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Comparison of Specific and Cross-Reactive Antigens of Alphaviruses on Virions and Infected Cells

DAVID GATES,† ARTHUR BROWN, AND CARL J. WUST*

Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37916

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Rabbit hyperimmune antisera against Sindbis (SIN) or Semliki Forest (SF) virus were absorbed with purified SIN virus or SIN virus-infected cells, or with SF virus or SF virus-infected cells. Residual antibody titers were determined by hemagglutination inhibition (HAI) and antibody-dependent, complement-mediated cytolysis (ADCMC) assays. It appeared that absorption with virus-infected cells removed ADCMC-detectable cross-reactive antibody much more efficiently than did absorption with either virus. HAI assays with the same absorbed antisera indicated that both virus and virus-infected cells removed HAI-detectable cross-reactive antibody. On the basis of these and other data, there appeared to be a cross-reactive antigen present on virus-infected cells which was detectable by ADCMC and was distinct from the cross-reactive antigen assayed by HAI.

Sindbis (SIN) virus and Semliki Forest (SF) virus are two closely related alphaviruses. SIN and SF virions contain two glycoproteins, E1 and E2, and, in the case of SF virus, a third small glycoprotein, E3 (7, 18). The corresponding E3 in SIN-infected cells is not incorporated into the virion, but is released into the medium (22). Maturation of either virus involves the insertion of partially glycosylated E1 and the immediate precursor of E2 and E3, PE2/NVP62, into the host cell membrane as a noncovalently bound dimer (1, 6, 7, 21). This complex undergoes extensive conformation changes before incorporation into the free virion, involving proteolytic cleavage from PE2 to E2 and E3 (8, 20) and glycosylation (11, 19). Since cleavage of PE2 is rate limiting, taking up to 20 min (9, 18), it seems likely that there is a significant delineation between the immature PE2-E1 complex on the cell surface and the mature E1-E2-(E3) complex found on the free virion. The conformational differences between PE2-E1 and E1-E2-(E3) manifest themselves as differences in antigenic presentation (10, 11).

We have been interested in elucidating the mechanism of cross-protection in vivo and immunological cross-reactivities in vitro between these two viruses (12–16). Using antibody-dependent, complement-mediated cytolysis (ADCMC), hemagglutination inhibition (HAI), and neutralization assays, we are attempting to determine and compare the virus-induced antigens recognized in these cross-reactions. Furthermore, by using antisera absorbed with infected cells or purified virions, we are exploring the differences in antigen presentation between the virus-specific glycoproteins on infected cells and those on free virions. On the basis of our data, there appear to be two cross-reactive subpopulations of antibody in antisera to SIN or SF virus sera. One subpopulation, detected by ADCMC, recognizes antigenic determinants predominantly, if not exclusively, on infected cells, whereas the second population, detected by HAI, recognizes determinants on both infected cells and mature virus.

† Present address: Department of Microbiology, Health Sciences Center, State University of New York, Stony Brook, NY 11794.

MATERIALS AND METHODS

Cells and cell culture. Primary chicken embryo cells (CEF) were prepared from 9- to 10-day-old embryos as previously described (12).

Virus purification. Roller bottles (450 cm^2; Corning Glass Works) were seeded at 10^6 cells/ml in 100 ml of medium 199 containing 5% calf serum (Flow Laboratories, Inc.) and incubated for 3 to 4 days at 37°C until confluent. After the monolayers were washed with Hanks balanced salt solution (HBSS), they were infected at a multiplicity of infection of 0.01 in 0.1 ml of brain heart infusion (Difco Laboratories) medium with either SIN, strain AR339, or SF, Casals prototype strain. Virus was allowed to adsorb for 45 min, after which 20 ml of medium 199 plus 1% calf serum was added. After 18 h for SIN or 16.5 h for SF, the virus-containing medium was removed and centrifuged at 3,000 × *g* for 10 min, and the clarified supernatant was frozen at −70°C. After approximately 1,500 ml had been collected, the virus-containing supernatant was thawed, and virus was precipitated by addition of 10% (wt/vol) polyethylene glycol and solid NaCl to give a final concentration of 0.5 M NaCl. After stirring at 4°C for 1 h, the virus suspension was centrifuged at 10,000 × *g* for 45 min, and the pellet was suspended in TNE

