JOURNAL OF VIROLOGY, Mar. 1986, p. 893–898
0022-538X/86/030893-06$02.00/0

Vol. 57, No. 3

Rubella Virus Antigens: Localization of Epitopes Involved in Hemagglutination and Neutralization by Using Monoclonal Antibodies

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Monoclonal antibodies (MAbs) against the rubella virion were used to locate epitopes involved in hemagglutination and neutralization. The MAbs exhibiting high-level hemagglutination-inhibiting activity were shown by Western blot analysis to be specific for the E1 polypeptide; this is consistent with the presence of the hemagglutinin on the E1 polypeptide. Some of the E1-specific MAbs also neutralized viral infectivity. However, hemagglutination-inhibiting activity and neutralizing activity did not always correlate. Three distinct functional epitopes were identified on the E1 polypeptide by competition analyses: (i) one which reacted with MAbs with high-level hemagglutination-inhibiting activity and with neutralizing activity, (ii) one which reacted with MAbs with low-level hemagglutination-inhibiting activity and with neutralizing activity, and (iii) one which reacted with MAbs with only hemagglutination-inhibiting activity. A MAb specific for the E2 polypeptide exhibited neutralizing activity. This E2-specific MAb and two E1-specific MAbs with neutralizing activity were capable of precipitating intact virus which indicates that at least three epitopes involved in neutralization are accessible on the surface of the virion.

Rubella virus (RV) is the sole member of the genus *Rubivirus* and is in the family *Togaviridae*; it was isolated and propagated in cell culture in 1961. RV replication and protein synthesis are much like replication and protein synthesis in the alphaviruses in that the structural protein genes are located in the 3′ portion of the 40S genomic RNA, and the structural proteins are translated from a 24S messenger RNA (13). The virion consists of three major structural polypeptides, designated E1, E2, and C; E1 and E2 are glycosylated. The E2 glycopolypeptide migrates as a diffuse band in polyacrylamide gels. Oker-Blom et al. (12) detected two bands, designated E2a (molecular weight, 47,000) and E2b (molecular weight, 42,000). Since tryptic maps of E2a and E2b were identical, they proposed that the molecular weight difference resulted from heterogeneous glycosylation of the same gene product. These data were supported by intrinsic labeling experiments with [^{3}H]mannose which showed that mannose was more efficiently incorporated into E2b than into E2a. Waxham and Wolinsky (27) showed by isoelectric focusing that the E2 polypeptide can be broken into a broad range of molecules that span the molecular weight range from E2a to E2b. They also attributed this heterogeneity to posttranslational modification of the same gene product. The two glycopolypeptides, E1 (molecular weight, 63,000) and E2, compose the stubby spikelike projections on the virion envelope (15, 24). The capsid is constructed from the nonglycosylated C polypeptide (molecular weight, 30,000).

Similar to those of other enveloped viruses, the RV glycoproteins appear to contain antigens responsible for the elicitation of immunity in the host (2, 19). The E1 glycopolypeptide, which was associated also with the hemagglutinin (HA) (8, 26), was associated with the binding of hemagglutination-inhibiting (HI) antibody (3, 16, 26, 28) and hemolysis-inhibiting antibody (27). The E2 glycopolypeptide contains strain-specific antigens (3) and was linked indirectly with neutralization (NT) (8).

Since it has proven difficult to isolate the protein subunits from RV without destroying their biological activity (23), the polypeptide constituents and epitope locations of the antigens responsible for NT have not been characterized, and it has not been determined whether HI and NT antibody functions correlate. Our research located and characterized antigens involved in the binding of HI and NT antibodies and determined whether HI antibody also neutralizes viral infectivity. To accomplish this, a panel of hybridomas which secreted monoclonal antibodies (MAbs) specific for RV was constructed. MAbs were isolated which exhibited HI or NT activity or both, and these MAbs were found to be specific for the E1 polypeptide. A MAb was also isolated which was specific for the E2 polypeptide and exhibited NT activity.

