Antibody-mediated Early Death in vivo after Infection with Yellow Fever Virus

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SUMMARY

The phenomenon known as antibody-dependent enhancement (ADE) has been demonstrated in vitro but its significance in viral pathogenesis is uncertain even though it has been associated with dengue shock syndrome. Here we report for the first time the enhancement of virus virulence in mice using monoclonal antibodies (MAbs) prepared against yellow fever (YF) viruses. Our results show that the average survival time of mice was reduced by up to 33% (i.e. 6.7 to 4.5 days) and that ADE is both antibody dose-dependent and antibody- and virus strain-specific. A total of 12 YF viruses and 11 MAbs were examined and of these only three YF viruses (FNV, Asibi and B11) could be enhanced in vivo by only two MAbs (427 and 126). A particular combination of virus and antibody is required for ADE to take place.

Antibody-dependent enhancement (ADE) is a phenomenon where infection of Fc receptor-bearing cells, usually macrophages, is increased by the presence of antiviral antibodies. This enhancement is usually seen as an increase in the yield of infectious virus from infected cells. Most studies of ADE have been performed in vitro using the Fc receptor-bearing mouse macrophage cell line P388D1 and viruses from the family Flaviviridae including dengue (DEN), yellow fever (YF) and West Nile viruses, (Porterfield, 1982). Enhancement of virus infectivity is associated with antibodies that react with the surface of the virus but occurs independently of other surface phenomena such as neutralization and haemagglutination inhibition (Porterfield & Cardosa, 1984). A high proportion of monoclonal antibodies (MAbs) enhance infectivity in vitro with the viruses that they recognize antigenically (Brandt et al., 1982; Halstead, 1981; Halstead et al., 1980, 1983; Peiris et al., 1982; Schlesinger & Brandriss, 1983). Whether ADE takes place in vivo is not known although increased viraemia was seen in DEN-2-infected monkeys which had been pretreated with serum containing DEN-2 antibodies (Halstead, 1979). It has also been proposed, from immunosuppression studies, that the 'early death' syndrome seen in rabies-infected mice may be due to ADE (Prabhaker & Nathanson, 1981), and a similar phenomenon has been reported for feline infectious peritonitis virus (Weiss & Scott, 1981).

We have recently prepared a panel of MAbs against YF viruses (Gould et al., 1985). One of these (MAb 126) was prepared against the Asibi wild-type strain and another (MAb 427) against the French neurotropic vaccine (FNV) strain of YF virus. These recognize different antigenic determinants in the viral envelope glycoprotein of YF virus (Cammack & Gould, 1986a), and in general do not neutralize YF viruses efficiently when tested by plaque reduction neutralization. MAb 126 has failed to neutralize any YF virus examined to date while MAb 427 will neutralize only 17D-UK (Buckley & Gould, 1985 and unpublished observations). By indirect immunofluorescence titration using acetone-fixed Vero cells infected with the appropriate homologous virus these antibodies have titres of at least 1/10000.

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We have now investigated the effect on young adult mice (3 to 4 weeks old; strain TO) of intraperitoneal injection with these antibodies and subsequent intracerebral infection with 50 LD₅₀ of different strains of YF virus. Both antibodies failed to protect the mice against infection by the majority of the YF viruses chosen for the investigation (Gould et al., 1986) or with Japanese encephalitis (JE) virus which has been shown by indirect immunofluorescence to react with these antibodies. On the other hand the results (Table 1) show that administration of undiluted MAb 126 to mice 24 h prior to infection with FNV virus consistently resulted in mice dying 1.6 days earlier (average survival time 5.4 ± 0.3 days) than those treated with a control YF non-reactive MAb (7.0 ± 0.3 days). Similarly, with MAb 427 a significantly shorter time to death (by 1.7 days) was observed when FNV virus was used for intracerebral challenge (5.2 ± 0.3 compared to 7.0 ± 0.3 days). Repeating the experiment with a different clone of MAb 427 showed that the results were reproducible and the differences in average survival time were found to be statistically highly significant (P < 0.01). Similar results to those with FNV viruses were observed with Asibi and B11 viruses but none of the nine other YF viruses tested or JE virus were affected in this way. Nine other MAbs (110, 140, 411, 612, 813, 825, 843, 864 and 868) with molecular specificity for the YF viral envelope glycoprotein (Gould et al., 1985) failed to cause early death with any of the viruses tested above. In these cases the results with antibody-treated mice were either indistinguishable from those in non-treated mice or mice were protected from death as reported previously (Gould et al., 1986).

