Bivalent Attachment of Antibody onto Poliovirus Leads to Conformational Alteration and Neutralization

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The treatment of nonsaturating, neutralizing antibody-poliovirus complexes with papain generally led to the loss of viral neutralization and to the loss of the neutralization-associated change in the isoelectric point (pI) of the virion. Subsequent treatment with anti-immunoglobulin G antibodies restored the neutralization of the virus and the alteration of the viral pI. It appears that, under nonsaturating conditions, poliovirus neutralization by an antibody is dependent upon the ability of the antibody to bivalently attach to the virion. Exceptions are monospecific neutralizing antibodies with an affinity for capsid protein VP3.

In a recent study on the functional basis of antibody-dependent poliovirus neutralization, we examined seven anti-poliovirus type 1-monospecific antibodies for their role in the inhibition of viral infectivity (4). Each of these antibodies binds to a unique epitope which is functionally independent of the epitopes to which the remaining antibodies bind. Six of the antibodies are monoclonal and attach to epitopes located on the VP1 structural protein (3, 3a, 4). The remaining antibody, anti-VP4/VP3, was obtained from a rabbit which had been inoculated with the purified structural protein VP4. This serum contains a monospecific population of antibodies which neutralize by binding to a single site on the VP3 structural protein of the intact virion (2).

Each of the seven monospecific neutralizing antibodies can significantly inhibit (>99%) the infectivity of poliovirus type 1 upon attachment of as few as four antibody molecules per virion (4). In fact, Icenogle et al. (4a) have presented evidence that a minimum of four immunoglobulin G (IgG) molecules are required to neutralize the poliovirus virion, a study that was carried out with a single monoclonal antibody. Under nonsaturating conditions, six of the seven antibodies studied by us fail to inhibit either viral attachment or penetration into the host cell (4). (One monoclonal antibody appears to inhibit viral attachment.) Despite uptake, viral macromolecular synthesis does not occur. It appears

that the block to viral replication is related to a neutralizing antibody-dependent alteration in the structural conformation of the virion. Each of the six monoclonal antibodies, when bound under nonsaturating conditions, alters the isoelectric point (pI) of the intact virion from 7.0 to ca. 3.5 (4), an observation in agreement with that reported previously in studies with hyperimmune sera (5). An antibody which binds to the virion, but that does not neutralize it, has no effect on the pI of the virion. Hence, viral neutralization may be mediated by antibodies that interact with capsid protein VP1 through a mechanism of virion conformational change that could, in turn, inhibit the uncoating and subsequent expression of the viral RNA. Surprisingly, neutralization mediated by the monospecific anti-VP4/VP3 activity fails to change the viral pI. These antibodies have an affinity for an epitope on VP3 and may, therefore, neutralize by a unique mechanism (4). In this communication, we report experiments that will help to further elucidate the mechanism(s) of antibody-dependent poliovirus neutralization.

As mentioned above, the alteration of the conformation of the virion and the subsequent viral neutralization occur with as few as four antibody molecules bound per virion. It was of interest to determine whether such profound antibody-mediated changes in virion structure involve bivalent attachment of intact antibody molecules or whether simple binding to specific virion sites is sufficient. To this end, three neutralizing monoclonal antibodies (H3, D3, and A13) which bind to three independent epitopes

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TABLE 1. Effect of papain and of subsequent treatment with anti-IgG on poliovirus neutralization by antibody

Strain ^a	Antibody ^b	Neutralization ^c		
		+	+	+
		Antibody	Antibody and papain	Antibody, papain, and anti-IgG
Mahoney	H3 (B)	+	—	+
Mahoney	D3 (A)	+	—	+
Mahoney	A13 (C)	+	—	+
Mahoney	Anti-VP4/VP3 (G)	+	+	ND
Sabin 1	BA12-1	+	—	+

^a Strain of poliovirus type 1.

^b H3, D3, A13, and 1 BA 12-1 are monoclonal antibodies. Anti-VP4/VP3 is a monospecific neutralizing antiserum (see text). The letters in parentheses refer to the epitope group to which the antibody belongs (4).

