Neutralization and Sensitization of Lactate Dehydrogenase-elevating Virus with Monoclonal Antibodies

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SUMMARY

Monoclonal antibodies directed against VP3, the envelope glycoprotein of lactate dehydrogenase-elevating virus (LDV), were found to neutralize a large proportion of the virus population. This effect of monoclonal anti-VP3 antibodies was significantly increased by a murine monoclonal rheumatoid factor, indicating that the same antiviral antibodies can either neutralize or sensitize different fractions of the virus. This observation could be explained by heterogeneity in LDV particles, resulting in diverse responses to antibodies and therefore to the persistence of the virus in vivo.

Lactate dehydrogenase-elevating virus (LDV) is unique among murine viruses in inducing a life-long viraemia in healthy adult animals (Riley et al., 1960). To date, no deficiency in the humoral immune response of the infected host has been demonstrated. Indeed, significant amounts of anti-LDV antibodies are produced after infection. These have an antigenic specificity largely restricted to VP3, the viral envelope glycoprotein (Notkins et al., 1966; Porter et al., 1969; Cafruny & Plagemann, 1982a; McDonald et al., 1983; Coutelier et al., 1986b; Cafruny et al., 1986). Like most murine antibodies elicited by virus infections, anti-LDV antibodies are predominantly of the IgG2a subclass (Coutelier et al., 1987). Several reports have indicated that polyclonal anti-LDV antibodies can neutralize the virus (Rowson et al., 1966; Notkins et al., 1966; Cafruny & Plagemann, 1982b; Cafruny et al., 1986). Moreover, LDV neutralization can be increased by anti-mouse IgG or IgA, but not by anti-mouse IgM antibodies. This phenomenon, which has been called sensitization, was shown to correspond to the binding of antibodies that do not directly neutralize a fraction of the virus (Notkins et al., 1966, 1968; Cafruny et al., 1986).

As most of the previous studies on LDV antibodies were performed with polyclonal antisera raised in infected mice or in animals immunized with whole virus, little is known of the antigenic specificity of the antibodies responsible for viral neutralization and sensitization. In this report, we have addressed this question using a collection of monoclonal antibodies (MAbs) recently developed from both infected and immunized mice (Coutelier et al., 1986b).

LDV (Riley strain, American Type Culture Collection) was grown and concentrated as described previously (Coutelier & Van Snick, 1985). Polyclonal anti-LDV immunoglobulins were prepared from sera of chronically infected mice by precipitation with ammonium sulphate. To decrease the possibility of contamination by viruses contained in the serum of these animals, antibodies were treated for 1 h at pH 2-6 (Crispens, 1965). Anti-LDV MAbs were produced and characterized as reported by Coutelier et al. (1986b), and were purified by affinity chromatography in Protein A–Sepharose.

For neutralization experiments, samples of concentrated LDV (approx. $10^{12} ID_{50}$/ml) were incubated with antibodies for 15 min at 37 °C and 1 h at 4 °C in 50 to 100 μl of Hanks' medium.
Fig. 1. Neutralization and sensitization of LDV with anti-VP3 MAbs. LDV samples were first incubated with an irrelevant IgG2a MAb (A6202F4), with anti-VP3 IgG2a MAbs [B6503E7, B6504F4 and C1307D10 react only with the 27K to 38K material of VP3, as described previously (Coutelier et al., 1986b); B6501A4, B6506A7 and B6505H9 recognize also the 25K band of the protein] or with murine polyclonal anti-LDV Ig (MIS, 1 mg). Reduction of LDV infectivity titre (ID$_{50}$/ml) was measured after a second incubation with medium alone (open columns) or with a monoclonal anti-IgG2a RF (B304G6, shaded columns).

supplemented with 5% foetal bovine serum, followed by a second incubation after addition of medium or 50 µg of a monoclonal rheumatoid factor (RF; B304G6, anti-IgG2a IgM derived from a CBA mouse immunized with chicken lysozyme). After injection of serially diluted samples into groups of six female NMRI mice and determination of lactate dehydrogenase levels in plasma 4 to 5 days later, LDV titres were calculated according to Rowson & Mahy (1975).

Preliminary experiments with IgG2a and IgG3 anti-VP3 MAbs showed that some neutralization was obtained with 5 µg of antibody and reached a plateau with 50 µg of MAb (data not shown). This dose was therefore used for each experiment. Our results indicate that a maximum of 91 to 98% of infectious virus particles was neutralized after incubation with anti-VP3 IgG2a MAbs, which correspond to the prevalent antibodies in infected mice, whereas polyclonal antibodies were able to cause a much greater reduction in the LDV titre (Fig. 1). In addition, when the antibody-treated virus was incubated with RF MAb, approximately 90% of the remaining virus was neutralized. Surprisingly, the proportion of sensitized LDV particles was similar after incubation with either monoclonal or polyclonal anti-LDV antibodies (Fig. 1). That this phenomenon corresponded to a genuine sensitization by anti-LDV antibodies rather than to a non-specific effect of RF was indicated by the fact that RF failed to neutralize the virus after incubation with an irrelevant MAb.

