Antigenic and Immunogenic Properties of Defined Physical Forms of Tick-Borne Encephalitis Virus Structural Proteins

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Polymeric, delipidated glycoprotein complexes of defined size and composition were prepared from tick-borne encephalitis virus by solubilization with Triton X-100 or cetyltrimethylammonium bromide, followed by centrifugation into detergent-free sucrose density gradients. The antigenic reactivities and immunogenicities of these complexes were compared with those of complete inactivated virus. These glycoprotein preparations induced hemagglutination-inhibiting and neutralizing antibodies which proved to be protective in passive mouse protection tests and monospecifically reacted only with the viral envelope and not with the internal core. In a competitive radioimmunoassay the glycoprotein complexes revealed about 10-fold higher antigenicity than whole virus when tested at equal protein concentrations. The important implications of these results with respect to antigen quantification in vaccines are discussed. As shown in the mouse challenge potency test, glycoprotein complexes prepared after Triton X-100 solubilization actively protected mice almost as well as did complete inactivated virus at the same protein concentration, whereas those prepared after cetyltrimethylammonium bromide solubilization had a somewhat lower protective activity per microgram of protein.

The Flavivirus genus of togaviruses comprises several members which cause severe human diseases, including yellow fever, Japanese encephalitis, dengue hemorrhagic fever, Saint Louis encephalitis, Rocio and tick-borne encephalitis (TBE) viruses. TBE is the most important arthropod-transmitted disease in Europe, and in certain countries like Austria or Czekoslovakia it represents a major public health problem. Similar to other flaviviruses, TBE virus contains only three structural proteins (V₁, V₂, V₃) with molecular weights of 8,000, 15,000, and 55,000, respectively (7). V₃, which is the only structural glycoprotein, forms the viral envelope with V₁ and lipid, and V₂ is the only protein constituent of the core (8) which also contains single-standard ribonucleic acid of messenger polarity (for review, see reference 16). Treatment of the purified virus with nonionic detergents separates the envelope from the core. At low detergent-to-protein ratios large fragments are obtained in which the lipid still remains associated with V₁ and V₃, whereas at higher concentrations delipidation of the envelope proteins occurs and a dimeric, hemagglutinating glycoprotein complex can be isolated by density gradient centrifugation in the presence of detergent (8). Ionic detergents like sodium deoxycholate or cetyltrimethylammonium bromide (CTAB) not only solubilize the envelope but also cause disintegration of the core (8).

The physical form is one of the critical determinants for the immunogenicity of antigens, and large polymeric structures are usually more immunogenic than small monomeric proteins (4, 15, 22). Using sodium deoxycholate, Della-Porta and Westaway (5) prepared large membrane fragments of 100 to 120S from another flavivirus, Kunjin, which proved to be immunogenic, whereas small envelope fragments obtained at higher detergent concentration did not elicit an immune response. As we have shown in experiments using protease treatment of purified virus (7), V₃ from TBE virus is an amphiphilic protein with a hydrophobic tail of molecular weight 4,000 to 6,000 which is located inside the lipid bilayer. After solubilization by detergents, such amphiphilic proteins can be reassociated by centrifugation into detergent-free density gradients to form water-soluble, polymeric protein complexes of defined size and sedimentation behavior which contain very little lipid and detergent (11, 19). We have recently used this method to prepare exactly characterized multimeric glycoprotein complexes from TBE virus after solubilization with different detergents (9), and the present paper deals with the antigenic and immunogenic characterization of such preparations.
derived from TBE virus.

MATERIALS AND METHODS

Growth and purification of TBE virus. A TBE virus strain (term ed Neudorf) isolated from a tick in Austria was plaque purified and used throughout this study. Essentially as described previously (6), the virus was grown in primary chicken embryo cells, harvested at about 40 h postinfection, concentrated by ultracentrifugation, and purified by rate zonal centrifugation followed by equilibrium density gradient centrifugation in sucrose gradients. The buffer used throughout was 0.05 M triethanolamine–0.1 M NaCl, pH 8.0 (TAN). The purified virus from the sucrose gradient was dialyzed against TAN and stored at −80°C until used.