MATERIALS AND METHODS

Cells and virus. RV strain HPV-77 was plaque purified three times in Vero cells. The virus was purified from the culture fluid of infected Vero cells by a modification of the procedure described by Gravell et al. (6).

Construction of hybridomas. BALB/c mice were each immunized intraperitoneally with 100 μg of purified RV. After 30 days, each mouse received an intraperitoneal booster injection of 100 μg of virus, and the spleen cells were harvested for hybridoma production after an additional 3 days.

For production of hybridomas, the spleen cells were fused with SP2/0-Ag14 murine myeloma cells (20) at a ratio of 3:1 in 35% (wt/vol) polyethylene glycol by the method of Gefter et al. (5). After an overnight incubation at 37°C, the cells were suspended in medium containing hypoxanthine (0.027 mg/ml), aminopterin (0.00036 mg/ml), and thymidine (0.015 mg/ml). Screening of the culture fluids was done 2 to 3 weeks later by a modification of the enzyme-linked immunosorbent assay (ELISA) described by Gravell et al. (6) with purified

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RV immobilized on polyvinyl microtiter plates (Costar) and rabbit anti-mouse immunoglobulins conjugated with alkaline phosphatase (Sigma Chemical Co.) at a 1:1,000 dilution. Positive colonies were expanded and cloned twice in soft agar.

Production of ascites fluids and determination of MAb isotypes. Ascites preparations containing high titers of MAbs were produced by intraperitoneal injection of approximately 5×10^6 hybridoma cells into each BALB/c mouse primed 3 weeks before with 1 ml of pristane (Sigma). Ascites was also prepared with hybridoma cells which secreted immunoglobulins nonspecific for RV for use as negative ascites controls. The ascites fluids were collected after 1 to 2 weeks, clarified by low-speed centrifugation, and stored at −70°C. MAb isotypes were determined by ELISA with class- and subclass-specific antisera by using the MonoAb-ID EIA Kit (Zymed Laboratories).

Determination of protein specificity of MAbs. The polypeptide specificity of each MAb was determined by using a modification of the Western blot technique described by Burnett (1). Purified RV was disrupted in sample buffer containing 0.05% sodium dodecyl sulfate (SDS) in the presence or absence of 2-mercaptoethanol as a reducing agent, and the solubilized polypeptides were electrophoresed through an 8.8% polyacrylamide gel at 20 mA for 5 h at 4°C using the buffer system of Laemmli (9). The proteins were then transferred to nitrocellulose sheets in an electroblot apparatus (Bio-Rad Laboratories) at 30 V overnight in 25 mM Tris buffer (pH 8.3) containing 192 mM glycine and 20% methanol (21). The nitrocellulose sheets were then soaked in 20 mM Tris buffer (pH 7.5) containing 500 mM NaCl (TBS) and 3% gelatin for 1 h at room temperature with rocking to block any remaining adsorption sites. Each nitrocellulose sheet was cut into strips (width, 1 cm), and each strip was placed in a solution of TBS containing 1% gelatin and a 1:5 dilution of hybridoma culture fluid or a 1:500 dilution of mouse hyperimmune serum and incubated at room temperature overnight. After being washed for 20 min with TBS containing 0.05% Tween 20, the strips were immersed in a 1:1,000 dilution of biotin-conjugated goat anti-mouse immunoglobulin (Cooper Biomedical, Inc.) and incubated for 2 h at room temperature with rocking. The strips were then washed as before and incubated for 1 h at room temperature in a 1:1,000 dilution of peroxidase-labeled avidin (Sigma) in TBS containing 1% gelatin. The strips were again washed, and the bands were visualized by the addition of peroxidase color development reagent (Bio-Rad).

