Virus versus antibody

PM Colman

Variation in the proteins produced by animal viruses allows the virus to reinfect the same host, but is constrained by the requirement to maintain critical viral functions, in particular engagement with cellular receptors. The fundamental characteristics of proteins and their interactions with each other suggest that this may not be so much of a constraint at all.

Address: Biomolecular Research Institute, 343 Royal Parade, Parkville, Victoria 3052, Australia.

E-mail: peter.colman@mel.dbe.csiro.au

Structure 15 May 1997, 5:591–593
http://biomednet.com/elecref/0969212600500591

Antigenic variation permits a virus to reinfect its host. Structural studies of the influenza virus and of rhinoviruses reveal a separation between antigenically variable and functionally conserved sites. If antibodies were directed at the conserved sites of the virus, reinfection could not occur. So, how do viruses conceal functionally critical structural elements from surveillance by the host's antibodies?

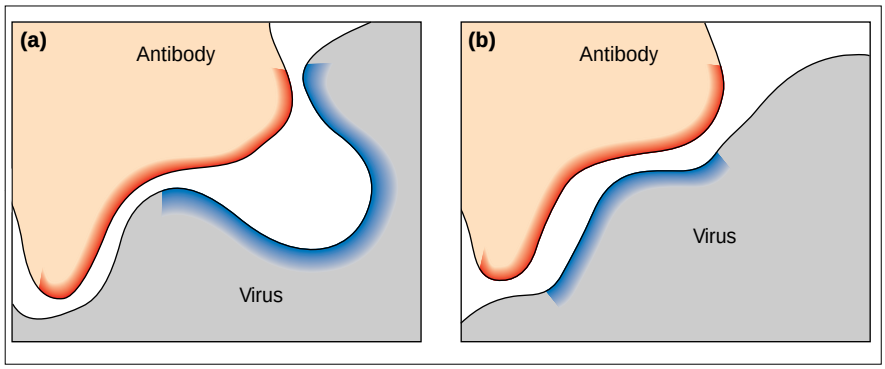
Antibodies and viral proteins

A structural solution to this problem was proposed in 1983, when the structure of influenza virus neuraminidase, an antigen embedded in the viral envelope, was first described [1]. Three possible explanations were proposed that might allow for the preservation of the functionally important catalytic centre in the face of immune selection pressure [1]. Firstly, it was proposed that the catalytic site is inaccessible to antibodies (Fig. 1a); this proposal was subsequently popularized as the 'canyon hypothesis' [2]. It stemmed from the observation that the neuraminidase active site, like most enzyme centres, is an invaginated structure. Antibody penetration of that site seems unlikely, as it did for the canyon-like structure of rhinoviruses. The second proposal suggested that the site is accessible to antibodies, but only in such a way that catalytically nonessential amino acids also form part of the antigen–antibody complex (Fig. 1b). The central idea here is that the 'antibody footprint' is larger than the functional site on the virus, so it must therefore extend to include functionally unimportant amino acids on the virus. Because a single amino acid substitution can effectively abolish an antibody–antigen interaction, mutations outside the functional site allow the virus to escape from an antibody whilst preserving viral function. At the time these proposals were made [1], although the structures of antibodies in complex with macromolecular antigens were unknown, the likely size of the interacting surface could be estimated from the known structure of the complementarity-determining regions of an antibody — the region of amino-acid sequence that is hypervariable and forms part of the antigen-binding site. This area was found to be of the order of 700 $Å^2$, a figure reminiscent of other protein–protein interactions but larger than the active sites of enzymes. The first high-resolution structural studies of antibodies in complex with proteins (lysozyme [3] and influenza virus neuraminidase [4,5]) substantiated this estimate, supporting the view that the size of the interacting surface — the antibody footprint — is a characteristic weakness of the antibody system that viruses might exploit. The third proposal was that the catalytic site is not antigenic; at the time there existed the possibility that the viral enzymes had identical active-site structures to those of host enzymes, and this might have rendered the catalytic site somehow unrecognizable by antibody.