Preparation of polymeric glycoprotein complexes, cores, and Formalin-inactivated virus. Glycoprotein complexes were prepared essentially as described in detail previously (9). Briefly, purified TBE virus (200 μg/ml) in TAN (pH 8.0) was treated with Triton X-100 (TX-100 or CTAB (Serva, Heidelberg, Germany) at final concentrations of 0.2% and 0.1%, respectively, for 1 h at room temperature. These samples were layered onto 10 to 50% (wt/wt) sucrose density gradients with a layer of 5% (wt/wt) sucrose containing half the amount of detergent used for solubilization. Centrifugation was carried out in a 6 × 14–

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Prepared cores were recovered from the same gradient as the pellet after TX-100 solubilization. They were suspended in TAN (pH 8.0), sonicated, and stored at −80°C. For the inactivation of purified virus, Formalin was added to a final dilution of 1:2,000 and the samples were incubated at 37°C for 24 h.

Competitive radioimmunoassay. Purified TBE virus was labeled either by the chloramine-T method (12) or with Bolton-Hunter reagent (1) obtained from the Radiochemical Centre, Amersham, England. The buffer used throughout this assay was 0.02 M tris(hydroxymethyl)aminomethane–0.13 M NaCl–0.5% bovine serum albumin–0.02% NaN3, pH 7.5 (RIA buffer).

(i) Antibody titration. A 100-μl volume of RIA buffer (instead of antigen) was mock-reacted with 50 μl of various dilutions of a rabbit immune serum against purified live TBE virus for 1 h at 37°C, and then 20,000 counts of chloramine-T-labeled or 5,000 counts of Bolton Hunter reagent-labeled TBE virus in 50 μl of RIA buffer was added and incubated overnight at 4°C. For the separation of bound from free label, 50 μl of Immunobeads (Bio-Rad Laboratories, Richmond, Calif.) containing goat anti-rabbit immunoglobulins was added according to the manufacturer’s instructions and incubated for 2 h at 37°C. The beads were then pelleted by centrifugation at 2,000 × g for 5 min at room temperature and washed three times in RIA buffer before being counted. From the antibody titration curve obtained, the dilution which resulted in 50% of the maximal binding was determined to be 1:100,000.

(ii) Competitive test. A 100-μl sample of test antigen diluted 10-fold in RIA buffer from 10 μg/ml to 1 ng/ml was incubated with 50 μl of antisera (diluted 1:100,000 to give 50% of maximal binding as determined by antibody titration) for 1 h at 37°C. Exactly the same procedure was then followed as described above for antibody titration.

Immunization of rabbits. One milliliter of antigen preparation was emulsified in complete Freund adjuvant and injected subcutaneously into a rabbit. Two booster doses 4 weeks apart were given, using the same antigen preparation emulsified in incomplete Freund adjuvant. After another 2 weeks, blood was taken by ear vein puncture, and the serum was stored at −20°C.

Mouse challenge potency test. Groups of 10 mice weighing about 10 g each were immunized subcutaneously with 0.2 ml of antigen diluted in threefold steps in phosphate-buffered saline (pH 7.2) and received a second dose after 1 week. The mice were challenged intraperitoneally with 100 to 1,000 50% lethal doses of TBE virus after a further 2 weeks. After 3 weeks of observation, results were calculated by the method of Reed and Muench (17).

Enzyme immunoassay. Polystyrene U-shaped microtiter plates (Nunc, Kamstrup, Denmark) were coated with either complete virus, glycoprotein complexes obtained after TX-100 and CTAB solubilization, or cores by adding 50 μl per well of each antigen diluted to 2 μg/ml in carbonate buffer (pH 9.6) and incubating overnight at 4°C. The plates were then emptied and phosphate-buffered saline (pH 7.5) containing 2% sheep serum was added and incubated for 1 h at 37°C. The wells were emptied again and to each well was added 50 μl of twofold dilutions of the test serum in phosphate-buffered saline (pH 7.4) containing 2% sheep serum, 2% Tween 20, and 0.02% NaN3. The sera were allowed to react for 2 h at 37°C. The plates were then washed three times with phosphate-buffered saline (pH 7.4) containing 0.2% sheep serum, and 50 μl of alkaline phosphatase-labeled anti-rabbit immunoglobulin G (Orion Diagnostika, Helsinki, Finland) in phosphate-buffered saline (pH 7.4)–2% sheep serum–2% Tween 20–0.02% NaN3 was added per well and incubated for 2 h at 37°C. The plates were again washed three times as described above, and then 100 μl of substrate (p-nitrophenylphosphate; Sigma 104 phosphatase substrate tablets, 1 mg/ml in 10% diethanolamine buffer, pH 9.8) was added per well. The enzyme reaction was allowed to proceed for 30 min at room temperature and was then stopped by the addition of 100 μl of 3 N NaOH. The absorbance at 405 nm was measured with a multichannel photometer (Miltiskan, Flow Laboratories, Bonn, Germany).