Immunoprecipitations. Purified RV was incubated in 0.1 M sodium phosphate buffer (pH 7.0) with or without 0.15% SDS as a disrupting agent for 15 min at room temperature. Ascites fluid (15 μl) was then added to the virus and allowed to react with the virus for 18 h at 4°C, and the immune complexes were precipitated with protein A insolubilized on Sepharose CL-4B beads (Sigma). After the beads were washed five times with 0.1 M phosphate buffer, the precipitated antigen was dissociated from the beads by the addition of sample buffer containing 0.2% SDS and 1% 2-mercaptoethanol. The polypeptides were separated by SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose sheets as described above. Visualization of antigens by Western blotting was accomplished by reacting them with rabbit hyperimmune serum and then with goat anti-rabbit immunoglobulins conjugated with peroxidase (Bio-Rad).

HI assays. HI assays were done by the procedure of Liebhaber (11) with ascites fluid pretreated with $CaCl_2$ and with dextran sulfate to remove nonspecific inhibitors. The HI titer is reported as the reciprocal of the last dilution of antibody which inhibited 4 hemagglutination units of virus.

NT assays. The NT activity of the MAbs was assayed against RV strain HPV-77. Serial dilutions of each ascites fluid were incubated with approximately 200 PFU of RV for 1 h at 37°C in the presence or absence of 5% guinea pig complement. Controls were included in which the virus suspension was incubated for 1 h at 37°C without ascites fluid or with negative ascites. Each mixture was then adsorbed onto two Vero cell monolayers for 1 h at 37°C, and an overlay of minimum essential medium with 1% agarose, 2% fetal bovine serum, 7.2 mM sodium bicarbonate, and 1 mM gentamicin was added. After 6 days the monolayers were fixed with 10% Formalin and stained with 1% crystal violet to visualize the plaques. The NT titer is expressed as the reciprocal of the ascites dilution which caused a 50% reduction (relative to controls) in the number of plaques.

Competition assays. The immunoglobulin G (IgG) fraction was purified from each ascites fluid by chromatography on protein A-Sepharose (4), and a portion of the IgG obtained was biotinylated by the procedure of Heggeness and Ash (7). The biotinylated antibody used in competition assays was quantitated by a modification of the ELISA described by Gravell et al. (6). Serial 10-fold dilutions of the biotinylated antibody in phosphate-buffered saline (0.01 M Na_2HPO_4, 0.14 M NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl) (PBS), pH 7.2, containing 1% bovine serum albumin (BSA) and 0.05% Tween 20 were incubated with the immobilized virus for 1 h at 37°C. After washing with PBS-BSA-Tween to remove unbound antibody, bound antibody was detected with avidin conjugated with alkaline phosphatase (Sigma); *p*-nitrophenyl phosphate (Sigma) was used as a substrate. The optical density of each well at 405 nm (OD_{405}) was recorded, and the dilution of biotinylated antibody required to produce approximately 1 OD_{405} unit was determined. For most of the MAbs, 1:1,000 dilutions of the biotinylated antibody portions were used in the competition assays. The competition assays were done by an ELISA similar to that subsequently described by Wagener et al. (25), in which serial 10-fold dilutions of the nonlabeled portion of the purified monoclonal IgG were allowed to react with the immobilized virus overnight at 4°C. After washing to remove unbound IgG, the diluted biotinylated antibody was added to each well, and the ELISA was continued. Each biotinylated MAb was competed with homologous nonlabeled MAb (positive control), with non-RV-specific IgG (negative control), and with each of the nonlabeled ascites IgG.

The percent competition was calculated as follows. Percent binding of biotinylated antibody in the presence of nonlabeled (competitive) antibody = (OD_{405} of biotinylated antibody in the presence of competitive antibody/OD_{405} of biotinylated antibody in the absence of competitive antibody) × 100. To determine the percent competition, this percentage was subtracted from 100%.