(0.05 M Tris, pH 7.6; 0.1 M NaCl; 0.001 M EDTA) plus 0.1% calf serum. After centrifuging at 500 × *g* for 10 min, 20 ml of virus suspensions was layered on top of 10 ml of 15% (wt/vol) sucrose in TNE plus 0.1% calf serum and centrifuged at 90,000 × *g* for 2.5 h onto a cushion of 6 ml of 60% sucrose in TNE plus 0.1% calf serum. The partially purified virus was collected from the 15 to 60% interface by monitoring absorbance at 240 nm through an Isco gradient collector. The collected peak was diluted 1:1 in TNE, layered on a 32-ml 20 to 60% continuous sucrose gradient in TNE, and centrifuged for 16 h at 90,000 × *g*. The virus band was collected as before, diluted 1:1 with TNE, pelleted at 100,000 × *g* for 2.5 h, resuspended in 1 ml of TNE, and sonicated for 30 s. Virus titer was then assayed on CEF monolayers, and viral protein concentration was determined by a modified Lowry procedure (17). Recovered infectious virus was usually 25 to 30% of the original titer. Approximately 10^{12} PFU corresponded to 2 mg of protein. Virus preparations were then frozen at −70°C until used.

Antisera. Two 250-μg injections of purified virus emulsified in Freund complete adjuvant were given to 6- to 8-week-old New Zealand white rabbits. The first injection was made intramuscularly into the gastrocnemius muscle, followed 3 weeks later by an intraperitoneal injection in Freund complete adjuvant. Three days later, an intravenous injection of 250 μg of purified virus in TNE without Freund complete adjuvant was given. Sera were collected 9 days later, heat inactivated at 56°C for 1 h, and absorbed twice with 10^8 fresh cells/ml.

Immunization leading to the production of homologous or cross-reactive antibody demonstrable in ADCMC assays necessitates the use of live virus. Similar immunization schedules with inactivated virus (formaldehyde or heat treated) do not give such antibody, although neutralizing or homologous HAI antibodies are produced. Cross-reactive HAI antibody is produced with inactivated virus only after prolonged hyperimmunization.

Antiserum absorption. Two milliliters of antiserum was absorbed two to three times either with 10^{11} PFU of the appropriate purified virus per ml or with 10^8 virus-infected cells/ml. Infected cells were obtained by infecting monolayers in 450-cm^2 roller bottles with a multiplicity of infection of 30 to 50. After 7.5 h, infected cells were washed once with HBSS and once with saline A (0.8% NaCl, 0.04% KCl, 0.1% glucose, 0.04% phenol red, and 0.5% $NaHCO_3$, pH 7.6) containing 1 mM EDTA, and then incubated with 10 ml of saline A and EDTA for 15 min at 37°C on a roller bottle apparatus. After the cells were removed by flushing the roller bottle with a syringe and 14-gauge cannula, they were washed in suspension three times with HBSS. Antisera were absorbed for 1 h at 37°C with rocking and then overnight at 4°C. Residual virus was pelleted out of the antisera by centrifugation at 100,000 × *g* for 2 h, whereas cells were removed by centrifugation at 800 × *g* for 20 min. Absorbed antisera were removed and frozen at −70°C until used. No particular difference in absorption profiles was seen between cells obtained by EDTA removal and cells obtained by scraping monolayers directly into HBSS.

