Future Opportunities for Passive Immunity Against Viral Diseases

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(See the article by Schepens et al, on pages 1692–701.)

Antibodies are key effector molecules for protection against viral infections. The protection afforded by immunization with licensed antiviral vaccines is mediated by vaccine-induced antibody in most cases. Passively administered antibodies have been shown to be effective against diseases caused by a wide variety of RNA and DNA viruses, and polyclonal immunoglobulin products have been licensed for clinical use for cytomegalovirus, hepatitis A and B viruses, measles virus, poliovirus, rabies virus, respiratory syncytial virus, and varicella zoster virus. Thus, it is surprising that of the 29 licensed monoclonal antibody (mAb) products developed over the last 25 years, only 1 is for use against a microbial pathogen, respiratory syncytial virus (RSV). The rest are for autoimmune diseases, malignancy, asthma, or angioedema.

Palivizumab is a humanized immunoglobulin G1 (IgG1) mAb derived from the original murine mAb 1129 [1], and is licensed for the prophylactic treatment of premature infants to prevent severe disease from RSV [2]. Palivizumab and its higher-affinity derivative, motavizumab, recognize an epitope in the RSV fusion (F) glycoprotein (antigenic site II) and the atomic structure of the interaction has been characterized [3]. In this issue of the Journal of Infectious Diseases, Schepens et al. have reported the development of Nanobodies (Ablynx) specific for the palivizumab epitope and have evaluated their potency in vivo in a murine model [4]. Nanobodies are proteins representing the variable domain of the heavy chain from antibodies produced by members of the family Camelidae (camels, llamas, and alpacas). Cartilaginous fish (eg, sharks, skates, and rays) and camelids have uniquely developed heavy-chain-only immunoglobulin molecules that recognize and bind antigenic sites with just the single variable domain at the tip of the heavy chain. The basis for this adaptation is unknown, but there is evidence of convergent evolution [5]. The variable domains tend to have relatively long antigen-binding loops and because the light chain present in human antibodies is not in the way, they are ideally suited for reaching epitopes residing in clefts or pockets. For example, llama-derived heavy-chain variable domains (VHH) have been discovered that can reach into the CD4 binding pocket of human immunodeficiency virus (HIV) gp120, resulting in broad neutralizing activity [6]. Heavy-chain-only antibodies tend to have more charged and polar residues in the framework 2 region of the variable domain, which in classic human immunoglobulins, has highly conserved hydrophobic sequences that are adjacent to the light chain. The VHH also has a relatively high frequency of cysteine residues that allow intradomain disulfide bonds, and together with the polar interactions between loops help to stabilize the antigen-binding sites, perhaps to compensate for the lost stabilizing effect of a light chain [5, 6].

The investigators immunized llamas with a trimeric transmembrane-deleted form of the F glycoprotein, and then cloned VHH sequences into a phagemid vector. The resulting phage library was screened for binding to F, and sequences were identified that competed for binding with palivizumab. Microneutralization assays against the RSV Long strain (a prototypic subtype A laboratory-adapted isolate) showed that the monovalent VHH-designated RSV-D3 is about 3-fold more potent than the palivizumab antigen-binding fragment (Fab). Interestingly, a bivalent version of RSV-D3 made with Gly-Ser linkers is about 40-fold more potent than the bivalent palivizumab mAb. One of the important characteristics of palivizumab is that it is equally potent against subtype B RSV strains. Surprisingly, the bivalent RSV-D3 was about 600-fold less potent against the RSV B1 strain than it was against the subtype A virus [7]. The reason for the difference in cross-reactivity...
is intriguing because neutralization escape mutations for palivizumab also escape RSV-D3, suggesting similar contact residues. It may be related to the size of the interaction area, or it is possible that the VHH is a relatively rigid, preorganized structure that gives it higher affinity, but makes it less flexible for adjusting to minor variations in epitope structure. Defining the crystal structure of the interaction may be informative for understanding cross-neutralization and other fundamental aspects of viral neutralization. It may also provide insight as to why the structurally conserved, scaffold palivizumab epitope can elicit antibodies that bind F, but do not neutralize RSV [8].

The RSV-D3 bivalent Nanobody neutralizes by inhibiting fusion and not by blocking attachment. This is also true for palivizumab and motavizumab, so it is not surprising. The epitope appears to be present throughout the fusion process, based on modeling of the prefusion F trimer and structure of the postfusion trimer [9]. The assumption based on structural analysis is that palivizumab and motavizumab inhibit fusion by binding a prehairpin fusion intermediate structure, thereby interfering with formation of the final 6-helix bundle that pulls the virus and target cell membranes together [9]. While the motavizumab Fab appears to clash with the adjacent oligomer in the prefusion trimer model [3], the small size of the VHH may provide an advantage of Nanobodies over mAb because of the potential for binding F in the prefusion state. This would be another reason to evaluate the crystal structure of the RSV-D3 bound to F or to the palivizumab epitope.

Size matters, not just for accessing cryptic epitopes but also for biodistribution and pharmacokinetics. Schepens and colleagues treated mice with RSV-D3 Nanobodies both before and after RSV challenge. Nasal administration was chosen because there is relatively rapid renal clearance of the monovalent (15 kilodaltons [Kda]) and bivalent (30 Kda) VHHs when given parenterally. They showed there is bioactive VHH in lung homogenates up to 72 hours after intranasal delivery. When anesthetized mice received 100 µg in 50 µL intranasally 4 or 24 hours prior to RSV challenge, bivalent VHH-treated mice had a similar outcome as palivizumab-treated mice, with no detectable virus in lung at 3 and 5 days postchallenge and no weight loss. Delivered at 4 hours prechallenge, a dose of 12 µg partially suppressed virus titer. As in vitro, bivalent VHHs were more potent in vivo than monovalent VHHs. Therefore, size matters, but so does avidity, dose, delivery route, and timing. For passively administered antibodies or Nanobodies, it is important to determine the tissue-specific half-life and what factors may affect the persistence of active product when given by a particular route. For example, if given topically, what is the susceptibility of Nanobodies to proteases found in mucosal secretions?

Using antibodies to treat viral infections has historically been less successful than using antibodies prophylactically. For example, treatment of RSV-infected patients with RSV immune globulin or palivizumab has not shown clinical benefit. Nevertheless, there are examples of success, including the finding that treatment of patients infected with the Junin arenavirus (Argentine hemorrhagic fever) with immune serum reduces mortality when given up to 8 days after the onset of symptoms [10]. Also, patients with antibody deficiencies who are infected with enteroviruses have been shown to benefit from immunoglobulin treatment [11].

In the current study, Schepens et al. treated mice 4 hours, 24 hours, or 48 hours postchallenge and then evaluated RSV titers and RNA in lung homogenates at day 5. While inflammatory cell counts in BAL and RSV titers appear to be partially reduced, there was little reduction in viral RNA, and some of the plaque reduction may be confounded by in vitro neutralization from residual RSV-D3 in lung. The requirements for antibody-mediated functions to effectively treat a virus infection may extend beyond those needed to prevent infection. Activities such as antibody-dependent cellular cytotoxicity, antibody-dependent cell-mediated virus inhibition, or antibody-mediated complement-dependent cytolysis may be useful for clearing virus-infected cells, but require fragment crystallizable region (Fc region)—mediated interactions with cellular effectors. Nanobodies do not support these functions, and appear to neutralize primarily by having strong binding affinity to antigenic sites that can interfere with viral entry. Another challenge for treatment of self-limited virus infections in general is that it is difficult to recognize clinical manifestations and diagnose infection soon enough for antiviral therapy to make a difference.

Notes

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