^c Antibody was bound to the viruses under nonsaturating conditions (ca. four antibody molecules per virion determined as described previously [4]). The virus-antibody mixture was allowed to incubate in phosphate-buffered saline at room temperature for 60 min. Virus-antibody complexes were treated with 50 µg of papain (Sigma Chemical Co., St. Louis, Mo.) per ml for 3 h at room temperature in phosphate-buffered saline containing 10 mM cysteine. The reaction was terminated by the addition of iodoacetamide (Sigma) to 1.5 mM. Virion-F(ab) complexes were incubated with a 1:10 dilution of goat anti-mouse IgG (Cappel Laboratories, Cochranville, Pa.) in phosphate-buffered saline for 60 min at room temperature. Samples of the treated virus were set aside after each procedure and used to simultaneously infect monolayer HeLa cell cultures. The virus concentration in each sample was adjusted so that the multiplicity of infection in all cases was 1 to 5 PFU per cell. The infected cells were incubated at 37°C and microscopically inspected for the appearance of cytopathic effect (CPE). The degree of CPE in each infection was determined at the time of appearance of at least 25% CPE in the untreated virus-infected control cells. +, No CPE due to neutralized virus; —, CPE present to a degree similar to that seen in the control cells (virus was, therefore, not neutralized); ND, not done.

on the intact virion (4) were used to neutralize the Mahoney strain of poliovirus type 1 under nonsaturating conditions. Once bound, each of the three virion-antibody complexes was treated with papain to generate monovalent F(ab) fragments. This treatment led to a reversal of neutralization (Table 1). The F(ab) fragments remained attached to the virion since subsequent incubation of the latter complexes with goat anti-mouse IgG led, once again, to neutralization. This treatment did not cause viral aggregation as determined by analysis of the complexes by velocity gradient centrifugation (data not shown). Similar results were obtained by Icen-

gle et al. (4a), using a single neutralizing monoclonal antibody. These observations provide evidence for a neutralization model in which bivalent attachment of at least four antibody molecules across the surface of the virion is essential.

Identical results were obtained with a neutralizing monoclonal antibody, 1 BA 12-1, specific for the Sabin strain of poliovirus type 1 (Table 1). 1 BA 12-1 is of particular interest since this antibody binds to both the Mahoney strain and the Sabin strain but is capable of neutralizing only the latter (1). The inability of 1 BA 12-1 to neutralize the Mahoney strain of virus may be for one of two reasons: the monoclonal antibody is unable to bind bivalently to the Mahoney virion or the monoclonal antibody binds bivalently but structural changes of the virion do not occur. The following observations favor the first explanation. The binding of 1 BA 12-1 to the Mahoney virion does not change the pI of the complex (4). Treatment of the antibody-virion complex with goat anti-mouse IgG, however, resulted in the neutralization of the virus concomitant with a decrease in the pI (see below). Once again, significant aggregation of the virus-anti-IgG complex was not noted (data not shown).

It was shown previously (4) that saturation of the virion surface with an antibody resulted in virus neutralization by the inhibition of viral adsorption. Such inhibition was interpreted as being due to steric hindrance. If such hindrance does play a role in viral inhibition, then the hindrance should also be noted in the presence of excess F(ab) fragments. An experiment, identical to that summarized in Table 1, was conducted in the presence of saturating amounts of either H3, D3, or A13 antibody. In all cases, treatment with papain did not lead to the loss of virus neutralization.

Finally, the effect of papain on neutralization by nonsaturating amounts of anti-VP4/VP3 antibodies was tested. Unlike the results obtained with the monoclonal antibodies, anti-VP4/VP3 F(ab) fragments were found to be capable of neutralizing the virus even under non saturating conditions (Table 1). The observation supported the view that the anti-VP4/VP3 activity neutralizes poliovirus by a mechanism different from that employed by the monoclonal antibodies which bind to sites on VP1.

