Properties of Monoclonal Antibodies Directed Against the Glycoproteins of Sindbis Virus

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SUMMARY

Four monoclonal antibodies that react with Sindbis virus glycoproteins have been examined for (i) their effects on virus infectivity, (ii) their ability to recognize conformational changes in glycoprotein structure, and (iii) their cross-reaction with several different alphaviruses. Two of the monoclonal antibodies reacted with the native forms of the E1 glycoprotein but did not neutralize virus infectivity. One of the anti-E1 antibodies formed an infectious virus–antibody complex. The other two monoclonal antibodies reacted with the E2 glycoprotein in both an unfolded as well as a native structure. One of these antibodies was very effective for blocking virus infectivity. None of these antibodies reacted with Semliki Forest virus. The anti-E1, but not the anti-E2, antibodies cross-reacted with three serologically related equine encephalomyelitis viruses.

In a recent communication (Roehrig et al., 1980), we described the isolation and partial characterization of 11 hybridoma clones that produced antibodies directed against the three structural proteins of Sindbis virions (Strauss & Strauss, 1977). Six of these clones made antibodies against virus capsid and four secreted antibodies (IgG) that reacted with the virus glycoproteins. One clone produced an IgM antibody that appeared to react with many proteins, including bovine serum albumin; it has not been studied further. We have extended our studies on the anti-glycoprotein monoclonal antibodies to determine how they affect virus infectivity and how they react against different structural conformations of the glycoproteins. In addition, we have used these monoclonal antibodies in a limited survey to determine common antigenic structures among five alphavirus serological groups. The results of this work are presented here.

Monoclonal antibodies were prepared from high titre ascitic fluids that were obtained from isogenic Balb/c mice primed with pristane (Aldrich Chemical Co.) 7 days prior to injecting $10^7$ hybridoma cells intraperitoneally. Mice were repeatedly tapped, beginning 5 to 7 days after injection (when visible ascites developed) until the animals died. Fluid was clarified by a low-speed centrifugation and antibody was partially purified by precipitating with 50% saturated ammonium sulphate three times. Salt was removed by dialysis and antibody solutions stored at $-70\degree\text{C}$. Repeated freezing and thawing destroyed these antibody activities (clone 7 was particularly sensitive); therefore, samples were frozen in several aliquots.

The antibodies from ascitic fluids showed the same properties of monoclonality as were previously found with hybridoma cell products (Roehrig et al., 1980) when tested in isoelectric focusing gels (data not shown). Furthermore, the antigenic specificity of the ascitic fluid antibody preparation was retained as demonstrated by enzyme-linked immunosorbent assay (ELISA) and radioimmune precipitation (see below). The titres of three anti-glycoprotein antibody preparations from clones 2, 3 and 7 had roughly the same amount of antibody as determined by ELISA, but clones 1 and 5 had respectively, 5 to 12 times greater amounts of antibody.
When tested for antiviral activity, the clone 7 anti-E2 antibody showed a 50% inhibition of p.f.u. at a dilution of 1/20000 (Fig. 1b). None of the other monoclonals was as effective in neutralization. Clone 3, the other anti-E2 antibody, required 100-fold more material to neutralize virus. The anti-E1 antibodies could only neutralize virus if an anti-mouse IgG was also included in the reaction (Fig. 1a). Even so, rather high amounts of the anti-E1 antibody were needed for formation of this kind of virus–antibody complex. No direct effect on virus neutralization was detected by the anti-E1 monoclonal IgGs alone. Previous results (Dalrymple et al., 1976; Symington et al., 1977) showed that anti-E2 serum from rabbits was more effective for neutralization of Sindbis virus, and anti-E1 serum was more effective in complexing but not neutralizing virus. Our data support those earlier observations and provide unambiguous evidence for a role of E2 in virus infectivity.

Although these monoclonal antibodies were initially classified as either anti-E1, anti-E2 or anti-capsid, we discovered that the conformational state of the virus glycoprotein folding significantly influenced reactivity with them. These effects were most clearly seen when we tested samples of purified [35S]methionine-labelled Sindbis virus that had been treated with detergents which could unfold the proteins. For these studies we considered that the treatment of virus with Triton X-100 would disrupt the particle but not significantly alter the tertiary structure of the glycoproteins. Reaction with SDS, however, would partially unfold the protein and the addition of a sulphydryl reducing agent would completely unfold the molecule. Clones 1 and 2 showed strong reactivity with E1 from native and Triton-disrupted virions, and virtually no recognition of the SDS-treated virus protein (Fig. 2a, b). The anti-E2 monoclonal IgGs (clones 3 and 7) recognized determinants accessible on the SDS-treated, as well as on the Triton-disrupted and native forms of the glycoprotein, although there was a weaker reaction of both clones with the more fully unfolded form of E2 (Fig. 2c, d). Clone 3 antibody appeared to recognize determinants on the unfolded forms of both E2 and E1, and clone 7 appeared to recognize to a very limited extent a determinant on native E1. In many of these radioimmune precipitations, the capsid protein ‘sticks’ non-specifically to the pre-
Fig. 2. SDS–polyacrylamide gel patterns of Sindbis virus glycoproteins precipitated with monoclonal antibodies. (a) Clone 1; (b) clone 2; (c) clone 3; (d) clone 7. Samples (5 μl) of purified [35S]methionine-labelled Sindbis virus containing approx. 25 000 ct/min (Schmidt et al., 1979) were treated as follows: lane 1, diluted with 95 μl buffer containing 0.05 M-tris-HCl pH 7.4, 1 mM-EDTA and 0.1 M-NaCl (TNE); lane 2, diluted with 95 μl buffer containing 0.2 M-tris-HCl pH 8, 0.5 % Triton X-100; lane 3, mixed with 5 μl 2% SDS, incubated for 15 min at 23 °C and diluted with 90 μl TNE; lane 4, mixed with 5 μl 2% SDS and 1 μl β-mercaptoethanol, incubated for 15 min at 23 °C and diluted with 90 μl TNE. Ten μl samples of each of the above were mixed with 10 μl of monoclonal antibody preparations, and radioimmune precipitations performed and analysed as described previously (Roehrig et al., 1980) except that the treated virus samples were not preadsorbed to the S. aureus. Lane M is 5 μl virus that was not treated with antibody.