HA and HAI. Hemagglutination (HA) and HA inhibition (HAI) tests were performed at pH 6.4 using goose erythrocytes essentially as described by Clarke and Casals (2).

Neutralization test. The neutralization test was performed in flat-bottomed microtiter tissue culture plates (Nunc). Twofold dilutions of rabbit antiserum (25 μl) were mixed with an equal volume of TBE virus
diluted to yield 100 to 1,000 50% tissue culture infective doses. After an incubation period of 3 h at 37° C, 0.1 ml of secondary chicken embryo cells (10⁶ cells per ml) was added and further incubated at 37° C. Results were read after 5 days. Each serum dilution was tested in triplicate.

**Passive protection test.** Fourfold dilutions of rabbit immune sera were injected intraperitoneally into groups of 10 mice weighing 15 g each (0.2 ml per mouse). After 24 h the mice were challenged intraperitoneally with 100 to 1,000 50% lethal doses of TBE virus. The results were calculated by the method of Reed and Muench (17) after 3 weeks of observation.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 15% slab gels was performed using the buffer system described by Laemmli and Favre (14). For sample preparation the proteins were precipitated with 10% trichloroacetic acid, washed twice with acetone, suspended in sample buffer, and placed in a boiling water bath for 5 min. Gels were stained with Coomassie brilliant blue R-250 (0.2% in 10% acetic acid–50% methanol) and destained in 10% acetic acid–10% methanol.

**Protein determination.** Protein was determined by the method of Schaffner and Weissemann (18), using bovine serum albumin as a standard.

**Electron microscopy.** One drop of each sample was placed on grids with carbon-coated Formvar films and allowed to adsorb for 1 min. The specimens were then negatively stained with 1% aqueous uranyl acetate (pH 4.4) for 1 min and examined in a Zeiss (Oberkochen, Germany) electron microscope (EM10).

**RESULTS**

**Preparation and characterization of polymeric glycoprotein complexes and cores.** Purified TBE virus was treated with TX-100 or CTAB and subjected to centrifugation into detergent-free sucrose density gradients for reassociation of the solubilized glycoprotein and the formation of polymeric complexes (“rosettes”) as described in Materials and Methods. As we have shown previously (9), rosettes obtained after TX-100 solubilization contain less than 1% lipid and bound detergent by weight and sediment at 15 to 16S. After CTAB solubilization the lipid content is about 2%, and the complexes sediment at 11 to 12S. Purified cores were recovered from the pellet of the gradients after TX-100 solubilization.

Figure 1 shows the polypeptide composition of the different preparations. Purified TBE virus revealed the three structural proteins V⁰, V₂, and V₃ (Fig. 1a). Rosettes obtained after TX-100 solubilization contained both V₃ and V₁ (Fig. 1b), whereas V₁ was not detectable in those obtained after CTAB treatment (Fig. 1d). V₂ was found exclusively in the core preparation (Fig. 1c).

Electron micrographs of negatively stained purified TBE virions revealed particles with a diameter of about 50 nm carrying a fringe of small surface projections which, however, are much less prominent than those found with myxoviruses (Fig. 2A). Isolated cores showed a diameter of about 29 nm (Fig. 2B). Rosette preparations obtained after TX-100 as well as CTAB solubilization both revealed roundish particles with a diameter of about 17 nm in which single subunits are radially arranged (Fig. 2C and D). As shown by Simons et al. (19), such complexes most likely resemble detergent micelles, with the apolar moiety of the protein located in the interior and the hydrophilic part forming the surface.

