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Authors

Smith, TJ
Olson, NH
Che, ZW
et al.

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Thomas J. Smith, Norman H. Olson, Zhiwei Che, Elaine Chase, Holland Cheng, and Timothy S. Baker
Department of Biological Sciences
Purdue University
West Lafayette, Indiana 47907

Anne Mosser, Donna Leippe, and Roland R. Rueckert
Institute for Molecular Virology
University of Wisconsin
Madison, Wisconsin 53706

Described here are recent experiments that examined the mechanism of antibody-mediated neutralization of human rhinovirus 14 (HRV-14). Human rhinovirus is the major cause of the common cold in humans and is a member of the picornavirus family that includes foot-and-mouth disease virus, poliovirus, and hepatitis A. The HRV-14 capsid is composed of 60 copies each of four virally encoded structural proteins: VP1, VP2, VP3, and VP4. The first three viral proteins (~30 kD each) are the major components of the capsid, whereas the small VP4 lies at the RNA/capsid interface. The structure of HRV-14 has been determined to atomic resolution (Rossmann et al. 1985) and displays a number of features with functional significance. Canyons surround each of the icosahedral fivefold axes (Rossmann et al. 1985), and the base of this canyon includes the binding sites for the cell receptor, ICAM-1 (intercellular adhesion molecule 1). Four of the outermost protrusions on the virion surface form the four neutralizing immunogenic sites (NIm sites): NIm-IA, NIm-IB, NIm-II, and NIm-III (Rossmann et al. 1985; Sherry and Rueckert 1985; Sherry et al. 1986).

Antibodies are a major component of the adaptive immune system that combats virus infections in mammals. A great number of studies during the past few decades have been carried out to gain a better understanding of the mechanism of antibody-mediated neutralization of virus infections. With regard to the picornaviruses, several mechanisms have been proposed. Early studies suggested that antibodies caused a large drop in pI of the viral capsid upon binding, which suggested that neutralization might be mediated by large conformational changes in the viral capsid induced by antibody binding (Mandel 1976; Emini et al. 1983). Such conformational changes might inhibit one or more steps of the virus infection pathway. Since there was not always a correlation between these pI changes and neutralization (Brioen et al. 1985), it was suggested that antibodies might neutralize by aggregating the virions (Brioen et al. 1983). Aggregation would decrease the number of independent infectious units and might increase the clearance rate in vivo. Whether the concentrations of virus and antibody in vivo are sufficient to cause this immunoprecipitation reaction is unknown.

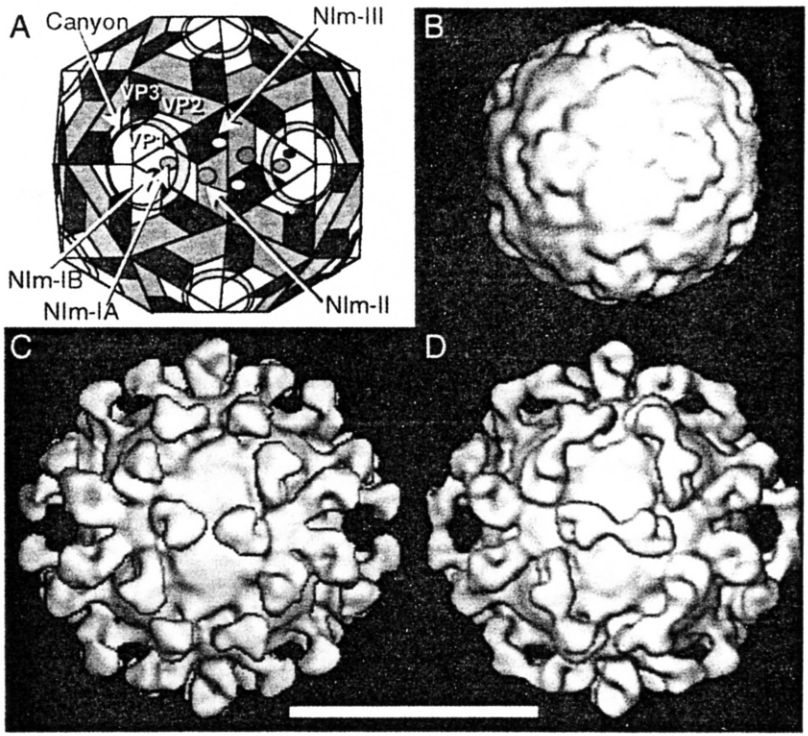

Figure 1
(*A*) Schematic diagram of HRV-14 in the same orientation as *B*, *C*, and *D*. (*B*) Surface rendering of HRV-14 at 25 Å using the atomic coordinates. (*C*) Fab17-IA/HRV-14 complex. Note the proximity of the Fab constant domains at the twofold axes and the nonradial orientation of the bound Fabs. (*D*) mAb17-IA/HRV-14 complex. Note the strong connection at the twofold axes. Bar, 250 Å.

