Characterization and Specificity of Humoral Immune Responses to Theiler's Murine Encephalomyelitis Virus Capsid Proteins

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SUMMARY

Humoral antibody responses to Theiler's murine encephalomyelitis virus (TMEV) capsid proteins were examined. Rabbit antisera produced against the native BeAn strain of TMEV and against the isolated capsid proteins (VP1, VP2 and VP3) were tested for their ability to bind or neutralize virus and to inhibit the virus-induced haemagglutination of human O+ erythrocytes. Western immunoblotting analysis showed that isolated VP1, VP2 and VP3 each primed for a specific antibody response, but that native virions primed for antibodies specific for VP1 and VP2, but not VP3. Virus neutralization studies revealed that a dominant TMEV neutralizing determinant(s) lay on VP1, as did the haemagglutinating determinant. The possible location of the neutralizing epitopes are discussed on the basis of molecular modelling of the predicted amino acid sequence of TMEV from that of the closely related Mengo virus for which the three-dimensional structure is known.

The Theiler's murine encephalomyelitis viruses (TMEV) are members of the cardiovirus group of the picornavirus family (Pevear et al., 1987). Following intracerebral inoculation, certain mouse strains develop a chronic, inflammatory demyelinating disorder of the central nervous system beginning 1 to several months post-infection (Theiler, 1937; Theiler & Gard, 1940; Lipton, 1975; Dal Canto & Lipton, 1976; Lehrich et al., 1976). TMEV-induced demyelinating disease has been considered one of the best experimental models of human multiple sclerosis (MS) due to histopathological and genetic similarities to MS, its immune-mediated effector nature (Lipton & Dal Canto, 1976; Roos et al., 1982a, b; Clatch et al., 1985) and to epidemiological studies favouring a viral aetiology of MS (Nathanson & Miller, 1978; Kurtzke & Hyllested, 1986). Although TMEV-infected mice display chronically elevated systemic virus-specific neutralizing antibody responses, low levels of infectious virus can be isolated for virtually the lifetime of the mouse (Lipton, 1975; Lehrich et al., 1976; Lipton et al., 1984).

TMEV, like all picornaviruses, is composed of a single-stranded, positive polarity RNA encapsidated in an icosahedral protein coat formed from 60 copies of each of four proteins: VP1, VP2, VP3 and VP4. Essentially nothing is known about the contribution of the three external capsid proteins (VP1, VP2 and VP3) to induction of humoral antibody responses for the cardioviruses. VP4 is buried beneath the surface of the virion in close association with the viral RNA and is thus presumed to be inaccessible to antibodies (Luo et al., 1987). Previous studies of other picornaviruses have shown that, depending on the virus, one, two or all three of the external virus proteins can be the target of neutralizing antibody responses (Bachrach et al., 1975; Beatrice et al., 1980; Bittle et al., 1982; Blondel et al., 1982; Cartwright et al., 1982; Chow...
Short communication

Fig. 1. Specificities of anti-TMEV capsid protein antisera by Western blot analysis. Purified BeAn virions (a) or DA-infected cell lysates (b) were electrophoresed, blotted onto nitrocellulose paper and reacted with the following antibodies: rabbit antisera prepared against native BeAn virus (lanes 1); rabbit antisera prepared against the purified BeAn capsid proteins, VP1 (lanes 2), VP2 (lanes 3) and VP3 (lanes 4); (c) normal rabbit serum. Thereafter the immunoblot was overlaid with peroxidase-conjugated anti-rabbit IgG and developed with diaminobenzidine and hydrogen peroxide.

& Baltimore, 1982; Dernick et al., 1983; Emini et al., 1983; Laporte et al., 1973; Meloen et al., 1979; Strohmaier et al., 1982; van der Marel et al., 1983). In this report, we examine the capacity of monospecific antisera, generated against each of these three external TMEV capsid proteins, to bind or neutralize virus, and to inhibit the virus-induced haemagglutination of human O+ erythrocytes.

The DA and BeAn 8386 strains of TMEV were grown in BHK cells and purified by isopycnic centrifugation on Cs2SO4 gradients as previously described (Lipton & Friedmann, 1980; Roos et al., 1982b). Individual BeAn capsid proteins were isolated by electroelution from SDS-polyacrylamide gels as previously described (Stralfors & Belfrage, 1983). Monospecific antisera were prepared as follows. Two-hundred μg of BeAn or individual BeAn capsid proteins emulsified in complete Freund’s adjuvant were injected subcutaneously into rabbits. Three 100 μg booster injections in incomplete Freund’s adjuvant were given at 4 week intervals. The rabbits were bled prior to immunization and 10 days following each booster immunization and the sera were stored at −80 °C.