Labeling conditions. Fresh cells were diluted to 5 × 10^7 cells/ml in medium 199, and 150 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear Corp.) in 0.85% saline was added per ml. Cells were incubated in suspension for 1 h at 37°C with occasional shaking. Labeled cells were then counted and diluted to 3 × 10^6 cells/ml, and 1 ml was plated per well in 24-well plates. Cells were allowed to form monolayers overnight at 37°C and then used in ADCMC assays as described below.

Serological assays. (i) HAI. HAI assays were done in microtiter plates essentially as described by Clarke and Casals (2). The HAI titer was taken to be the highest dilution which caused complete inhibition of hemagglutination by 4 U of infected mouse brain antigen in triplicate samples. A unit of antigen was taken to be the last dilution of antigen which caused complete hemagglutination in prior hemagglutination assays. Controls included uninfected mouse brain antigen, normal serum, antiserum without addition of antigen, and buffer alone.

(ii) Neutralization. Titration of virus-neutralizing activity was done by the plaque reduction assay. Twofold dilutions of antisera were made in medium 199 without calf serum, and an equal volume of virus at approximately 1,000 PFU/ml was added. The virus-antibody mixture was allowed to incubate at room temperature for 1 h, after which 0.2-ml portions were added to monolayers in 60-mm-diameter plates. Virus was allowed to adsorb for 30 min at 37°C and then overlayed with Lac-10 agar (0.5% lactoalbumin hydrolysate, 0.1% yeast extract, 1.1% agar, 125 U of penicillin, 125 μg of streptomycin, 10% HBSS, 15 mg of cysteine, 12 mg of histidine, and 0.14% $NaHCO_3$, pH 7.4). Plates were incubated for 48 h at 37°C and stained with neutral red. The number of viral plaques at each dilution was counted in triplicate samples, and the neutralizing titer was taken as the reciprocal of that dilution which caused a 50% reduction in plaque number compared with plates containing virus alone or virus plus preimmune or normal serum.

(iii) ADCMC. ADCMC assays were performed as follows. ^{51}Cr-labeled monolayers in 24-well plates were infected at a multiplicity of infection of 10 with the appropriate virus. At 7.5 h postinfection, the infected cells were washed with HBSS, and 0.5 ml of antiserum at the appropriate dilution and 0.5 ml of guinea pig complement (Flow Laboratories) (1:15, final dilution) were added. After 1 h of incubation at 37°C, the supernatant was removed, the monolayer was washed with HBSS, and the cells were suspended in 1 ml of saline A plus EDTA containing 0.25% trypsin. The supernatant and cells were counted in a Beckman 4000 gamma spectrometer, and the specific ^{51}Cr release was determined according to the following formula:

$$\left(\frac{\text{cpm in supernatant}}{\text{cpm in supernatant plus cells}}\right)_{exp} - \left(\frac{\text{cpm in supernatant}}{\text{cpm in supernatant plus cells}}\right)_{control} \times 100$$

Controls included noninfected cells plus a 1:5 dilution of antiserum and complement, infected cells plus antiserum plus heated complement, infected cells plus complement alone, and infected cells plus medium 199 alone.

RESULTS

Specific antibody against SIN or SF virus was removed to a greater extent by homologous absorption than by heterologous absorption. Specific antibody was assayed in SIN antisera by performing ADCMC assays against ^{51}Cr-labeled monolayers which had been infected with SIN virus. When SIN antiserum was absorbed with SIN virions, a fourfold reduction in titer and a 34% decrease in the plateau ^{51}Cr release were seen (Fig. 1A). Similarly, when SIN antiserum was absorbed with SIN-infected cells, an eightfold reduction in titer and a 40% decrease in the plateau ^{51}Cr release were seen. Absorption of SIN antiserum with SF virion or SF-infected cells did not reduce the titer or the level of ^{51}Cr release to the same extent (no reduction in titer and 11% reduction in ^{51}Cr release for antiserum absorbed with SF virions; a twofold reduction in titer and 15% reduction in ^{51}Cr release for antiserum absorbed with SF-infected cells). The same general pattern of titer reduction was seen in reciprocal experiments using anti-SF (Fig. 1B). Both the titers and the amount of ^{51}Cr release were relatively unaffected after heterologous absorption (anti-SF absorbed with SIN virus or SIN-infected cells). Absorption of anti-SF with SF-infected cells resulted in a sevenfold reduction in titer and a 40% decrease in ^{51}Cr release. Absorption with SF virions was much less efficient in removing specific antibody (twofold reduction in titer and 16% decrease in ^{51}Cr release).

