Lack of Quantitative Correlation between the Neutralization of Poliovirus and the Antibody-mediated pI Shift of the Virions

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SUMMARY

The effect of mono- and polyclonal antibodies on the infectivity and pI (isoelectric pH) of type 1 poliovirus was studied. According to Mandel's hypothesis, the isoelectric pH of poliovirus should change to about pI 4 upon neutralization. However, several antibodies did not follow this rule. Moreover, when antibodies did shift the pI, no quantitative correlation existed between the extent of neutralization and the amount of poliovirus shifted to low pI.

Native poliovirus focuses at approximately pH 7. The pI (isoelectric pH) is lowered to 4-0 to 5-5 (B zone of the pH gradient) after acidification before focusing, after mild heating or u.v. inactivation (Mandel, 1971), and after treatment with a neutralizing antiserum (Mandel, 1976). Based on the latter finding, Mandel (1976, 1978) hypothesized that the neutralization of poliovirus occurred by means of stabilization of the virions in a biologically inactive conformation focusing in the B zone.

According to this model, three predictions can be made. When partly neutralized virus is submitted to isoelectric focusing, (i) virus recovered from the A zone should be fully infectious, (ii) virus recovered from the B zone should be non-infectious, and (iii) the fraction of virus focusing in the A zone should be equal to the virus survival ratio. Mandel (1976) reported an excellent correlation between the amount of virus focusing in the A zone and the survival ratio, in agreement with prediction (iii). However, the quantitative data concerned only a single serum.

Recently, it was reported (Emini et al., 1983a, b; Icenogle et al., 1983) that the pI shift could also be induced by monoclonal antibodies (MoAbs); however, no quantitative data were presented to test for Mandel's hypothesis. Moreover, these reports also mentioned cases of neutralization by both mono- and polyclonal antibodies without concomitant shift in the isoelectric pH of the virus.

The apparent absence of a pI shift with some neutralizing antibodies prompted us to investigate the quantitative correlation between the antibody-mediated pI shift and the neutralization of poliovirus. Five different antibody preparations will be discussed. We used Mandel's original focusing technique, slightly modified by preformation of the pH gradient before introduction of the virus (Vrijseren et al., 1983).

The N1-specific IgG2α-κ antibody, MoAb 35-1f4 (Rombaut et al., 1983; Brioen et al., 1982), was shown to neutralize poliovirus by antibody-mediated aggregation (Brioen et al., 1983). Immunoglobulins were purified from ascitic fluids by ammonium sulphate precipitation. Part of this preparation was 14C-labelled by reductive methylation (A. A. M. Thomas et al., unpublished), and the fraction of poliovirus-specific immunoglobulin was determined by ultracentrifugation of a mixture of antibody and excess poliovirus. As 58% of the 14C co-sedimented with the viral material, this fraction of the immunoglobulin was considered to represent poliovirus-specific antibody.

At the antibody/virus ratio of 12:1, the virus was neutralized by 95%, but its pI was not shifted (compare Fig. 1a and 2a). Free 35-1f4 antibody focused at pH 7-3 (Fig. 1b). In the presence of poliovirus (Fig. 1a), the immunoglobulin yielded a profile with two peaks. The
Fig. 1. Isoelectric focusing of poliovirus neutralized with MoAb 35-1f4 (a) and of free MoAb 35-1f4 (b). Purified [3H]leucine-labelled type 1 poliovirus (strain 1a/S3) used in this study was prepared as described by Vanden Berghe & Boeyé, 1973. 14C-labelled antibody was added at the molar ratio of 12 molecules of specific antibody per poliovirion (the final virus concentration was 12 nm) in PBS (150 mM-NaCl, 13 mM-KCl, 0-3 mM-CaCl2, 0-5 mM-MgCl2, 10 mM-phosphate, pH 7.2). The mixture was incubated for 1 h at 37 °C and samples collected for infectivity titration and isoelectric focusing. Similar mixtures were made containing either antibody alone (Fig. 1 b) or virus alone (see Fig. 2 a). Isoelectric focusing columns were filled with a 1 to 39% sucrose gradient containing 3% ampholytes (Ampholine 3.5 to 10. LKB) essentially as described by Mandel (1971). A pH gradient was formed during a 2 h prerun at 4 °C under a constant power (4 W) and variable voltage (up to 2000 V). After lowering a catheter into the column to the pH 8 level, a 300 μl sample was removed, to which 100 μl of neutralization mixture was added. The mixture was then reintroduced into the column at the original level. After focusing for 5 h under the same conditions as the prerun, 400 μl fractions were collected, and their pH and radioactivity were determined. (a) 3H-labelled virions. ○, 14C-labelled MoAb 35-1f4 antibody.