RESULTS

Polypeptide specificities of MAbs. The polypeptide specificity of each of the MAbs was determined by Western blot analysis. Each of the MAbs was reacted on a Western blot with RV antigens which had been reduced prior to electrophoresis and with antigens which had not been reduced. MAbs demonstrating specificity for the E1, E2, and C polypeptides were isolated. The MAbs specific for the C polypeptide and those specific for the E2 polypeptide reacted with either reduced or nonreduced antigens. However,

some of the E1-specific MAbs reacted only with antigens which had not been reduced prior to electrophoresis. Western blot reactions of selected E1-specific MAbs are shown in Fig. 1. These five MAbs (lanes 2 to 5) reacted only with nonreduced E1 antigens and with the glycoprotein dimers that contain E1. Lane 1, developed with mouse hyperimmune serum, shows the three RV structural proteins and the two glycoprotein dimers, E1:E1 and E1:E2 (8, 26). Under the nonreducing conditions used, the capsid protein migrated as a disulfide-linked dimer of the C polypeptide (12, 27).

Immunoprecipitations. Immunoprecipitations of purified intact RV or SDS-disrupted, nonreduced RV were done to determine whether the E1- or E2-specific MAbs reacted with antigens exposed on the external surface of the virion (Fig. 2). Purified RV (lane 1) was used as a marker for the locations of the precipitated polypeptides. Under the reducing conditions used, there was an apparent decrease in the amount of the glycoprotein dimers, and the C protein migrated as the monomeric C polypeptide. The E1-specific MAbs 1-6 and 2-6 precipitated whole virus (lanes 3 and 5, respectively) as indicated by the presence of all three structural polypeptides in the precipitate. MAb 1-6 precipitated the E1 polypeptide from SDS-disrupted virus (lane 2), whereas MAb 2-6 did not (lane 4), although both reacted with nonreduced E1 antigens on Western blots. Apparently the solubilization of the virus with 0.15% SDS adversely affected the binding of MAb 2-6 to soluble E1 antigens. The reactivity of this MAb on Western blots may indicate that E1 antigens renature when they are adsorbed to the solid phase.

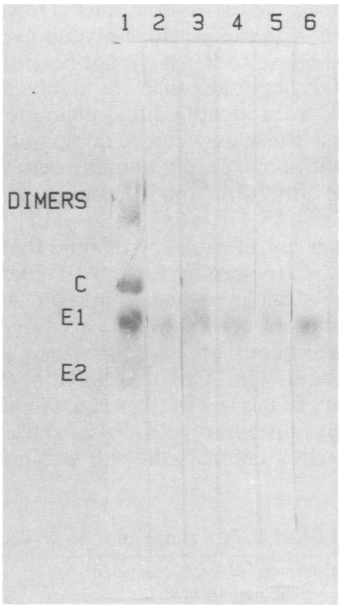

FIG. 1. Western blot analysis of binding of E1-specific MAbs to E1 polypeptide of RV. Purified RV (5 μg per lane), disrupted in the absence of a reducing agent, was subjected to electrophoresis through an 8.8% polyacrylamide gel and transferred to nitrocellulose as described in the text. The lanes were excised, and after a blocking step, incubated with a 1:5 dilution of hybridoma supernatant fluid or with a 1:500 dilution of mouse anti-RV hyperimmune serum. After being washed, the lanes were incubated with biotin-conjugated anti-mouse immunoglobulins and then with peroxidase-labeled avidin. The specific signal was developed with HRP color development reagent. Lanes: 1, mouse anti-RV hyperimmune serum; 2, MAb 2-6; 3, MAb 2-42; 4, MAb 3-8; 5, MAb 2-12; 6, MAb 2-13.

