epitope, may well have somewhat different angles of approach to their target and may present a somewhat different array of Fcs to an effector cell with potential differences in effector function. For example, the importance of Ab arrays is suggested to be critical in complement triggering (Diebolder et al., 2014; Strasser et al., 2019). Furthermore, this discussion refers to mAbs. Polyclonal Abs, as induced by vaccination, may exhibit more complex behavior and be more difficult to cover with general rules.

But do the caveats discussed matter greatly in considering passive Abs and/or vaccine design? Shouldn't one attempt to maximize Fc effector function in any case, other than possibly instances where Ab-dependent enhancement may be a concern? The answer may well be "yes," but will likely require careful evaluation in humans before the answer can be given with any certainty. As to how effector function can be maximized, for passive Abs this may be relatively straightforwardly achieved by Ab engineering to enhance binding to Fc receptors and/or complement (Saunders, 2019) once more is understood about protection mechanisms in each instance. The cancer field has pioneered Abs with enhanced effector function. For vaccines, enhancement may

depend upon immunization strategies. In the paper, the authors argue that the use of protein scaffolds as immunogens may be advantageous.

In conclusion, Earnest et al. (2019) describe a thorough study of Ab protection against an alphavirus in a mouse model (note that several different aspects of the study are not covered in this overview). It will be interesting to see the next stages of the work in terms of understanding the molecular origins of the observations made. Indeed, the exploration of mechanisms of protection, particularly in humans, may be critical in the optimal use of passive Abs and vaccine design against pathogens in general in the future.

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