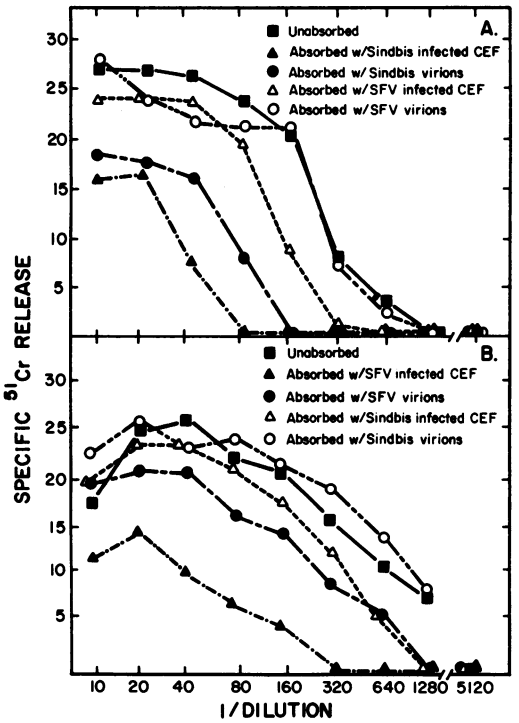

FIG. 1. Titration of specific antibody by ADCMC. Anti-SIN (A) and anti-SF (B) were assayed for specific antibody by ADCMC against SIN-infected (A) or SF-infected (B) ^{51}Cr-labeled CEF monolayers. Antisera were absorbed twice with 10^8 infected CEF/ml or twice with 10^{11} PFU of purified virus/ml before assay.

These data suggested that homologous absorption was more efficient in removing specific antibody than was heterologous absorption. However, the inability to exhaustively absorb out specific antibody, coupled with the two- to fourfold difference in titer reduction between antiserum absorbed with homologous virus and antisera absorbed with cells infected with the homologous virus, necessitated additional experiments to further substantiate the ability of homologous virus or cells infected with homologous virus to remove specific antibody. Therefore, antisera were diluted 1:4 in TNE buffer and absorbed three times with either SIN virions, SIN-infected cells, SF virions, or SF-infected cells. After each absorption, specific HAI antibody titers were assayed. Only homologous absorption removed antibody (Table 1). Heterologous absorption had no effect on specific antibody titers, even after three absorptions. The inability of heterologous absorption to remove specific antibody was further corroborated by ADCMC (data not shown). Diluted antisera absorbed with heterologous virus or with cells infected with heterologous virus still contained detectable specific antibody after three rounds of absorption. In contrast, specific antibody was below the limits of detection in antisera absorbed with the homologous virus or with cells infected with the homologous virus.

Cross-reactive antibody detectable by ADCMC was removed more efficiently by cells infected with either SIN or SF than by either free virus. In contrast to the titer reduction pattern seen when specific antibody was assayed, these same absorbed antisera showed a different pattern when assayed for cross-reactive antibody, e.g., anti-SIN assayed against ^{51}Cr-labeled cells infected with SF. Anti-SIN sera absorbed with either SF-infected cells or SIN-infected cells did not contain ADCMC-detectable cross-reactive antibody (Fig. 2A). However, anti-SIN sera absorbed with either SF or SIN virions showed much greater amounts of residual cross-reactive antibody (titers of 80% of the unabsorbed titers and plateau ^{51}Cr release values within 20% of the unabsorbed values). Reciprocal experiments using anti-SF sera demonstrated the marked ability of virus-infected cells to remove cross-reacting antibody (Fig. 2B). Again, although there was no detectable antibody in infected cell-absorbed antisera, antisera absorbed with either virus still

