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MINIREVIEW

Antibody and Virus: Binding and Neutralization

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During infection with an enveloped virus, antibodies are elicited to envelope proteins. Some of these antibodies bind to envelope spikes on the virion, some bind to nonvirion forms of the envelope ("viral debris"), and some bind to both. The relative amounts of different antibody types elicited varies from virus to virus. There is general agreement that only anti-envelope antibodies that bind to the envelope spike on the virion will be neutralizing or show antiviral activity. However, there is less agreement about whether all antibodies that bind to the envelope spike will neutralize virus, i.e., are there antibodies that bind well to envelope spikes but do not neutralize virus? The question is more than academic. If all antibodies that bind neutralize, then the envelope spike has the requisite antigenic properties of an ideal vaccine candidate. On the other hand, if the envelope spike can induce nonneutralizing antibodies, then it may not be an optimal antigen. In particular, the induction of nonneutralizing antibodies that can bind to envelope spikes and inhibit the binding of neutralizing antibodies would be undesirable.

Our experience, most particularly in studies on human monoclonal antibodies to HIV-1, RSV, and Ebola virus, has been that there is an excellent correlation between binding to envelope spikes and neutralization, which in these studies was measured as binding to infected cells (i.e., cell-associated virus). We have not encountered monoclonal antibodies that bind well to envelope spikes on infected cells but do not neutralize virus. Furthermore, we have generally seen a close correlation between half-maximal antibody binding and antibody concentration required to give 50% neutralization suggesting that neutralization is directly related to occupancy of sites on the virion. For HIV-1, neutralization is incremental with increasing antibody occupancy, irrespective of the epitope recognized, leading to increased inhibition of infectivity. This is consistent with a multihit neutralization model rather than with a single-hit model or models which predict a neutralization threshold. We have viewed antibody neutralization as a process in which virions become coated with antibody and are thereby sterically inhibited from attachment to the target cell or fusion with the membrane of the target cell. The antibody molecule is typically similar in size to an envelope spike, e.g., for HIV-1 the extracellular trimer has a molecular weight of about 450 kDa, similar to that of three IgG molecules, making steric obstruction a likely scenario. In this view, it is relatively difficult to imagine a virion coated with nonneutralizing antibodies.

However, in contrast to these observations and this discussion, there is a strong tradition in virology of "binding (i.e., to the virion) but nonneutralizing" antibodies. We have wondered why we have not found evidence for such antibodies. Of course this could simply reflect their absence in the systems that we have studied. We question the evidence for nonneutralizing antibodies that bind well to the virus.

Early studies described a "nonneutralizable" fraction of virus which persisted even at high antibody concentrations. Addition of anti-antibody could reduce infectivity of this fraction. It seems that virus aggregation may have been responsible for this phenomenon. We also note that nonneutralizable fractions have been described for hepatitis A and hepatitis C as a result of virus association with lipids or lipoproteins.

Undoubtedly, some descriptions of binding, nonneutralizing antibodies arose because of a failure to appreciate that antibodies that bind to isolated envelope molecules do not necessarily, and very often do not, bind to envelope spikes. A classic example is HIV-1 where many antibodies have been described which bind with high affinity to monomeric gp120 or unprocessed gp160, very few of these however showing substantial affinity for envelope spikes.

Antibody-mediated enhancement of infection is a phenomenon that at first glance illustrates the existence of nonneutralizing antibodies since the antibodies involved must bind to virions. However, a key observation here is that enhancement, when described, appears to occur for neutralizing antibodies at subneutralizing concentra-

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tions. Neutralization and enhancement of infection appear to be two different biological outcomes of the interaction of an antibody with virus at different levels of occupancy. Enhancement in particular is very sensitive to the target cell. A classical example is enhancement of dengue virus infection which is dependent on the interaction between virion-bound antibody and Fc receptors expressed on the target cell. In typical assays, neutralization is observed at relatively high concentrations, whereas enhancement is observed at lower concentrations. Neutralization of dengue virus therefore also appears to result from relatively high levels of antibody occupancy following multihit kinetics, whereas enhancement may occur at very low levels of occupancy. In Fc-dependent enhancement, Fc receptor-mediated endocytosis of virion immune complexes may lead to internalization of virus and infection. An alternative is that binding to Fc receptors brings the virion and target cells closer together permitting interaction of the envelope spike and virus receptor at low antibody coating of the virion. At higher coating this interaction is inhibited. The latter scenario appears more consistent with enhancement occurring only at subneutralizing antibody concentrations. In some cases, such as HIV-1, the enhancement does not require the Fc part of the antibody molecule. It is suggested that the low-level coating may trigger conformational changes in the envelope that, for example, favor fusion or such coating may nonspecifically reduce repulsion between the virion and target cell surfaces.

Overall, therefore, enhancement is not, in our view, a demonstration of binding but nonneutralizing antibodies as the terminology is classically used. The phenomenon does suggest however a potential problem for a vaccine that induces low levels of neutralizing antibodies.

A number of elegant and detailed studies have been carried out by Flamand and colleagues on antibody neutralization of rabies virus. These studies support the general notion that neutralization is the result of coating the virion with a large number of antibody molecules. However, three monoclonal antibodies were identified which bound to the virus without neutralization. Two of these were subsequently shown to bind to a minor population of envelope spikes in an altered acidic conformation of the envelope. Coating of the virion via this altered spike was too low to permit neutralization. If the virion was maintained at lower pH to convert most spikes to the acidic form, then more antibody bound and the virus was neutralized. One monoclonal antibody, which effectively neutralized wild-type virus, appeared to saturate the envelope spikes of a neutralization escape variant but did not neutralize it. The antibody-coated virus was still able to attach to target cells. The authors considered it unlikely that this attachment was occurring through the envelope because of the thickness of the antibody shell coating the virus. This case appears to constitute a bona fide example of effective antibody coating of virion without neutralization.

Problems do exist in attempts to measure antibody binding to virions that may complicate data interpretation. In many virus preparations, infectious particle:total particle ratios are low introducing an element of uncertainty with regard to envelope homogeneity. Measurement of binding to the envelope at the surface of infected cells may therefore be preferable. Envelope spikes such as those of HIV-1 can shed protein providing opportunities for artifactual observation of nonneutralizing but virus binding antibodies. We believe that anti-HIV gp41 antibodies that bind but do not neutralize probably fall into this category, i.e., they bind to inactive envelope spikes in which gp41 is exposed following gp120 shedding. A second type of artifact in HIV-1 is provided by antibody binding to shed gp120 in interaction with CD4; nonneutralizing antibodies apparently bind to the cells but this does not reflect binding to envelope spikes.

In summary, we remain of the opinion that antibody neutralization and binding to envelope spikes are very closely related. A very few convincing instances of nonneutralizing but spike binding antibodies have been reported. They merit further attention.

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