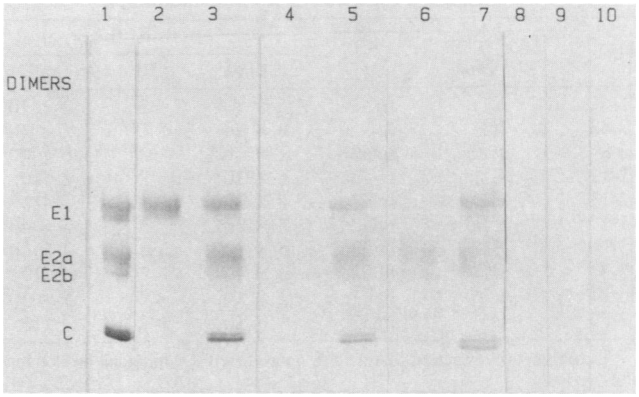

FIG. 2. Immunoprecipitation of RV structural polypeptides by MAbs. Purified RV, either intact or disrupted with 0.15% SDS, was reacted with ascites fluids containing MAbs specific for the E1 (MAbs 1-6 and 2-6) or E2 (MAb 26-24) polypeptide. The immune complexes were precipitated with protein A-Sepharose beads, and the precipitated antigens were dissociated from the beads with SDS and subjected to SDS-polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose and visualized by reaction with rabbit hyperimmune serum as described in the text. Lanes: 1, virus only; 2, MAb 1-6 reacted with SDS-disrupted RV; 3, MAb 1-6 reacted with intact RV; 4, MAb 2-6 reacted with SDS-disrupted RV; 5, MAb 2-6 reacted with intact RV; 6, MAb 26-24 reacted with SDS-disrupted RV; 7, MAb 26-24 reacted with intact RV; 8, negative ascites fluid reacted with SDS-disrupted RV; 9, negative ascites fluid reacted with intact RV; 10, protein A-Sepharose beads reacted only with intact RV.

An E2-specific MAb, 26-24, precipitated whole virus (lane 7) as well as the E2 polypeptide from SDS-disrupted virus (lane 6). No antigens were precipitated by negative ascites fluid incubated with disrupted (lane 8) or whole (lane 9) virus. An additional control consisted of whole virus incubated with insolubilized protein A (lane 10).

Functional activities of MAbs. Western blot analysis showed the polypeptide specificity of each of the MAbs and revealed that MAbs specific for each of the three structural polypeptides of RV had been isolated. Immunoprecipitations indicated the location of some of these epitopes within the virion. To determine the location of the sites for hemagglutination and NT, each of the MAbs was tested for HI and NT activities (Table 1). Some of the E1-specific MAbs exhibited high-level HI activity (MAbs 2-6, 2-42, 3-8, 2-12, and 2-13), whereas others exhibited comparatively low-level activity (MAbs 1-6 and 3-7) or had no activity (MAb 2-34). Although the ELISA titer for each of the MAbs was approximately the same, there was at least a 3-log difference in the HI titers of the high-level and low-level HI MAbs. This variation in HI activity implies that different sites on the E1 protein bind HI antibody since some HI MAbs appear to function in this activity more effectively than others. The E1 MAbs exhibiting high-level HI activity reacted on Western blots only with nonreduced E1 antigens (Fig. 1), whereas those exhibiting low-level activity reacted with reduced or nonreduced E1 antigens (data not shown). The E2-specific MAb (26-24) did not exhibit HI activity. However, at low dilutions (1:8 and 1:16) it did cause an aberrant pattern in the virus hemagglutination reaction. To determine whether the HI and NT activities correlated, the MAbs were tested for NT activity in an in vitro plaque reduction assay. The results of typical NT assays using a MAb with NT activity (MAb 2-6) and a MAb without NT activity (MAb 2-13) are shown in Fig. 3. The MAbs with NT activity were active in the

TABLE 1. Properties of RV-specific MAbs

MAb	Polypeptide specificity[a]	Isotype[b]	Activity (titer)		
			ELISA[c]	HI[d]	NT[e]
2-6	E1	IgG2a	5×10^6	2^{21}	2×10^4
2-42	E1	IgG2a	8×10^6	2^{17}	2×10^4
3-8	E1	IgG2b	1×10^6	2^{21}	8×10^2
2-12	E1	IgG2a	6×10^6	2^{20}	$<10^2$
2-13	E1	IgG2a	8×10^6	1^{17}	$<10^2$
1-6	E1	IgG2a	5×10^7	2^8	5×10^3
3-7	E1	IgG2a	5×10^6	2^7	4×10^3
2-34	E1	IgG2a	1×10^6	$<2^3$	$<10^2$
26-24	E2	IgG1	1×10^6	$<2^3$	5×10^3
2-59	C	IgG2b	1×10^6	$<2^3$	$<10^2$