TABLE 1. Specific HAI antibody titers of diluted antisera before and after absorption with virus or infected cells[a]

No. of absorptions	Antibody titer							
	Anti-SIN absorbed with:				Anti-SF absorbed with:			
	SIN virus	SIN-infected cells	SF virus	SF-infected cells	SIN virus	SIN-infected cells	SF virus	SF-infected cells
Unabsorbed	80	80	80	80	640	640	640	640
1	<40	<40	80	80	320	640	<40	40
2	<40	<40	80	80	640	640	40	<40
3	<40	<40	80	80	320	640	<40	<40

[a] Antisera were identical to those used in ADCMC assays shown in Fig. 2, except that antisera were absorbed with 50 μl of gander erythrocytes and an equal volume of kaolin before assay. HAI assays were done in microtiter by the method of Clarke and Casals (2). The pH of SIN HAI antigen was 6.0; that of SF virus HAI antigen was 6.2. Data are average of triplicate samples.

had titers of 80% of the unabsorbed antisera and ^{51}Cr release values within 15% of the ^{51}Cr release values of unabsorbed antisera.

There may be two cross-reactive antibody populations present in anti-SIN and anti-SF sera. Other investigators (3) have shown that mature virus contains cross-reacting determinants on E1 which are demonstrable by HAI. We therefore ran HAI assays on the same absorbed antisera used in ADCMC assays (Table 2). Specific antibody was removed more efficiently by homologous absorption, in agreement with ADCMC assays. When cross-reactive antibody titers were assayed, a different pattern of titer reduction was observed. Cross-reactive antibody titers were reduced fourfold or greater after absorption with cells infected with either virus. However, in contrast to ADCMC results, absorption with the heterologous virus (anti-SIN absorbed with SF virus, or anti-SF absorbed with SIN virus) also resulted in a reduction of cross-reactive HAI titers equal to those of antisera absorbed with infected cells. Taken together, these findings are consistent with the existence of two cross-reactive antibody populations. One population, assayable by ADCMC, was removed most efficiently by virus-infected cells. The other population, assayable by HAI, was removed to a similar extent by either infected cells or free virus.

Enhanced ability of virus-infected cells to remove cross-reactive antibody was not due to an intrinsic deficiency in the ability of free virus to remove antibody. It was noticed throughout these assays that, in general, absorption with infected cells decreased titers to a greater extent than absorption with free virus. The possibility existed that the failure of free virus to remove ADCMC-detectable cross-reactive antibody was due to some difference in the ability of free virus to remove antibody during absorption. The comparison of HAI titers between virus-absorbed and infected cell-absorbed antisera (Table 2) showed similar degrees of specific antibody reduction. Furthermore, we assayed for residual neutralizing antibody, which is specific and recognizes non-cross-reacting determinants on E2 (2), after virion or infected-cell absorption. Absorption of antisera with virus reduced the neutralizing antibody titer to a similar, if not greater,