The canyon hypothesis [2] implicated an invaginated surface on rhinoviruses in receptor binding, and this

Figure 1

Antibody–virus interactions. **(a)** Schematic showing an antibody unable to penetrate an invaginated surface structure on a viral protein. The functionally important (antigenically invariant) surface on the viral protein is indicated (blue), as is the antigen-binding surface of the antibody (red). **(b)** Schematic showing an antibody interacting with all of the conserved elements of a viral protein, but also overlapping with functionally unimportant amino acids on the viral protein. Colouring as in (a).



aspect of the hypothesis has been substantiated by electron microscope images [6] of rhinovirus in complex with its cognate cellular receptor [6]. However, it now emerges that antibodies can also bind in the canyon [7], so the issue of antibody surveillance in this case has shifted back to the antibody footprint argument [7]. A structural description of an influenza haemagglutinin–antibody complex has also relied upon that argument to rationalize the preservation of the receptor-binding sites of influenza virus [8].

Although the presence of an enzyme activity on the surface of a virus is uncommon (but well characterized for orthomyxoviruses and paramyxoviruses), all viruses have some form of surface binding site for cellular receptors. These receptors range from simple sugars (e.g. sialic acid — in the context of its linkage stereochemistry — in the case of influenza virus) to macromolecules (e.g. ICAM-1 for rhinoviruses, or CD4 for HIV). In the latter cases, where protein–protein interactions are involved, it is plausible to consider that the receptor footprint on the virus might be as large as, or larger than, an antibody footprint.

Are viruses restricted in their design so that functionally important structural elements are either smaller than antibody-binding sites or sterically inaccessible to antibodies? Probably not. Three lines of evidence, taken together, suggest a mechanism for viral survival even in cases, for example, where the receptor-binding region on the virus is both larger than an antibody footprint and highly accessible to antibody.

Monoclonal variants

The first line of evidence is from *in vitro* antibody-selected viral variants. These variants show that subtle stereochemical alterations (e.g. serine to alanine) within the antibody-binding site can allow the virus to escape neutralization [9]. On the other hand, quite dramatic amino acid substitutions (e.g. isoleucine to arginine) within the antibody-binding site are sometimes tolerated by antibodies [9,10]. The ability of the antibody to bind where there is an Ile→Arg substitution on the antigen, depends on the structural plasticity in the antibody–antigen interface. In this case in particular, not only does an antibody residue shift by 1.3 Å with respect to its position in the wild-type complex, but also the substituted arginyl residue on the antigen is shifted by nearly 3 Å from its position in the uncomplexed mutant [10]. The effect of any given amino acid substitution within an interface depends on the structural context of the substitution. There are obvious parallels in the need to consider structure in evaluating the effects of mutation both on protein–protein interactions and on protein folding.

The energetic epitope

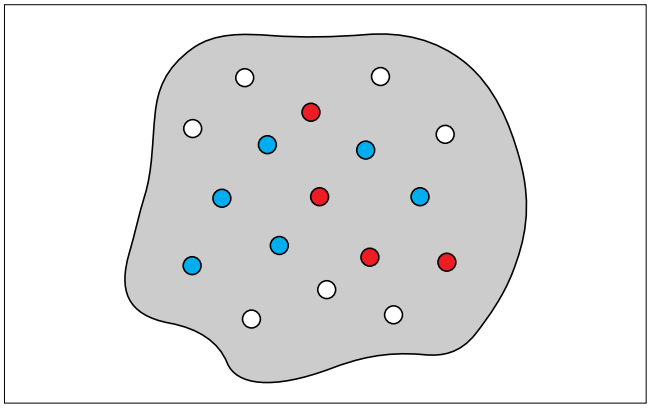
The second line of evidence suggesting that viral design is not constrained by antibody-binding considerations comes from various approaches to estimating the residue-by-residue contributions to the binding energy of a protein–protein complex. The results of alanine-scanning mutagenesis [11] imply that fewer than half of the amino acids within a hormone–receptor binding interface contribute significantly to complex formation. Mutagenesis of influenza neuraminidase amino acids located in the binding site of the NC41 antibody showed that antibody binding was more tolerant of substitution at some sites than at others [12].