 precipitated Staphylococcus aureus A protein despite washing of the precipitate with detergents and salt.

We have tested these antibodies for their reactivity with cellular forms of the Sindbis glycoproteins, and found that both of the anti-E2 antibodies reacted with the PE2 cellular precursor of E2. When comparing pulse-labelled E1 with E1 labelled after a 30 min chase, the anti-E1 antibodies reacted more strongly with the late forms of E1. Kaluza et al. (1980) have reported antigenic changes in Semliki Forest virus glycoprotein structures during intracellular maturation and attributed this to oligosaccharide alterations. Our preliminary data suggest that modifications other than carbohydrate attachment are involved in changing the Sindbis virus E1 conformation.

Sindbis is a member of the Western equine encephalomyelitis virus (WEEV) complex (Karabatsos, 1975; Chanas et al., 1976; Trent & Grant, 1980) and we were interested to determine the extent to which our monoclonal antibodies could react with WEEV strains as well as other alphaviruses. For these assays, 4 μg of purified virus was used as antigen and 1/100 dilution of the antibody was tested. Assays were performed in quadruplicate and the results statistically evaluated by a two-tailed t-test with α = 0.01. The ELISA analyses were performed as described previously (Roehrig et al., 1980) except that a Titertek Multiskan (Flow Laboratories) was used to record the p-nitrophenol produced. Sindbis virus was from E. Pfefferkorn (Pfefferkorn & Hunter, 1963) and is a stock in use in this laboratory since 1971. The other alphaviruses were from stocks maintained at the Center for Disease Control, U.S. Public Health Service, Fort Collins, Co., U.S.A. The anti-E1 antibodies cross-reacted with Western (MacMillen strain) as well as Venezuelan (strain TC-83) and Eastern (New Jersey strain) equine encephalomyelitis, but not with Semliki Forest viruses. Both the anti-E2 monoclonal antibodies were highly specific for Sindbis. Most of the anti-capsid monoclonal antibodies resembled clone 5 and cross-reacted with all the alphaviruses. The latter result is not surprising since the nucleocapsid structure and capsid proteins are considered to be conserved among these viruses; thus, the capsid is a ‘group-specific’ antigen. Although there
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There is considerable homology in the amino acid sequences of Sindbis and Semliki Forest virus E1 protein (Rice & Strauss, 1981), neither of the Sindbis anti-E1 monoclonal antibodies reacted with Semliki Forest virus. The close antigenic relation of E1 among the other alphaviruses is noteworthy. This protein has a haemagglutinating activity (Dalrymple et al., 1976) but its role in virus infection is unclear.

There is an increasing amount of information accumulating about the antigenic determinants on virus proteins as a result of the application of monoclonal antibody technology. Monoclonal antibodies have been made most recently against influenza virus strains (Koprowski et al., 1977; Gerhard et al., 1978; van Wyke et al., 1980; Hackett et al., 1980), murine leukaemia viruses (Lostrom et al., 1979), herpes simplex virus (Zweig et al., 1980), mouse mammary tumour viruses (Massey et al., 1980), simian virus 40 T antigens (Gurney et al., 1980) and rabies virus (Flamand et al., 1980a, b). Elegant work has been reported on utilizing monoclonal antibodies to study antigenic drift and selecting for variants of influenza viruses (Webster et al., 1979; Laver et al., 1979a, b; Lubeck et al., 1980). Topological mapping of antigenic determinants has been carried out on influenza and murine leukaemia virus proteins (Webster & Laver, 1980; Stone & Nowinski, 1980), and antigenic polymorphism studied with monoclonal antibodies against these viruses (Stone et al., 1979). The anti-Sindbis virus glycoprotein monoclonal antibodies are shown here to identify both structural characteristics of these proteins as well as aid in alphavirus serology. They also may contribute significantly in identifying subtle changes occurring during maturation of these glycoproteins in virus replication.

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