Key words: Bunyaviridae/enhancement/antibodies

Relationship between Glycoproteins of the Viral Envelope of Bunyaviruses and Antibody-dependent Plaque Enhancement

(Accepted 22 June 1982)

SUMMARY

Hamster antisera against three parental bunyaviruses, Batai, Bunyamwera and Maguari viruses, and six recombinant viruses which carried the nucleocapsid protein of one parent and the glycoproteins of the other, have been tested for their interaction with each of the nine viruses under study by two assays, plaque reduction neutralization and antibody-dependent plaque enhancement. Neutralization was clearly related to the specificity of the parental glycoproteins rather than the nucleoprotein, but the antibody-dependent plaque enhancement assay showed greater cross-reactivity.

The phenomenon of antibody-mediated enhancement of virus infectivity (Hawkes, 1964; Halstead & O’Rourke, 1977; Peiris & Porterfield, 1979, 1981) is still not completely understood. The most probable explanation is that virus–antibody complexes bind to Fc receptors, after which virus is internalized and replicated, the yield of virus being greater than that produced by the same amount of virus in the same cells in the absence of antibody. Since it is antibody, rather than virus, which is responsible for the initial phase of attachment, the specificity of the antibodies that are capable of binding to virus without neutralizing infectivity is a matter of considerable interest. This question has been examined in relation to Togaviridae by the use of both conventional and monoclonal antibodies (Chanas et al., 1982; Peiris & Porterfield, 1982; Peiris et al., 1982). An entirely different approach to the same problem is through the use of viruses with segmented genomes in which the genome segment coding for determinants responsible for neutralizing activity is separable from genome segments controlling other virus functions. Such is the case with Bunyaviridae (Bishop et al., 1980), a family of viruses which have three species of RNA, large (L), medium (M) and small (S). The M virus RNA codes for the two envelope glycoproteins (G1 and G2), which are known to elicit neutralizing antibodies (Gentsch et al., 1980), while the S virus RNA codes for the internal nucleocapsid protein (N) (Gentsch & Bishop, 1978) and the L virus RNA probably codes for the large, internal, nucleocapsid-associated protein (L). Iroegbu & Pringle (1981) prepared a number of recombinants between Batai, Bunyamwera and Maguari viruses, three serologically related but distinct members of the Bunyavirus genus in which they were able to show that the glycoproteins were derived from one parent, while the nucleocapsid protein was derived from a different parent. When they tested the three wild-type and six recombinant viruses against antisera prepared against the three parental strains, they found a pattern of neutralization which followed the specificity of the glycoproteins rather than the nucleocapsid proteins.

We obtained from Dr C. R. Pringle the three wild-type bunyaviruses and six recombinant viruses. In the subsequent description each virus is defined by a six letter cryptogram, in which the first three letters indicate the origin of the virus glycoproteins, and the second three letters the origin of the nucleocapsid protein. Thus, ‘BUN.BUN’ represents the wild-type Bunyamwera virus, whilst ‘BAT.MAG’ represents a recombinant having its glycoproteins derived from Batai virus and its nucleocapsid protein derived from Maguari virus. We prepared antisera against each of the three wild-type viruses and the six recombinant viruses by giving hamsters a single intramuscular inoculation of live virus without adjuvant, the animals being bled on day 28 (Lindsey et al., 1976). We used all nine viruses and nine antisera to examine homologous and heterologous virus antibody interactions using two different assays. One was a conventional plaque reduction neutralization test, carried out in the pig kidney (PS) (Madrid & Porterfield, 1969) or monkey kidney (Vero) cell line, and the other was the antibody-dependent plaque enhancement (ADPE) assay (Peiris & Porterfield, 1981), carried out in the P388D1 line of...
mouse macrophages, which carry receptors for the Fc portion of immunoglobulin. The results of these tests are shown in Fig. 1 and 2.