**Antigenic reactivity.** In a competitive radioimmunoassay, live virus, Formalin-inactivated virus, and glycoprotein rosettes obtained after TX-100 and CTAB solubilization were allowed to compete with radiolabeled live virus for a limited amount of rabbit antiserum against live virus (Fig. 3). This test therefore compares the reactivities of the antigenic determinants present on the surface of live virus with those after Formalin treatment or solubilization and reassociation of glycoproteins. Since iodination by itself chemically alters the native protein and therefore theoretically could obscure differences occurring at the reactive site involved, we used two different iodination methods which label the protein at different reactive groups: chloramine-T predominantly mediates iodination of tyrosine, and the Bolton-Hunter reagent reacts with lysine and terminal amino groups. Formalin-inactivated virus showed exactly the same competition curve as live virus (Fig. 3), irrespective...
of whether the virus was labeled with chloramine-T or the Bolton-Hunter reagent, indicating excellent preservation of antigenic reactivity. Although used at the same protein concentration, the glycoprotein complexes were much more efficient competitors than whole virus. As deduced from their competition curves, only about one-tenth the amount of protein was needed to give the same degree of competition as whole virus. The slopes of the curves were the same, indicating equal affinities of reacting antigenic determinants for the antiserum against live virus. Slight differences could be observed between the reactivities of rosettes obtained after TX-100 and CTAB solubilization (Fig. 3A and B). When the chloramine-T method was used for radioiodination, the competition curve for CTAB rosettes was slightly shifted to the left, and competition is probably not 100% as with TX-100 rosettes. In the case of labeling with the Bolton-Hunter reagent, the curves were superimposed, but at high concentrations again a small fraction of antibodies did not seem to react with CTAB rosettes.

**Immunogenicity: immunization of rabbits.** To obtain high-titer antibodies to be used for the qualitative serological characterization of the immunizing antigens, rabbits were immunized as described in Materials and Methods with Formalin-inactivated whole virus, with glycoprotein complexes obtained after TX-100 or CTAB solubilization, or with cores. The specificity of the resulting immune sera was assayed in an enzyme immunoassay in which the solid phase was coated with the different antigens used for immunization (Fig. 4). Antiserum against whole virus contained antibodies against the glycoprotein as well as the internal core (Fig. 4A), whereas antisera against glycoprotein complexes were monospecific and gave a positive reaction only when the solid phase was coated with glycoprotein preparations or whole virus (Fig. 4B and C). Conversely, the core antiserum monospecifically reacted only with the core but not with the viral glycoprotein preparations (Fig. 4D). From the antibody titration curves it appears that antisera to complete virus (Fig. 4A) and TX-100 rosettes (Fig. 4B) exhibited a some-

![Fig. 2. Electron microscopy of preparations derived from TBE virus for immunization. (A) Complete virus; (B) viral core; glycoprotein complexes obtained (C) after TX-100 solubilization and (D) after CTAB solubilization. Negative staining with uranyl acetate was performed as described in the text. Bar, 100 nm.]
what weaker reactivity against CTAB rosettes than against their homologous antigens, whereas no differences were observed with the immune serum against CTAB rosettes (Fig. 4C). This may be an indication of some slight damage in the native antigenic pattern of the glycoprotein, caused by CTAB solubilization. These immune sera were also tested in HAI, neutralization, and passive mouse protection tests. As can be seen from Table 1, the antisera against glycoprotein complexes contained HA-inhibiting and neutralizing antibodies similar to the serum against complete inactivated virus, and these also proved to passively protect mice from lethal challenge. No such antibodies were induced by purified cores. These results have shown qualitatively that neutralizing and protective antibodies can only be induced by glycoprotein-containing structures.

The preparations used for immunization represent exactly defined physical forms of the viral structural proteins arranged either as complete virus, rosette-like glycoprotein complexes, or spherical (most likely icosahedral) core. It was therefore of great interest to quantitatively compare their immunogenicities when used at equal protein concentration.

First, groups of 10 mice received three 0.2-ml doses of antigen 2 weeks apart at a protein concentration of 10 μg/ml either without or with 2 mg of Al(OH)₃ per ml as adjuvant (Table 2). The collected immune sera were tested individually in the HAI test for the calculation of geometric mean titers and as a pool in the neutralization test. Glycoprotein complexes induced almost equally high antibody titers as complete virus when used at the same protein concentration; complexes obtained after CTAB solubilization probably had a slightly decreased immunogenicity. An adjuvant effect of Al(OH)₃ could be demonstrated in the HAI test when individual mouse sera were tested but did not show up in the neutralization test when serum pools were used. Again the purified core did not induce detectable HA-inhibiting or neutralizing antibodies.

The immunogenic activities were also compared in a direct mouse challenge potency test. The results shown in Table 3 represent the mean values of three individual tests. Mice could be actively protected by complete virus as well as by glycoprotein preparations, which, however, seemed to be slightly less efficient: those obtained after CTAB solubilization had a lower immunogenic activity per microgram of protein than did complete inactivated or TX-100-solubilized virus. Mice could not be protected by the core when used at the same protein concentration.