To determine whether these (or other) mechanisms of neutralization are responsible for antibody-mediated neutralization, the neutralization properties of a large panel of antibodies to HRV-14 were examined in detail (Mosser et al. 1989; Leippe 1991). Neutralizing antibodies to rhinovirus may be broadly classified as either strong or weak neutralizers. Strongly neutralizing antibodies neutralize virus infectivity over a broad range of concentrations with little to no precipitation of virus. In contrast, weakly neutralizing antibodies are most efficacious over a narrow range of antibody concentrations and precipitate most of the virus at these optimal concentrations. Binding studies demonstrated that ~30 and ~60 strongly and weakly neutralizing antibody molecules, respectively, bind to virions at saturating concentrations (Mosser et al. 1989; Leippe 1991). These results suggested that strongly neutralizing antibodies bind bivalently to the virion surface, whereas weakly neutralizing antibodies bind monovalently. Therefore, antibodies are most efficacious when allowed to cross-link viral subunits within individual capsids. Such studies, however, did not elucidate a structural mechanism for neutralization. For example, bivalent attachment of antibodies to virions could either stabilize or destabilize the capsid.

Several structural techniques can be used to help ascertain the mechanism of antibody-mediated neutralization. The crystal structures of several Fab/peptide complexes have been determined in which the peptide represents viral immunogenic sites. Such studies have been performed on several picornaviruses, including poliovirus (Wien et al.

1995), FMDV (Verdaguer et al. 1994), and HRV-2 (Tormo et al. 1994). Some of these atomic resolution studies showed structural differences between the bound peptide and the corresponding immunogenic loop on the viral capsid. This suggested that the antibodies might induce conformational changes upon binding to the viral surface. These studies provide important atomic details of paratope/epitope interactions, yet they do not unequivocally prove that similar conformational changes occur in the intact virion or that such changes are responsible for neutralization. In addition, although the position of the peptide in the hypervariable region of the Fab fragment may provide clues for docking the Fab to the intact virion, a great deal of uncertainty remains in these modeling studies.

We have used a combination of molecular biology, electron microscopy, and crystallography to examine aspects of antibody-mediated neutralization. Our goal is to examine the interactions of antibodies in intact virions at high resolution. Structural analyses of virus-antibody complexes are aimed at determining whether large conformational changes occur upon antibody binding, how antibody binding induces neutralization efficacy, and what kinds of interactions dominate paratope-epitope interfaces. We have primarily concentrated initial studies on the NIm-IA site since this is the only site to which both strongly and weakly neutralizing antibodies bind, and thus allows a direct comparison of antibody binding and neutralization efficacy. NIm-IA is defined by natural escape mutations that occur at residues 91 and 95 of each copy of VP1, on the βB-βC loop that lies between the fivefold axis and the north rim of the canyon.

We have determined the atomic structure of a Fab fragment (Fab17-IA) from a strongly neutralizing antibody by X-ray crystallography (Liu et al. 1994) and the low-resolution (~25 Å) structure of the Fab/HRV-14 structure by cryo-electron microscopy and three-dimensional image reconstruction (Smith et al. 1993b). This Fab crystal structure, in conjunction with the previously determined atomic structure of HRV-14, allowed us to interpret the image reconstruction to pseudo-atomic resolution. Comparison of the Fab/HRV-14 capsid density with the atomic structure of HRV-14 and with electron microscopy reconstructions of HRV-14 alone showed no gross deformations in the viral capsid upon Fab binding. This implied that the pI changes that occur upon Fab and monoclonal antibody binding are not necessarily correlated with gross conformational changes in the virion.

Fab17-IA binds in a nonradial orientation on the HRV-14 surface, with the variable domain at the NIm-IA site and the constant domain lying toward the nearest twofold axes. This "leaning" of the Fab toward the twofold axes strongly suggests that the intact antibody binds bivalently to the virion surface with the Fc region bisecting an icosahedral twofold axis. Indeed, this hypothesis concurs with biochemical data which showed that mAb17-IA binds with a stoichiometry of approximately 30 monoclonal antibodies per virion and was a poor precipitator of virions in solution (Leippe 1991). Preliminary modeling studies suggested that electrostatic charge interactions dominated the paratope/epitope interface and that residues other than D91 and E95 (e.g., K85, K97, and K236) were important for Fab17-IA binding (Smith et al. 1993b). The atomic structures of HRV-14 and Fab17-IA, fitted into the electron microscopy density, confirmed these modeling studies. Furthermore, mutation studies showed that the positively charged residues K97, K85, and K236 of VP1 were all important for Fab17-IA binding (Smith et al. 1993b).