Computational approaches to this problem also suggest the existence of an 'energetic' subset of all interface residues. The calculated contributions to the binding energy in several different antibody–antigen interactions [13] suggest that few residues contribute most of the interaction energy, although the sum of the computed residue-by-residue interaction energies often does not agree with measurements of the association constants. Nevertheless, the trends observed from experimental mutagenesis have been captured in some calculations [14].

One target, two bullets

The third line of evidence against antibody-imposed viral constraints is provided by the analyses of situations where two different proteins or protein surfaces bind a common target surface. Familiar examples of such situations are: firstly, two different antibodies in an antiserum binding the same site on an antigen; secondly, an antibody (Ab1) that binds both to its cognate antigen and to anti-idiotopic antibodies (Ab2) raised against it; and thirdly, an antibody raised to a pathogen antigen which cross-reacts with host antigen, as in some auto-immune diseases. In such cases, it is instructive to consider the

Figure 2



Schematic showing a surface of seventeen amino acids on a viral protein that form the interface with both the viral receptor and an antibody. Red residues contribute most of the binding energy for the receptor, and blue residues for the antibody. Mutations elsewhere are permissive.

similarity between the two surfaces that bind the common third surface.

Examination of the three-dimensional structures of two different antibodies in complex with a common target area on influenza neuraminidase [15] suggested that the antibodies are dissimilar, in the sense that antigen residues are not always in the same type of chemical environment in the two antibody complexes. A study of the electrostatic interactions in these two complexes [16] has also concluded that although both complexes display a measure of electrostatic complementarity, which is similar to that found in other protein–protein complexes, there is no similarity in the electrostatic potentials of the two antibodies, implying that the complementarity of the two complexes involves different surface patches within the antigen interface. This suggests that at least the electrostatic contribution to the binding energy of the two complexes is differently distributed among the neuraminidase residues common to the two complexes.

An anti-idiotope (Ab2)–antibody (Ab1) complex has been compared structurally with an antigen (lysozyme)–antibody (Ab1) complex, leading to the conclusion that similar binding interactions occur in the two complexes [17], but only over the hydrophilic parts of the interface [18]. Alanine-scanning mutagenesis of the anti-lysozyme antibody (Ab1) has found that the antibody interacts with lysozyme and the anti-idiotypic antibody through two different 'energetic' subsets of residues [19]. In two cases, alanine substitutions on Ab1 reduce the binding affinity to the anti-idiotope by more than 4 kcal mol^{-1}, whereas the reduction in antigen-binding affinity is approximately 0.5 kcal mol^{-1}. A third example reduces binding to antigen by 2.7 kcal mol^{-1}, whereas a reduction in anti-idiotope binding is by only 0.3 kcal mol^{-1}.

The results of both of these studies [16,17] point to the existence of mutations in the common target surface that will compromise one of the two interactions in question, but leave the other essentially unaffected (Fig. 2).

Conclusion

Thus, antibodies may select amino acid substitutions on the viral protein which can compromise binding with that particular antibody, but have no effect on receptor binding, even though the receptor- and antibody-binding sites are otherwise identical. On this basis, it is unnecessary for functional sites on viral proteins to be restricted in any way, either by size or by location, because antibody surveillance of such sites on rapidly mutating viruses is fundamentally compromised by the very nature of protein–protein interactions. Only in cases where the antibody and the receptor bind to the virus in energetically identical ways will the potential of the virus to evade antibody surveillance be threatened.