**DISCUSSION**

The isolation of membrane proteins in a native conformation by the use of detergents critically depends on the type of detergent used and the ratio of detergent to protein applied for solubilization (10). Conformational changes due to detergent binding will result in changes of antigenic determinants, and for the isolation of membrane proteins in an immunogenic form, especially mild conditions have to be selected which maximally preserve biological activity. In most instances nonionic detergents conserve native structures much better than ionic detergents.
The second important point determining the capability of an antigen to induce a strong immune response seems to be its presentation to the immune system in a multimeric form (4, 15). The importance of these parameters is also reflected in different reports on the isolation of immunogenic subunits from flaviviruses. Stohlman et al. (20) compared the antigenicity of membrane-bound V₃ from dengue virus with that after purification by binding to concanavalin A and elution in a sodium deoxycholate-containing buffer. The solubilized form of the glycoprotein had lost its capability to block neutralizing antibodies, and, as suggested by the authors, this is most likely due to conformational changes caused by detergent binding. Also Kitano et al. (13), working with Japanese encephalitis virus, observed a strong reduction of HA activity after treatment with sodium deoxycholate and obtained much better results with the nonionic Nonidet P-40. Upon CsCl centrifugation, however, the solubilized membrane preparation proved to be heterogeneous and could be separated into denser and less dense components presumably due to different lipid contents. Della-Porta and Westaway (5) prepared large, incompletely dissociated membranous fragments sedimenting at 100 to 120 S from Kunjin virus by disrupting the virus with low concentrations of sodium deoxycholate and compared their immunogenicity with small fragments obtained at high sodium deoxycholate concentrations. Only the large fragments elicited a detectable immune response in rabbits. Glycoproteins from different flaviviruses prepared by isoelectric focusing in the presence of the nonionic detergent Triton N-101 showed excellent preservation of their immunological reactivity (21).

We have prepared multimeric glycoprotein complexes of defined size, shape, sedimentation rate, and chemical composition from TBE virus by using complete solubilization of the envelope with different detergents, followed by reassociation of the delipitated glycoproteins via their hydrophobic ends by means of centrifugation into detergent-free sucrose density gradients (9). Comparison of the antigenicity of such complexes obtained after TX-100 and CTAB solubilization by competitive radioimmunoassay revealed an excellent preservation of immunological reactivity. The slopes of the competition curves were identical to those obtained with live virus, indicating that no substantial damage had occurred to those antigenically reactive determinants which are also present on the surface of complete virions. In the case of CTAB rosettes,
but not TX-100 rosettes, a small percentage of antibodies present in the serum against whole virus seemed to persist that could not be bound by CTAB rosettes. As has been shown previously (9), at too high concentrations CTAB causes denaturation, and even at the mild conditions applied for the preparation of rosettes only about 50% of the total solubilized glycoprotein fraction is present in a state that can be reassociated into a polymeric form. Therefore, slight damage to a conformational antigenic site cannot be excluded. It is striking that in this competitive radioimmunoassay both glycoprotein complexes revealed about 10-fold higher antigenic reactivity per microgram of protein than complete virus. This effect cannot be explained by the lower relative amount of V3 present in whole virus, since, as determined from quantitatively evaluated sodium dodecyl sulfate-gel patterns, about 60 to 70% of the total mass of virus protein is represented by the glycoprotein. It is, however, likely that many of the possible antibody combining sites of V3 cannot react due to steric hinderance or are hidden when the molecule is present as integral membrane protein in the envelope of complete virions. In the form of glycoprotein rosettes, many more binding sites per microgram of protein are available, resulting in a higher specific antigenic reactivity. The immunogenicity of these complexes, however, never exceeded that of complete inactivated virus when used at the same protein concentration, and these results therefore have important implications when immunoassays are used for the quantification of “antigen” present in vaccines. The result will be extremely dependent on the physical state of the antigen(s) present in the vaccine; in our example, the rosette preparations would simulate a 10 times higher “antigenic content” than the whole virus preparation, even though the immunogenicities are at best identical. In less purified vaccines the presence of small soluble antigens in addition to complete virus may contribute much more to the antigenic reactivity than to the immunogenicity of the whole vaccine since the immunizing potential of such soluble antigens may be much lower than that of the whole virus. The results from immunoassays for the quantification and standardization of the amount of antigen in vaccines have therefore to be treated with great caution.