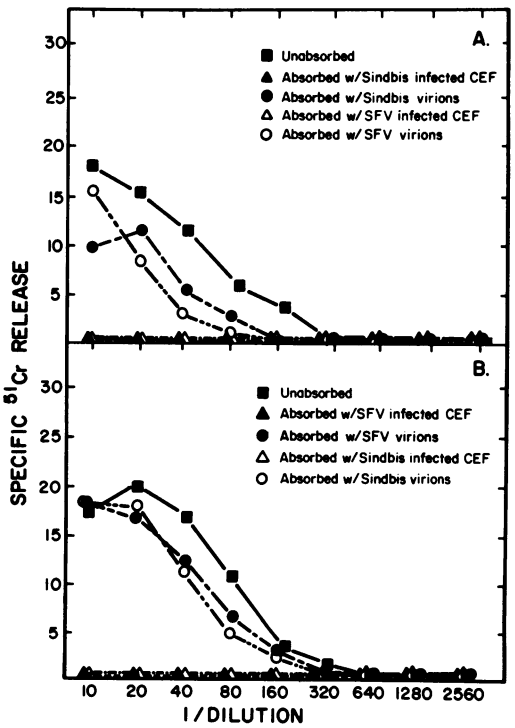

FIG. 2. Titration of cross-reactive antibody by ADCMC. Anti-SIN (A) and anti-SF (B) were assayed for cross-reactive antibody by ADCMC against SF-infected (A) or SIN-infected (B) ^{51}Cr-labeled CEF monolayers. Antisera were absorbed twice with 10^8 infected CEF/ml or twice with 10^{11} PFU of purified virus/ml before assay.

TABLE 2. HAI titers of virion- and infected cell-absorbed antisera[a]

Antiserum	HAI titer			
	Anti-SF		Anti-SIN	
	Specific	Cross-reactive	Specific	Cross-reactive
Unabsorbed	1,280	40	640	80
Absorbed with SF-infected cells	160	<10	320	<10
Absorbed with SF virions	320	20	640	20
Absorbed with SIN-infected cells	1,280	<10	80	10
Absorbed with SIN virions	640	<10	160	40

[a] Conditions were the same as those described in Table 1.

extent than did infected-cell absorption (Table 3).

It should be emphasized that homologous, or cross-reactive, antibody demonstrable in ADCMC assays was elicited only when live virus was used in the immunization. Inactivated virus produced neutralizing and homologous HAI antibody only.

If we assume that any cross-reactive antigenic determinants would be present on the same molecules as antigenic determinants recognized by specific antibody, it seems likely that differences in cross-reactive antibody reduction observed in ADCMC assays between infected cells and free virus apparently were not due to some deficiency in the ability of free virus to remove antibody.

Ability of virus-infected cells to remove cross-reactive antibody was not due to normal cell-associated antigens. Although antisera were absorbed twice with uninfected CEF before absorption with virus or virus-infected cells, it was possible that antigens on normal CEF were in some way responsible for removing cross-reactive antibody. To determine whether this was the case, antisera were absorbed twice with fresh, uninfected cells and then an additional three times with uninfected cells harvested in the same manner as virus-infected cells. Additional absorption with uninfected cells had little effect on either specific or cross-reactive ADCMC titers (Fig. 3).

DISCUSSION

Previous work by King et al. (12) suggested the possibility that HAI and ADCMC assays were detecting two separate antigen-antibody recognition systems. We decided to determine if this was the case by absorbing both anti-SIN and anti-SF sera with various combinations of infected cells or purified virus and assaying for remaining antibody by either HAI or ADCMC. Our results indicate that although absorption with homologous virus-infected cells or homologous virus removes antibody responsible for the specific ADCMC reaction more efficiently than does absorption with heterologous virus-infected cells or heterologous virus, cross-reactive antibody can be removed most efficiently by cells infected with either virus. Absorption with either free virus had a much less pronounced effect on cross-reactive ADCMC titers. When these same absorbed antisera were assayed by HAI, the pattern of titer reduction was somewhat different. As with ADCMC assays, HAI assays showed that homologous virus or cells infected with the homologous virus were best able to remove specific antibody. In contrast to ADCMC assays, heterologous virus absorption, as well as heterologous absorption with infected cells, significantly reduced cross-reactive titers.