Fig. 2. Isoelectric focusing of poliovirus neutralized with different mono- and polyclonal antibodies. For details of antibody input and residual infectivity, see Table 1. For incubation and focusing procedures, see legend to Fig. 1. (a) Control virus. (b) Virus neutralized with MoAb 35-1h2. (c) Virus neutralized with MoAb 36-5h2. (d) Virus neutralized with rabbit antibodies. A rabbit was immunized with 500 μg of purified type 1 poliovirus, boosted three times at monthly intervals, and bled 1 week after the last booster. Immunoglobulin was purified by two ammonium sulphate precipitations followed by dialysis against PBS. (e) Virus neutralized with guinea-pig antibodies. A guinea-pig was immunized with 10 μg purified type 1 poliovirus, boosted after 1 month with the same dose in incomplete adjuvant, and was bled 1 week later. The antibodies were prepared as in (d).

greater part focused with the virus at pH 6.7, and the remainder (presumably unspecific immunoglobulin) at pH 7.4. The material from the fraction at pH 6.7 was submitted to low-speed centrifugation (10 min at 11000 g), and 71% of the virus and 51% of the antibody were pelleted (less than 1% was pelleted in control experiments with free virus or free antibody). The antibody content of the pellet material was 9.6: 1, Ab/virion. When a control virus–Ab mixture was centrifuged without prior focusing, the pellet contained 76% of the virus and 58% of the immunoglobulin with an antibody/antigen ratio of 12:1 Ab/virion. We conclude that the
Short communication

Table 1. Isoelectric focusing of poliovirus neutralized with mono- and polyclonal antibodies: virus distribution and extent of neutralization

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Initial Ab/virus mixture</th>
<th>Electrofocused mixture</th>
<th>Specific Ab/virion (molar ratio)</th>
<th>Residual infectivity (%)</th>
<th>Figure showing profile</th>
<th>$^3$H virus distribution (%)</th>
<th>Specific infectivity†</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
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<tr>
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<tr>
<td>2</td>
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<td>5</td>
<td>1(a)</td>
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<td>17</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>3</td>
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<td>9</td>
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<td></td>
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<tr>
<td></td>
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<td>100</td>
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<tr>
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<td>MoAb 36-5h2</td>
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<td>90</td>
<td>1</td>
<td></td>
<td>11</td>
<td>89</td>
<td>ND</td>
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</table>

* Samples were diluted in PBS (see legend to Fig. 1) and plated as described by Brioen & Boeyé (1985).
† P.f.u./radioactivity ratio of virus recovered from focusing column, expressed as percent of same ratio for untreated virus.
‡ ND, Not determined.

Material that focused at pH 6.7 consisted of virus–Ab complexes. Comparison of the data before and after focusing allows the conclusion that the bulk of the virus was still aggregated after focusing, and that most of the antibody originally bound was still associated with the virions. It will be observed that the virus recovered from the A peak was still neutralized by 83%. A partial restoration of infectivity after focusing was observed by Mandel (1976) and by us with all the antibodies tested (Table 1), and regardless of whether the virus focused in the A or B zone. This may be explained by assuming that virus–antibody complexes are unstable under the conditions of focusing, which combine low ionic strength and a high potential gradient.

One might argue that large immune complexes lack sufficient mobility to reach their isoelectric pH. It was therefore verified that the virus migrated from the point of introduction at pH 8 to its final position in less than one-fifth of the time allowed for focusing. As shown by Vrijsen et al. (1983) the possible role of gravity on the mobility of large complexes can also be disregarded. Our findings extend those of Vrijsen et al. (1983) by showing that, whereas aggregates due to acidification always have a low pI, antibody-induced aggregates may focus in the A zone.