To determine the antigenic specificities of the anti-BeAn virus and anti-capsid protein antisera, Western immunoblotting analysis was carried out as previously described (Nitayaphan et al., 1985). As seen in Fig. 1 (a) antisera from rabbits immunized with VP1 (lane 2), VP2 (lane 3), or VP3 (lane 4) reacted specifically with their respective proteins from the BeAn strain. A
Table 1. Antibody titres of rabbit monospecific antisera to TMEV capsid proteins

<table>
<thead>
<tr>
<th>Immunization</th>
<th>Neutralizing antibody titre at post-immunization bleed*</th>
<th>ELISA titre†</th>
<th>HAI titre‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole BeAn</td>
<td>640 2560 10240</td>
<td>51200</td>
<td>6400</td>
</tr>
<tr>
<td>VP1</td>
<td>1280 2560 5120</td>
<td>1600</td>
<td>2400</td>
</tr>
<tr>
<td>VP2</td>
<td>80 160 160</td>
<td>1600</td>
<td>200</td>
</tr>
<tr>
<td>VP3</td>
<td>40 80 80</td>
<td>3200</td>
<td>200</td>
</tr>
<tr>
<td>Pre-immunization</td>
<td>≤40 ≤40 ≤40</td>
<td>≤200</td>
<td>≤200</td>
</tr>
</tbody>
</table>

* Sera for neutralizing assays were collected at 38 days (1st bleed), 66 days (2nd bleed) and 94 days (3rd bleed) following immunization.
† ELISA titres were determined on sera collected 94 days following immunization.
‡ HAI assays were determined on sera collected 94 days following immunization.

similar pattern was observed when the same antisera were reacted with the serologically cross-reactive DA strain (Fig. 1b). Anti-VP2 also reacted with the VP0 protein, the precursor of VP2 and VP4 (Rueckert & Wimmer, 1984), of both the BeAn and DA strains. Antisera to the intact BeAn virus reacted with VP0, VP1 and VP2, but failed to recognize VP3 (Fig. 1a and b, lane 1). The data thus reveal that isolated VP1, VP2 and VP3 each primed for a specific antibody response. However, in the context of the intact virion, VP3 played a minimal role in eliciting a rabbit antibody response as measured by Western immunoblotting. Similar results have been consistently observed with other rabbit anti-BeAn antisera as well as with some mouse antisera (data not shown).

We next assessed the ability of the rabbit antisera to neutralize live BeAn virus using a standard plaque reduction assay. Twofold dilutions of heat-inactivated (56 °C, 30 min) serum were incubated with 100 to 200 p.f.u. of BeAn virus at 24 °C for 60 min. Each mixture was assayed in duplicate on BHK-21 cell monolayers as previously described (Rabinowitz & Lipton, 1976). An 80% reduction in the number of plaques was considered significant neutralization. As seen in Table 1, antisera against intact BeAn virus and against isolated VP1 effectively neutralized live virus. The titres of both antisera increased with subsequent booster immunizations, indicative of an anamnestic response. Rabbit anti-VP2 antisera showed consistent neutralizing activity at low dilutions, but increasing titres were not observed upon repeated immunization. Anti-VP3 antisera failed to demonstrate significant neutralizing activity. These data suggest that a dominant TMEV neutralization site(s) resides on VP1 as determined by immunization with isolated VPs, but do not exclude the possibility that other conformational epitopes may be denatured by the VP isolation procedure.

In light of the poor to non-existent neutralizing activity of antisera raised to VP2 and VP3, we assessed the ability of these antisera to bind to TMEV using an ELISA (Lipton et al., 1983). Although it was clear that antisera raised against each of the isolated capsid proteins recognized viral determinants exposed under the mildly denaturing conditions of the ELISA (Table 1), in no case did the titre of any of the monospecific antisera approach the ELISA titre of antisera raised to intact virions. Interestingly, the ELISA titre of the monospecific anti-VP3 serum was equal to or greater than those of the sera raised against the other capsid proteins, although hyperimmune serum to intact virus did not react with VP3 on Western immunoblots (Fig. 1). This may suggest that a VP3 binding determinant, not accessible on intact virus, was exposed as a result of the VP isolation procedure.