As was expected, the neutralization tests showed a clear relationship to the specificity of the virus glycoproteins. This was most evident in the interactions between the three viruses with Bunyamwera-type glycoproteins (BUN.BUN, BUN.BAT and BUN.MAG) which were all neutralized by each of the corresponding three antisera (the central block of nine squares in Fig. 1), but by no other antisera. The three viruses which share Maguari glycoproteins were all neutralized by their three corresponding antisera, but while MAG.BAT virus showed no reactivity with any other serum, Maguari wild-type virus and the MAG.BUN recombinant both showed low-level cross-reactivity with four additional antisera. The three viruses which share BAT glycoproteins showed the least specificity in neutralization tests, but titres were highest with the three antisera prepared against viruses which have BAT glycoproteins. These findings are in agreement with those of Hunt & Calisher (1979) who found that Batai and Maguari viruses were more closely related to each other than either was to Bunyamwera virus, on the basis of plaque reduction neutralization tests carried out with rabbit antisera and mouse ascitic immune fluids. The hamster antisera used in our tests showed less cross-reactivity than was reported by Iroegbu & Pringle (1981) when they tested the same nine viruses against mouse ascitic fluids prepared against the three parental viruses.

When the same nine viruses and antisera were allowed to interact under the conditions of the ADPE assay, far greater cross-reactivity was apparent (Fig. 2). The three viruses with Bunyamwera-type glycoproteins were all enhanced by the three corresponding antisera out to dilutions of $10^{-6}$, except at those antibody dilutions at which neutralizing activity was seen. (Compare the

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**Fig. 1.** Interaction between three wild-type and six recombinant viruses and their respective hamster antisera as determined by plaque reduction neutralization tests. The filled-in fraction of each square represents those antiserum dilutions giving 50% plaque reduction, as shown in the key.
Fig. 2. Interaction between three wild-type and six recombinant viruses and their respective hamster antisera as determined by antibody-dependent plaque enhancement assays. The filled-in fraction of each square represents those antiserum dilutions (see key) that produce threefold or greater enhancement of plaque counts. Where lower antiserum dilutions failed to enhance, these dilutions are indicated by cross-hatching.

Of 27 interactions between pairs of viruses and antisera which shared common glycoproteins, all gave positive results by both neutralization and enhancement assays, whereas of the 54 interactions between viruses and antisera having different glycoproteins 26 showed neutralization but no less than 45 showed enhancement. Applying the same analysis to pairs of viruses and antisera which shared the same nucleoprotein, 19 out of 27 were positive by neutralization, and 20 out of 27 by enhancement; of the 54 pairs with different nucleoproteins, 24 were positive in neutralization tests and no less than 52 were positive by ADPE assay. These findings support the accepted view that neutralizing activity is associated with the specificity of the virus glycoproteins. They also indicate that although all antisera with neutralizing activity against a particular
virus are also capable of enhancing infectivity, many sera without neutralizing activity are also able to enhance. The results are compatible with the hypothesis that any antibodies that bind to virus are able to mediate virus infection of P388D1 cells through Fc receptor uptake.

Although the origins of glycoproteins and nucleoprotein were established by Iroegbu & Pringle (1981) by examination of [35S]methionine-labelled polypeptides in polyacrylamide gel electrophoresis, the contribution of the L RNA segment cannot be assessed since the segregation pattern of the third genome segment in the various recombinants is unknown. Nor is it possible to discriminate between the respective contributions of the G1 and G2 glycoproteins to either neutralization or enhancement since any recombinant will contain both glycoproteins derived from the same parent. Kingsford & Hill (1981) have reported that antibody prepared against the G2 glycoprotein of La Crosse virus failed to neutralize infectivity, but such an antibody would be expected to have enhancing activity. Mutants which affected the coding of only one of the glycoprotein genes without affecting the other, or monoclonal antibodies of appropriate specificity should clarify this point. The extent to which antisera against Bunyaviridae in other genera or in different serogroups within the same genus are capable of mediating ADPE is currently under investigation.

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REFERENCES


(Received 7 May 1982)