This pattern of titer reduction was seen with both anti-SIN and anti-SF sera. It did not seem to be due to some deficiency in the ability of

TABLE 3. Neutralization titers on virion- and infected cell-absorbed antisera[a]

Antiserum	50% Endpoint titer			
	Anti-SF		Anti-SIN	
	Specific	Cross-reactive	Specific	Cross-reactive
Unabsorbed	15,000	<10	15,000	<10
Absorbed with SF-infected CEF	3,200	NT[b]		
Absorbed with SF virions	2,000	NT		
Absorbed with SIN-infected cells			800	NT
Absorbed with SIN virions			139	NT

[a] Neutralization titers were obtained by the plaque reduction techniques explained in Materials and Methods.
[b] NT, Not tested.

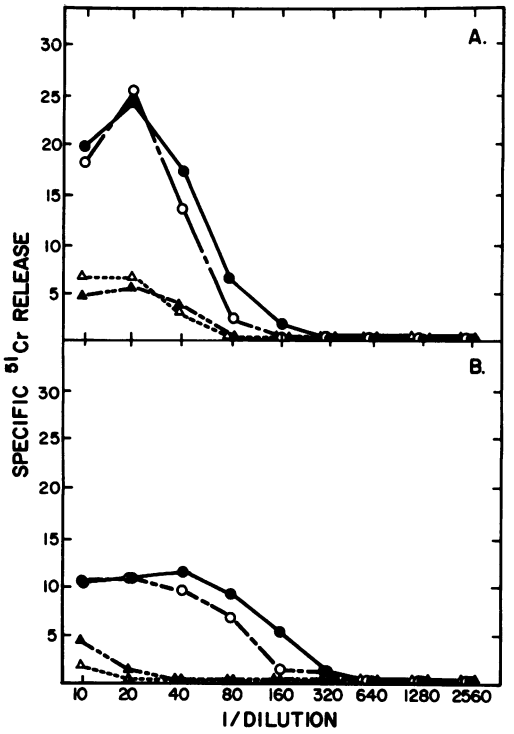

FIG. 3. Titration of antisera absorbed with normal CEF by ADCMC. Anti-SIN (A) and anti-SF (B) which had been absorbed three extra times with uninfected CEF (open symbols) were compared with antisera which had been absorbed in the standard manner (closed symbols). (○, ●), Titers for specific antibody; (△, ▲), titers for cross-reactive antibody.

virus to absorb out antibody, since neutralization titers of virion-absorbed antisera were reduced by similar, if not greater, amounts as virus-infected cell-absorbed antisera. In addition, antisera in which specific antibody titers were not completely removed by homologous virus or infected cells showed similar levels of titer reduction in HAI assays. These data suggest that both free virus and virus-infected cells are able to absorb out antibody and that the greater degree of cross-reactive antibody reduction seen after infected-cell absorption is not due to some intrinsic deficiency in the ability of virus to remove antibody.

Although absolute, qualitative distinctions between antigenic determinants on virus or virus-infected cells cannot be made, there do appear to be quantitative differences between the ability of infected cells and free virus to remove ADCMC-detectable cross-reactive antibody. The antisera used were against whole virus and virus-infected cells and would be expected to contain a broad range of antibody against various antigenic determinants. In this context, the small but detectable decrease in ADCMC-detectable cross-reactive antibody after virus absorption may have been due to removal of the same antibody population seen in HAI assays. Infected cells, on the other hand, may contain additional antigenic determinants and are thus able to remove both cross-reactive populations.

The apparent inefficiency of SF virus in removing specific antibody from undiluted anti-SF sera is of some concern, even though subsequent ADCMC assays on diluted antisera and HAI assays indicate that homologous virus is capable of removing specific antibody. One difference between SIN and SF viruses is the presence of E3 on the surface of SF virions. It is possible that E3 on SF virus in some way lowers the efficiency of antibody binding during absorption of antisera.

Rabbit hyperimmune antisera did show cytotoxicity to uninfected CEF by ADCMC. Therefore, all antisera were absorbed twice with fresh uninfected CEF before assays. It was still possible that the additional absorptions with infected cells might be further reducing some anti-CEF antibody. This possibility was ruled out by absorbing antisera an additional three times with uninfected cells harvested in the same manner as infected cells and demonstrating the lack of any change in titer.