Our present results have shown that by the methods applied we could prepare complexes from structural TBE virus components which were exactly characterized with respect to size, shape, sedimentation rate, lipid content, and polypeptide composition and which proved to

**TABLE 1. Immunization of rabbits: testing of resulting immune sera in HAI, neutralization, and passive protection tests**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>HAI test</th>
<th>Neutralization test</th>
<th>Passive protection test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete virus</td>
<td>640</td>
<td>160</td>
<td>57</td>
</tr>
<tr>
<td>Glycoprotein complexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX-100 rosettes</td>
<td>640</td>
<td>80–160</td>
<td>58</td>
</tr>
<tr>
<td>CTAB rosettes</td>
<td>1,280</td>
<td>320</td>
<td>60</td>
</tr>
<tr>
<td>Core</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

* Immunization of rabbits and assays were performed as described in the text.

* Results represent reciprocal of that serum dilution which protected 50% of the immunized animals.

* Formalin inactivated.

**TABLE 2. Immunization of mice without and with Al(OH)3 as an adjuvant: testing of resulting immune sera in HAI and neutralization tests**

<table>
<thead>
<tr>
<th>Antigen (10 µg/ml)</th>
<th>HAI test</th>
<th>Neutralization test</th>
<th>Passive protection test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No adjuvant</td>
<td>Al(OH)3</td>
<td>No adjuvant</td>
</tr>
<tr>
<td>Complete virus</td>
<td>44</td>
<td>71</td>
<td>80</td>
</tr>
<tr>
<td>Glycoprotein</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>complexes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX-100 rosettes</td>
<td>37</td>
<td>76</td>
<td>40–80</td>
</tr>
<tr>
<td>CTAB rosettes</td>
<td>33</td>
<td>45</td>
<td>40–80</td>
</tr>
<tr>
<td>Core</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

* Mice were immunized as described in the text.

* Results are expressed as geometric mean titer of 10 individual sera.

* Formalin inactivated.

**TABLE 3. Mouse challenge potency test**

<table>
<thead>
<tr>
<th>Antigen (15 µg/ml)</th>
<th>Titer</th>
<th>Dose (µg of protein) protecting 50% of mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete virus</td>
<td>58</td>
<td>0.1</td>
</tr>
<tr>
<td>Glycoprotein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>complexes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX-100 rosettes</td>
<td>45</td>
<td>0.14</td>
</tr>
<tr>
<td>CTAB rosettes</td>
<td>23</td>
<td>0.26</td>
</tr>
<tr>
<td>Core</td>
<td>&lt;3</td>
<td>—</td>
</tr>
</tbody>
</table>

* The test was performed as described in the text.

* Expressed as the reciprocal of that antigen dilution which protects 50% of the mice.

* Formalin inactivated.

* Core does not protect.
be excellent immunogens. Only the immune serum directed monospecifically against the viral glycoprotein contained HA-inhibiting, neutralizing, and protective antibodies. A quantitative comparison of the protective activity per microgram of protein in a direct mouse challenge potency test revealed that glycoprotein complexes obtained after TX-100 solubilization protected mice almost equally well as did complete inactivated virus, whereas a somewhat lower activity was observed when CTAB was used for solubilization. The amount of glycoprotein complexes (TX-100) applied in two doses which protected 50% of the mice from a lethal challenge was calculated to be 0.14 μg. Similar protection experiments with an octameric spike-glycoprotein complex from Semliki Forest virus revealed that 0.5 μg of that preparation applied in a single dose protected 50% of the mice (15). Taking into account that the same amount of antigen applied in two doses probably is more efficient than a single dose, these figures compare very well and are at least in the same order of magnitude. A dramatic increase in immunogenic activity for the isolated glycoprotein of rabies virus compared to whole virus was reported by Cox et al. (3). As little as 0.009 μg of a glycoprotein preparation obtained by isoelectric focusing in the presence of TX-100 was sufficient to protect 50% of the mice from lethal challenge, compared to 1.63 μg of complete inactivated virus. In the case of polymeric glycoprotein complexes from TBE virus, we never did observe such an increased immunogenic activity per microgram of protein compared to inactivated whole virus, which seems to represent an almost ideal form for immunization since the immunologically relevant glycoprotein is presented in multiple copies on a large lipid-containing carrier. A similar protective activity could also be achieved, however, by the use of delipidated reassOCIated glycoprotein preparations. Their effectiveness most likely depends on the use of mild conditions for the dissociation of the viral envelope and the reaggregation after solubilization into a multimeric protein complex.

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LITERATURE CITED