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The capacity of the rabbit antisera to inhibit haemagglutination of human type O+ erythrocytes by TMEV (Olitsky & Yeager, 1949; Lahelle & Horsfall, 1949; Lipton, 1978) was tested. The antisera were treated with a filtrate of a Pseudomonas species (Schmidt et al., 1964) to inactivate non-specific inhibitors of haemagglutination, and heat-inactivated at 56 °C for 30 min prior to determination of the haemagglutination inhibition (HAI) titre (Rosen, 1960). As seen in Table 1, antisera to whole virus and to VP1, but not to VP2 or VP3, exhibited significant
HAI activity. Thus, VP1 contained both a major neutralizing epitope(s) and a haemagglutinating determinant of TMEV. This may indicate that a previously described TMEV-specific monoclonal antibody having both neutralizing and HAI activity may be directed against VP1 (Nitayaphan et al., 1985). The haemagglutinating determinant of encephalomyocarditis virus, a close relative of TMEV, has recently been shown to recognize the glycophorin A protein on human erythrocytes (Angel & Burness, 1977; Allaway & Burness, 1986).

The Western immunoblot and ELISA data indicated that immunization of rabbits with all three external TMEV VPs, isolated by electroelution from SDS-polyacrylamide gels, led to the induction of potent monospecific anti-VP antibody responses. In contrast, immunization with intact TMEV led to easily demonstrable antibody responses against intact virions, VP1 and VP2, but not against isolated VP3. Virus neutralization studies utilizing the monospecific anti-VP antisera indicated that VP1, and possibly VP2, contain one or more neutralizing immunodominant determinants. However, the failure of antiserum raised upon immunization with isolated VP3 to neutralize TMEV does not prove that it does not contain neutralizing determinants as the isolation procedure may have altered or destroyed these putative neutralizing sites. Monoclonal antibody-resistant mutant isolation studies employing other picornaviruses have indicated that any of the external virion proteins may contain neutralization determinants. For example, poliovirus and rhinovirus have been shown to bear neutralizing epitopes on all three external virion proteins, and complex epitopes may be formed by more than one protein (Minor et al., 1986; Sherry et al., 1986). Studies employing isolated capsid proteins have yielded similar results. Poliovirus and foot-and-mouth disease virus (FMDV) bear neutralization epitopes on VP1, as immunization with purified VP1 protects animals from live virus challenge (Bachrach et al., 1975; Blondel et al., 1982; Cartwright et al., 1982; Chow & Baltimore, 1982; Dernick et al., 1983; Emini et al., 1983; Laporte et al., 1973; Meloen et al., 1979; van der Marel et al., 1983). Similarly, only VP1 from Mengo virus is capable of generating neutralizing antibody as measured by a plaque reduction assay (Lund et al., 1977). In addition, VP2 and VP3 of poliovirus, but not from FMDV, induce neutralizing antibodies in laboratory animals (Blondel et al., 1982; Chow & Baltimore, 1982; Dernick et al., 1983; Emini et al., 1983; van der Marel et al., 1983). Conversely, with respect to coxsackievirus B3, VP2 is apparently the only isolated capsid protein capable of inducing neutralizing antibodies in rabbits (Beatrice et al., 1980).

It is interesting to speculate on the possible location of the neutralizing epitopes on the TMEV capsid proteins. Preliminary data, based on alignment (Devereux et al., 1984) of the predicted amino acid sequence of TMEV (Pevear et al., 1987) with that of human rhinovirus 14 and the more closely related Mengo virus, whose three-dimensional structures have been solved (Luo et al., 1987), has allowed us to identify hypervariable regions on each external virion protein which are potential neutralizing determinants. The following potential sites have been identified: VP1, amino acids 50 to 70 and 74 to 110; VP2, amino acids 137 to 170; VP3, amino acids 56 to 64. Structurally, by alignment with Mengo virus, these areas are looped out from the virion surface and are thus prime targets for neutralizing antibodies (Rossmann et al., 1985; Sherry et al., 1986). It will be of great interest to compare the actual neutralizing determinants identified by a panel of neutralizing monoclonal anti-TMEV antibodies (study in progress) to those predicted by sequence alignments with Mengo virus.

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