Finally, the cross-reactivity described could not be attributed to contaminating virus during immunization or to inadvertent mixing of antisera, since cross-neutralization assays failed to detect cross-neutralizing antibody in high-titered serum.

The observation that HAI cross-reactive antibody is removed by heterologous virus but not homologous virus is puzzling. It is known that virions contain HAI cross-reactive determinants on E1 (5). In addition, preliminary radioimmune precipitation assays show that both viral and cellular E1 are precipitated by cross-reactive antibody. It seems that HAI cross-reactive antibody is present in the antisera but is not being absorbed out by homologous virus. Since absorption with homologous virus involves the binding of specific antibody as well as any cross-reactive antibody to the virus, it is possible that the additional binding of specific antibody in some way, either by steric hindrance or by increased avidity, blocks the binding of cross-reacting antibody. Heterologous absorption involves only the binding of cross-reactive antibody and so is not affected by such factors.

In contrast to HAI cross-reactive antibody, ADCMC cross-reactive antibody can be removed efficiently only by infected cells. Since virion-associated cross-reactive antigen can be demonstrated by HAI but not by ADCMC, it

seems probable that cross-reactive antibody may be composed of two subpopulations, one population recognizing cross-reactive antigens on both infected cells and virus, and a second population recognizing cross-reacting antigens predominantly on infected cells. In this context, it may be significant that ADCMC assays involved titration of cross-reactive antibody on whole infected cells, whereas HAI and radioimmune precipitation assays involve titration against extracted antigens.

There are a number of possible candidates for this cell-associated cross-reactive antigen. PE2/NVP62 is the immediate precursor to E2 and E3 and is known to be in the membrane of infected cells (1, 21). However, since this glycoprotein does not seem to be detectable by either lactoperoxidase iodination (20) or ferritin-conjugated antibody (21), it is unlikely that it would be accessible to recognition by lytic antibody. Evidence by Dalrymple et al. (4) has suggested the presence of three or four antigenic determinants on the surface of alphaviruses, based on radioimmune precipitation studies of SIN, western equine encephalitis, and eastern equine encephalitis viruses. In addition to an antigenic determinant recognized by specific antibody two, or possibly three, determinants appear to be recognized by cross-reaction between SIN and western equine encephalitis viruses. It may be the case in the SIN-SF system that one class of cross-reactive antibody recognizes antigens on both the intact virus and the infected cell and is assayable by HAI. A second class of cross-reactive antibody would recognize antigenic determinants on the surface of infected cells and would be assayable by ADCMC. However, for this hypothesis to be tenable, one would have to argue that this second class of cross-reacting antigen is much more efficiently recognized on the surface of infected cells compared with virions and that it is not due in some way to the presence of capsid antigens, which are known to be broadly cross-reactive (5).

Another more likely possibility is that conformational changes in one or both of the two main glycoproteins before budding sequester antigenic determinants on the mature virion which were initially exposed on the infected cell. The explanation is consistent with recent observations by Kaluza et al. (10, 11) in which antibody against infected cells was found to contain antibodies against antigenic determinants which were inaccessible on the mature virus. These antibodies were found to be directed against antigenic sites on E1 and E2 which were exposed on the cell surface before final glycosylation events, but were masked in mature virus due to carbohydrate-induced changes in conformation. It may be that this subpopulation of antibody is also responsible for cross-reactive ADCMC activity against infected cells.

There appear to be at least three populations of antibody present in anti-SIN or anti-SF antisera: a major population composed of antibody specific for the homologous virus, a cross-reactive population which reacts with antigenic determinants on mature, fully glycosylated E1 and is assayed by HAI, and a second cross-reactive population, assayed by ADCMC, that recognizes antigenic determinants primarily on infected cells, possibly partially glycosylated E1 or E2.

ACKNOWLEDGMENT

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