If the correlations among the antibody-induced virion conformational change, the change in the pI, and the antibody-induced viral neutralization are valid, the treatment of virus-antibody complexes with papain should lead to a restoration of the original conformation and pI of the virion. The experiments reported in Fig. 1 and Table 2 show that this was the case. The reacti-

vation of viral infectivity by the conversion of bivalent antibody molecules to monovalent F(ab) fragments was accompanied by the restoration of the pI of the virion to a point near that

of the untreated virion. Subsequent treatment of the virion-F(ab) fragment complexes with goat anti-mouse IgG led, again, to a change in the virion pI to ca. 3.5 (Table 2). This is identical to the pI change seen when the virion was treated with intact neutralizing antibody.

As mentioned before, treatment of the Mahoney strain of virus with 1 BA 12-1 neither neutralizes the virion nor alters its pI (4). It was found that the induction of neutralization by bivalent attachment with IgG was associated with a change in pI to 3.5 (Table 2). At this pI a poliovirion had lost, in all cases tested, its ability to initiate an infectious cycle.

The observations reported in this note strongly support the view of antibody-dependent poliovirus neutralization first proposed by Mandel (6). Under nonsaturating conditions (ca. four antibody molecules per virion), the inhibition of viral infectivity is mediated by an apparent antibody-induced alteration in the conformation of the virion. This apparent conformational change

FIG. 1. Representative isoelectric focusing profiles of (A) virus, (B) virus plus antibody A13, (C) virus plus antibody A13 plus papain, and (D) virus plus antibody A13 plus papain plus anti-mouse IgG. See footnotes to Table 1 for details of virus treatment. Virus was labeled with [35 S]methionine and purified (3). The pI was determined by isoelectric focusing as described by Emini et al. (4). Briefly, a 110-ml LKB focusing column was used. The column was composed of a 102.4-ml 0 to 50% (wt/vol) stepwise sucrose gradient containing 0.1% triton N-101 and 1.0% ampholytes (pH 3 to 10; Pharmalytes, Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The cathode (top electrode) buffer was 1.6% ethylenediamine. The anode (bottom electrode) buffer was 60% (wt/vol) sucrose-1.5% H_2SO_4 . The column was run overnight at a maximum voltage of 1,800 V, a maximum power of 15 W, and a maximum current of 15 mA. At the completion of focusing, the gradient was fractionated into 1.5-ml fractions. One hundred microliters of each fraction was assayed for radioactivity in a liquid scintillation counter with Aquasol-2 (New England Nuclear Corp., Boston Mass.). The pH of every fifth fraction was determined.

TABLE 2. Effect of papain and of subsequent treatment with anti-IgG on the pI of poliovirus

Strain ^a	Antibody ^b	pI ^c			
		No antibody ^d	+ Antibody	+ Antibody and papain	+ Antibody, papain, and anti-IgG
Mahoney	H3	7.0	3.5	6.6	3.5
Mahoney	A13	7.0	3.6	6.5	3.6
Sabin	1 BA 12-1	7.0	3.6	7.0	3.5
Mahoney	1 BA 12-1	7.0	7.0	ND	3.5

^a See Table 1, footnote a.

^b See Table 1, footnote b.

^c See legend to Fig. 1 for isoelectric focusing procedure. See Table 1, footnote c, for details of virus treatments. ND, Not done.

^d These pI values were determined by Emini et al. (4). They are listed here for comparison.

may be responsible for the loss of viral infectivity after viral penetration. Furthermore, it now seems that the apparent conformational change requires the bivalent attachment of the neutralizing antibodies to the virion. It is not clear, however, whether bivalent attachment per se will lead to conformational change. For example, polyclonal anti-VP1 antibodies bind to and precipitate virions, but they do not induce a change in the pI (4). It remains to be seen whether any of the antibodies in the anti-VP1 serum can bind bivalently to the surface of the virion.

Finally, it should be noted again that we have identified at least one exception to the proposed rule of bivalent attachment, and this is the anti-VP4/VP3 neutralizing activity. With this monospecific antibody population, the bivalent attachment of antibody molecules and the decrease in the pI of the virion appear not to be essential for neutralization. We have recently obtained evidence that the anti-VP4/VP3 neutralizing antibodies bind to a specific region within VP3 (E. A. Emini, B. A. Jameson, and E. Wimmer, unpublished data). Thus, the mechanism(s) of antibody-mediated poliovirus neutralization may depend upon which of the capsid polypeptides of the virion is involved in antibody binding.

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