The neutralizing activity of MAbs with different isotypes or specificity was also tested. As indicated in Table 1, anti-VP3 IgG3 MAbs reduced the viral titre by 10²- to 10⁴-fold, while IgM
Table 1. Neutralization of LDV by MAbS with different isotypes and specificity

<table>
<thead>
<tr>
<th>MAb†</th>
<th>Isotype</th>
<th>Specificity</th>
<th>LDV titre (log_{10})</th>
<th>Reduction of LDV titre (log_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Without MAb</td>
<td>With MAb</td>
</tr>
<tr>
<td>C3904H12</td>
<td>IgG3</td>
<td>VP3</td>
<td>11.70 ± 0.30</td>
<td>8.30 ± 0.36</td>
</tr>
<tr>
<td>C3901B3</td>
<td>IgG3</td>
<td>VP3</td>
<td>11.94 ± 0.34</td>
<td>9.18 ± 0.38</td>
</tr>
<tr>
<td>C7506B12</td>
<td>IgG3</td>
<td>VP3</td>
<td>11.80 ± 0.30</td>
<td>7.45 ± 0.45</td>
</tr>
<tr>
<td>C707H4</td>
<td>IgM</td>
<td>VP3</td>
<td>12.50 ± 0.30</td>
<td>12.40 ± 0.28</td>
</tr>
<tr>
<td>C701H5</td>
<td>IgM</td>
<td>VP3</td>
<td>11.70 ± 0.28</td>
<td>11.80 ± 0.30</td>
</tr>
<tr>
<td>B4102B1</td>
<td>IgM</td>
<td>VP3</td>
<td>12.64 ± 0.34</td>
<td>11.75 ± 0.31</td>
</tr>
<tr>
<td>C3506A9</td>
<td>IgG1</td>
<td>VP1</td>
<td>12.45 ± 0.38</td>
<td>12.15 ± 0.38</td>
</tr>
<tr>
<td>C3502C9</td>
<td>IgG1</td>
<td>VP1</td>
<td>12.90 ± 0.28</td>
<td>12.42 ± 0.39</td>
</tr>
<tr>
<td>C3502D1</td>
<td>IgG1</td>
<td>VP1</td>
<td>12.90 ± 0.28</td>
<td>12.30 ± 0.36</td>
</tr>
</tbody>
</table>

* LDV infectivity titre (ID_{50}/ml) ± S.D.
† Fifty µg.

antibodies recognizing the same protein did not efficiently neutralize the virus. In addition, IgG1 MAbS reacting with VP1, the nucleocapsid protein, had little effect on the virus. So far, it is not clear whether these differences in neutralizing activity, and especially the potent effect of anti-VP3 IgG3 MAbS, are related to the isotype rather than to a particular antigenic specificity of these antibodies.

Our results indicated that antibodies directed against VP3 can neutralize a large proportion of infectious LDV. However, a fraction of the virus was not neutralized after incubation with anti-LDV MAbS, even when an excess of antibody was used. As described previously (Coutelier et al., 1986b), anti-VP3 antibodies seem to recognize at least two different epitopes, corresponding in Western blots to the 27K to 38K heterogeneous material of the protein or including also the 25K band of VP3. Although further studies are needed to define exactly the different VP3 epitopes, no difference has so far been observed between MAbS displaying these two reacting patterns with the viral protein. Other authors have reported that several anti-VP3 MAbS derived from infected and immunized mice did not neutralize the virus (Harty et al., 1987a). However, recent evidence suggests that some of their MAbS could reduce LDV titres in the same way as those used in this study, confirming that VP3 is a target for neutralizing antibodies (Harty et al., 1987b). Since the major antibody response to LDV is directed against this protein (Coutelier et al., 1986b; Cafruny et al., 1986), it seems unlikely that the life-long viraemia produced by this virus is due to a deficiency in the antibody response. Moreover, the observation that the same MAbS can either neutralize or sensitize different fractions of the virus and that, even in the presence of an excess of antibody, a significant proportion of LDV is neither neutralized nor sensitized suggests that heterogeneity in viral particles, rather than other phenomena such as the production of blocking antibodies, can explain how LDV escapes the immune system of its host. In addition, binding of antibodies to the neutralization-resistant viral fraction could increase its infectivity by enabling LDV to infect more macrophages by binding their Fc receptors (Cafruny & Plagemann, 1982b) and therefore contribute to the persistence of the infection. The LDV variations that lead to these differences in responses to antibodies could correspond to the emergence of genetic mutants of the virus. However, the observation of Notkins et al. (1966) that the progeny of sensitized virus has the same susceptibility to neutralization as the parental virus does not fit well with this hypothesis. A phenomenon that could occur randomly at each virus generation, e.g. variable glycosylation of VP3, could therefore provide an alternative explanation of the different interactions of LDV with antibodies.

Finally, our results indicated that neutralization of sensitized virus is as efficient with RF MAb in a homologous murine system as with heterologous antibodies, such as anti-murine Ig antisera or human RF, used by others (Notkins et al., 1966; Ashe et al., 1971). Although it remains to be established whether RF production can actually interfere with LDV infection in vivo, our observations support the idea that murine RF could have a physiological role in defence.
against infections. Previous reports showing that isotype-specific RF is produced in normal secondary immune responses (Nemazee & Sato, 1983; Van Snick & Coulie, 1983) and during murine infection (Coutelier et al., 1986a) further strengthen this hypothesis.

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REFERENCES


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