Table 2 shows the results of experiments in which the input concentration of FNV virus was varied and MAb 427 and MAb 126 were titrated against the virus in a chequerboard fashion. As can be seen there was a decrease in the average survival time of mice treated with MAb 427 and then infected with FNV virus input at concentrations of either 50 (from 7.3 to 5.0 days, P > 0.001) or 500 LD₅₀ (from 6.7 to 4.5 days, P > 0.001). The maximum enhancement (i.e. shortest average survival time) was seen with the greatest concentration of MAb. For example, treatment with undiluted MAb 427 followed by infection with 500 LD₅₀ of FNV virus reduced the average survival time by 33% (i.e. 6.7 - 4.5 = 2.2 days). Similar results were obtained when MAb 126 was titrated against FNV virus in chequerboard fashion (Table 2). Attempts to increase enhancement by treatment of mice with both MAb 427 and MAb 126 together were unsuccessful and gave similar average survival times to those of mice treated with either MAb 427 or MAb 126 alone (data not shown).
Table 2. Average survival times of mice treated with different concentrations of either MAb 427 or MAb 126 and challenged with different doses of FNV virus

<table>
<thead>
<tr>
<th>Antibody concentration</th>
<th>Virus dose (LD&lt;sub&gt;so&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Undiluted</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>0.2</td>
<td>5.3 ± 0.5</td>
</tr>
<tr>
<td>0.04</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>0.0125</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td>Control</td>
<td>7.3 ± 0.3</td>
</tr>
</tbody>
</table>

MAb 427 MAb 126

* Groups of 24 mice were inoculated as described in Table 1 except that fivefold dilutions of MAb 427 or MAb 126 and two doses of FNV virus were used in a checkerboard titration. Average survival times (days ± S.E.M.) are shown.

The results presented show that under certain conditions, antibodies that recognize a virus may exacerbate the virus infection rather than assist the host in recovering. It is perhaps relevant that the three viruses which we have shown can be enhanced in vivo are neutralized, albeit poorly, by only one MAb (612) whereas the nine other YF viruses which were not enhanced can be neutralized by many of the MAbs used in this study (Buckley & Gould, 1985). It is possible that the particular virus–antibody combination may be important since one of two MAbs prepared against FNV, one of three against Asibi but none of six against the attenuated 17D vaccine virus were able to enhance the disease process. We have previously reported that none of the MAbs will passively protect mice against infection by FNV and Asibi viruses but will passively protect mice against infection with other YF viruses (e.g. 17D-UK and 17DD-Braz) (Gould et al., 1986). In the same paper it was shown that the lack of protection was related to the ability of those viruses to grow rapidly to high titre in the brain following intracerebral inoculation. Attempts to demonstrate enhancement following intraperitoneal inoculation of both FNV virus (10<sup>3</sup> p.f.u.) and MAb 427 were unsuccessful (data not shown). Thus at the present time enhancement in vivo could be expressed only using a virus–mouse neurovirulence model.

Experiments by Cammack & Gould (1986b) suggest that enhancement is not related to the affinity of a MAb for a virus since there was no correlation between the virus–antibody binding curves and the ability of viruses to be enhanced or not enhanced. However, Cammack & Gould (1986a) have recently performed a topographical analysis of epitopes on the envelope glycoprotein of 17D and Asibi viruses in which it was shown that the epitope recognized by MAb 427 was located in a different relative position on Asibi virus as compared with 17D virus. Thus ADE in vivo may reflect the presentation and position of epitopes on the surface of the envelope glycoprotein rather than their mere presence. Halstead et al. (1984) have made a similar suggestion from in vitro studies with DEN-2 virus. The experimental system described in this paper provides the possibility, for the first time, of correlating ADE in vivo with that in vitro, and of understanding this unusual phenomenon.

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REFERENCES


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