Although the orientation of bound Fab17-IA in the HRV/Fab complex seemed to confirm the biochemical data suggesting that mAb17-IA bound bivalently to the virion surface, there was no direct proof for this hypothesis; furthermore, this study could not answer the question of whether conformational changes occur upon monoclonal antibody binding. Hence, we examined the structure of the mAb17-IA/HRV-14 complex using electron microscopy reconstruction techniques (Smith et al. 1993a). Gross conforma-

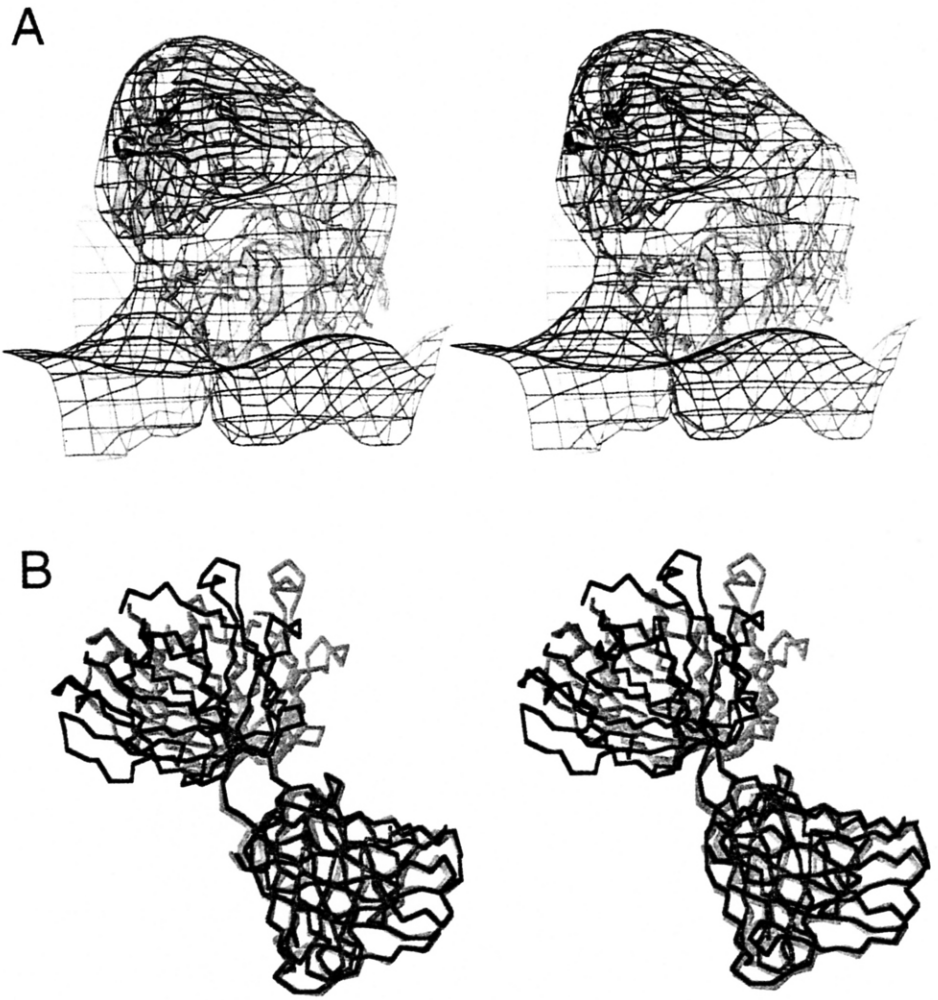

Figure 2
(*A*) Stereoview of a Fab17-IA ribbon model fitted into the cryo-electron microscopy image reconstruction density. The view is approximately from a twofold axis toward the nearest fivefold axis, with the viral suface toward the bottom of the diagram. (*B*) Stereodiagram of the C-α backbone of the bound Fab17-IA (*gray*) as fitted into the Fab17-IA/HRV-14 image reconstruction compared with the altered Fab17-IA structure (*black*) as fitted into the mAb17-IA/HRV-14 image reconstruction density. The view here is tangential to the virion surface with a fivefold axis toward the right, the nearest twofold axis toward the left, and the virus surface toward the bottom of the diagram. Note that the positions of the Fab and monoclonal antibody variable domains (the domains at the bottom) are identical, but the monoclonal antibody constant domains have been rotated about the elbow axis toward the virion surface. (Reprinted, with permission, from Smith et al. 1993a.)