To our knowledge, this is the first demonstration of an immune complex formed by poliovirus and a neutralizing antibody, which focused in the A zone. Similar observations seem to have been made by Icenogle et al. (1983), but the actual facts were not reported.

Even a great excess (1000 : 1, Ab/virion) of 35-1f4 antibody causing 99-98% neutralization failed to shift the pI of poliovirus (Table 1, expt. 2). The absence of the pI shift after reaction with MoAb 35-1f4 may reflect a neutralization mechanism not involving modifications in the virion conformation. This agrees with the antibody-mediated polymerization mechanism proposed by Brioen et al. (1983).

The IgG2a-κ antibody, MoAb 35-1h2, is N2-specific. Virus neutralized by MoAb 35-1h2 to 88% or even to 96% still focused in the A zone (Table 1, expt. 3 and Fig. 2b). However, when MoAb 35-1h2 was used in the extremely high Ab/virus ratio of 900 : 1, which caused more than 4 log$_{10}$ reduction of infectivity, the virus focused at pH 4.5 (Table 1, expt. 3). In this respect, MoAb 35-1h2 differed from 35-1f4. However, for both antibodies it is clear that the amount of virus shifted to low pI was not commensurate with neutralization.
MoAb 36-5h2, an N1-specific IgG3-κ antibody, differs from 35-1f4 as it does not mediate the formation of dimers and other distinct oligomers of poliovirions (A. A. M. Thomas et al., unpublished results). Whereas the results with MoAb 35-1h2 indicated that the pI shift lagged behind the neutralization, with MoAb 36-5h2 the opposite held true. At the input Ab/virion ratio of 5·4 : 1, neutralization was an insignificant 10⁻⁰ and yet 64⁻⁰ of the virus was shifted to pI 5·0 (Table 1, expt. 4). With an Ab/virion ratio of 37 : 1 and 8⁻⁰ residual infectivity, 100⁻⁰ of the virus focused in the B zone (Fig. 2c and Table 1, expt. 4).

In summary, the MoAbs tested caused either no pI shift at all (35-1f4), or a pI shift lagging behind neutralization (35-1h2), or one preceding neutralization (36-5h2). In none of the three cases tested was the amount of infectivity lost to neutralization related to the amount of virus in the B zone.

Isoelectric focusing experiments were also carried out after neutralization with the immunoglobulins prepared from two conventional antisera against type 1 poliovirus. The antibodies from a rabbit serum completely failed to induce any shift to the B zone, even at 99-98⁻⁰ neutralization (Fig. 2d and Table 1, expt. 5). The virus recovered after focusing still had a reduced specific infectivity, even though higher than expected from that of the mixture, again showing partial reactivation after focusing.

The antibodies purified from a guinea-pig serum caused both neutralization and pI shift, and there was a rough correlation between the amount of virus shifted to the B zone and the degree of neutralization (Table 1, expt. 6). This serum therefore somewhat resembled that described by Mandel (1976).

Mandel's hypothesis, although originally formulated with due prudence, has been widely accepted, and unfortunately, without further proof. Some electrofocusing experiments were performed with virus neutralized to more than 99⁻⁰ and the result cited in support of the hypothesis if and when this heavily neutralized virus happened to focus in the B zone (Emini et al., 1983a). The results obtained with MoAbs 35-1h2 and 36-5h2 (Table 1) exemplify the erroneous conclusions that may be drawn by testing only the highest Ab/virion ratios.

The following old and new evidence should be balanced against Mandel's unique observations. (i) His own finding that virus recovered from the B zone retained infectivity. (ii) The failure of our MoAb 35-1f4 to induce a pI shift at any antibody/virus ratio. This property can hardly be exceptional since other monoclonal antibodies also failed to shift the pI (Emini et al., 1983a), and our polyclonal rabbit serum, as well as an anti-VP3 serum reported by Emini et al. (1983a) were also found to neutralize without a concomitant pI shift. (iii) With none of our mono- or polyclonal antibodies was there a close correlation between the residual infectivity and the fraction of virus focusing at the original pI. In conclusion, Mandel's hypothesis can no longer be generally accepted. Remarkably, Mandel (1976) himself had the insight to recognize the possibility that "stabilization [in the B state] is a concomitant but not causally related, phenomenon of neutralization".

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