References

1. Colman, P.M., Varghese, J.N. & Laver, W.G. (1983). Structure of the catalytic and antigenic sites in influenza virus neuraminidase. *Nature* **303**, 41–44.
2. Rossmann, M.G., *et al.*, & Vriend, G. (1985). Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature* **317**, 145–153.
3. Amit, A.G., *et al.*, & Poljak, R.J. (1986). Three-dimensional structure of an antigen–antibody complex at 2.8 Å resolution. *Science* **233**, 747–753.
4. Colman, P.M., *et al.*, & Webster, R.G. (1987). The three dimensional structure of a complex of antibody with influenza virus neuraminidase. *Nature* **326**, 358–363.
5. Tulip, W.R., Varghese, J.N., Laver, W.G., Webster, R.G. & Colman, P.M. (1992). Refined structure of the influenza virus N9 neuraminidase-NC41 Fab complex. *J. Mol. Biol.* **227**, 122–148.
6. Olsen, N.H., *et al.*, & Rossmann, M.G. (1993). Structure of a human rhinovirus complexed with its receptor molecule. *Proc. Natl. Acad. Sci. USA* **90**, 507–511.
7. Smith, T.J., Chase, E.S., Schmidt, T.J., Olson, N.H. & Baker, T.S. (1996). Neutralising antibody to human rhinovirus 14 penetrates the receptor-binding canyon. *Nature*, **383**, 350–354.
8. Bizebard, T. *et al.* & Knossow, M. (1995) Structure of influenza virus haemagglutinin complexed with a neutralising antibody. *Nature* **376**, 92–94.
9. Webster, R.G., *et al.*, & Laver, W.G. (1987). Antigenic structure and variation in an influenza N9 neuraminidase. *J. Virol.* **61**, 2910–2916.
10. Tulip, W.R., Varghese, J.N., Webster, R.G., Laver, W.G. & Colman, P.M. (1992). Crystal structures of two mutant neuraminidase–antibody complexes with amino acid substitutions in the interface. *J. Mol. Biol.* **227**, 149–159.
11. Clackson, T. & Wells, J.A. (1995). A hot spot of binding energy in a hormone–receptor interface. *Science* **267**, 383–386.
12. Nuss, J.M., Bossart, P.J. & Air, G.M. (1993). Identification of critical contact residues in the NC41 epitope of a subtype N9 influenza virus neuraminidase. *Proteins* **15**, 121–132.
13. Novotny, J., Bruccoleri, R.E. & Saul, F.A. (1989). On the attribution of binding energy in antigen–antibody complexes McPC603, D1.3 and HyHEL-5 *Biochemistry* **28**, 4735–4749.
14. Tulip, W.R., Harley, V.R., Webster, R.G. & Novotny, J. (1994). N9 neuraminidase complexes with antibodies NC41 and NC10; empirical free energy calculations capture specificity trends observed with mutant binding data. *Biochemistry* **93**, 7986–7997.
15. Malby, R.L., *et al.*, & Colman, P.M. (1994). The structure of a complex between the NC10 antibody and influenza virus neuraminidase and comparison with the overlapping binding site of the NC41 antibody. *Structure* **2**, 733–746.
16. McCoy, A.J., Epa, V.C. & Colman, P.M. (1997) Electrostatic complementarity at protein/protein interfaces. *J. Mol. Biol.* **268**, 570–584.
17. Fields, B.A., Goldbaum, F.A., Ysern, X., Poljak, R.J. & Mariuzza, R.A. (1995). Molecular basis of antigen mimicry by an anti-idiotope. *Nature* **374**, 739–742.
18. Braden, B.C., *et al.*, & Mariuzza, R.A. (1996). Crystal structure of an Fv–Fv idiotope–anti-idiotope complex at 1.9 Å resolution. *J. Mol. Biol.* **264**, 137–151.
19. Dall'Acqua, W., Goldman, E.R., Eisenstein, E. & Mariuzza, R.A. (1996). A mutational analysis of the binding of two different proteins to the same antibody. *Biochemistry* **35**, 9667–9676.