tional changes in the HRV-14 capsid were not observed in this complex even though mAb17-IA was observed to clearly bind bivalently to the virion surface. The capsid exhibited no noticeable conformational changes, but the bound antibody had Fab arms with a larger elbow angle (~180°) than that seen in the virion-Fab complex (~165°). The

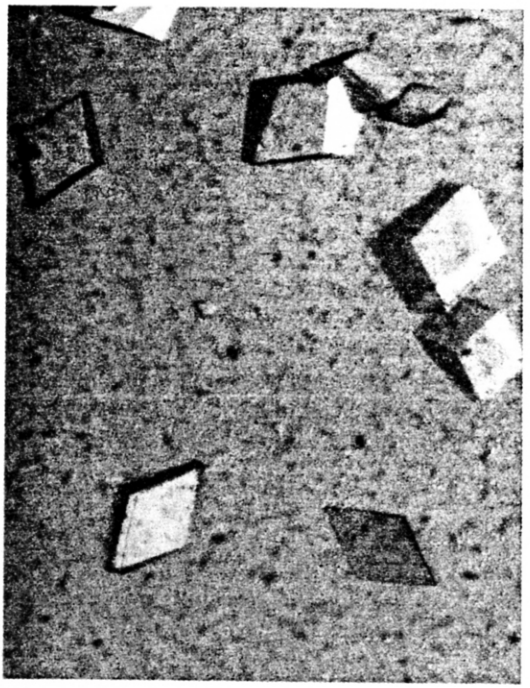

Figure 3
Crystals of the Fab17-IA/HRV-14 complex. Crystals usually grow to dimensions of ~0.8 mm and diffract to at least 4.5 Å resolution when frozen at liquid nitrogen temperatures. (Reprinted, with permission, from Smith et al. 1993a.)

larger elbow angle is necessary to bring the constant domains of the two Fab arms close enough to make bivalent attachment possible. All of these results suggested that strongly neutralizing antibodies bind bivalently but do not cause gross distortions in the capsid.

Our ongoing studies include image reconstruction analysis of HRV-14 complexed with other Fabs that bind to the NIm-IA site and crystal structure analysis of the Fab17-IA/HRV-14 complex. We have determined the cryo-electron microscopy structures of Fab1-IA/HRV-14 and Fab12-IA/HRV-14 complexes. Both these Fabs bind to the NIm-IA site, but Fab12-IA is a strongly neutralizing antibody, whereas Fab1-IA is a weakly neutralizing antibody. The orientation of Fab12-IA is almost identical to that of Fab17-IA and favors bivalent attachment of the corresponding mAb12-IA. Fab1-IA binds in a quite different orientation (~45° rotated about the long axis of the Fab). This confirms the hypothesis that neutralization efficacy is related to binding valency (which is in turn related to binding orientation). This also implies that since bivalently bound antibodies are expected to have affinity constants approximately 100–1000 times that of monovalently bound antibodies, neutralization efficacy is directly proportional to binding affinity. We are currently sequencing these and other Fab fragments to aid in the interpretation of the electron microscopy structures. To date, we have found that the charge interactions observed in the Fab17-IA/HRV-14 structure are conserved among the other Fabs despite differences in binding orientation. These modeling studies, and the crystal structure determination of Fab1-IA (work in progress), should help elucidate what aspects of the paratope govern Fab orientation.

Although the Fab17-IA/HRV-14 complex structure showed no large conformational changes in the virion structure, the possibility of smaller changes occurring upon Fab binding is not ruled out. Thus, the crystal structure determination of the Fab17-IA/HRV-14 complex will be quite important in elucidating changes that might occur. The X-ray data extend to approximately 4.5–5 Å resolution, which should be sufficient to determine main-chain positions in the proteins and give some indications of whether conformational changes do occur upon Fab binding. The pseudo-atomic model from the electron microscopy studies has provided a useful initial phasing model for structure determination which is currently progressing quite well at better than 6 Å resolution. The statistics from these preliminary crystallographic studies have clearly demonstrated the accuracy of the phases derived from the electron microscopy structure, which greatly facilitates the structure determination because heavy-atom derivative data collection becomes unnecessary. If large conformational changes are not observed in this crystal structure, then the notion that antibody binding to the virus is alone sufficient for strong neutralization would be proved. If conformational changes do occur, mutagenesis studies can be designed to help probe the relative importance of such changes.

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