[a] Determined by reacting MAbs with nonreduced RV antigens on Western blots.
[b] Determined by ELISA with anti-mouse class- and subclass-specific antisera.
[c] Expressed as the reciprocal of the ascites dilution which gave maximal reactivity by endpoint titration.
[d] Expressed as the reciprocal of the last dilution of antibody which inhibited 4 HA units of virus.
[e] Expressed as the reciprocal of the ascites dilution which caused a 50% reduction (relative to controls) in the number of plaques.

presence or absence of added complement (data not shown). The HI and NT titers of several MAbs are compared in Table 1. Four functional categories were apparent for the E1-specific MAbs: (i) MAbs with high-level HI activity and with NT activity, (ii) MAbs with low-level HI activity and with NT activity, (iii) MAbs with high-level HI activity and no NT activity, and (iv) MAbs with no HI or NT activity. These data demonstrate that HI and NT functions of antibodies may or may not correlate and that the E1 polypeptide contains epitopes which react with antibodies exhibiting HI or NT activity or both. The E2 polypeptide contains an epitope which reacted with an antibody exhibiting only NT activity. MAbs specific for the C polypeptide did not exhibit HI or NT activity.

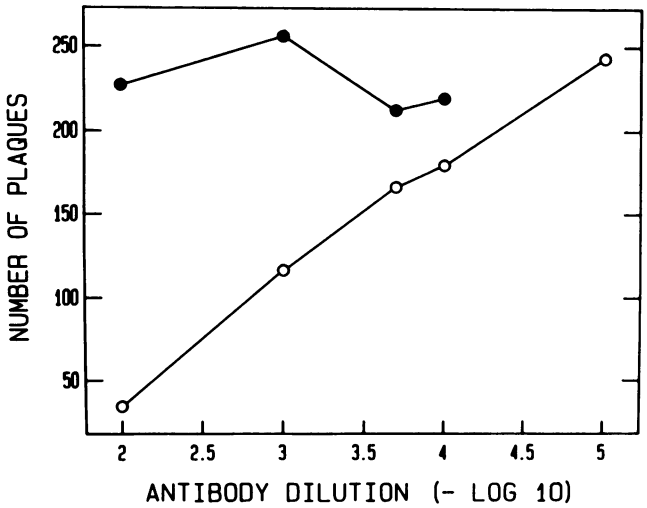

FIG. 3. NT activity of two E1-specific MAbs against RV. Serial dilutions of ascites fluid containing MAb 2-13 or 2-6 were reacted with approximately 200 PFU of RV for 1 h at 37°C before adsorption of the virus onto a Vero cell monolayer. An agar overlay containing medium, 2% fetal bovine serum, and antibiotics was added to the monolayers as described in the text; the monolayers were then incubated in a humidified air-CO_2 atmosphere for 6 days. The monolayers were fixed with Formalin and stained with crystal violet, and the number of plaques at each dilution of antibody was counted. MAb 2-6 (○-○) is representative of the E1-specific MAbs which demonstrated in vitro NT activity by reducing the number of plaques, whereas MAb 2-13 (●-●) is representative of the E1-specific MAbs which did not exhibit in vitro NT activity.

Identification of functional epitopes on the E1 polypeptide by competition analysis. Since HI and NT activities of the E1-specific MAbs did not always correlate, it appeared that the two activities involved different epitopes on the E1 polypeptide. To confirm this, the E1-specific MAbs were analyzed by competitive binding assays (Table 2). MAbs 2-6, 2-42, and 3-8, exhibiting NT activity and high-level HI activity, competed with one another. MAb 3-7, which exhibited NT activity and low-level HI activity, did not compete with any of the other MAbs, whereas MAb 1-6, which was similar to MAb 3-7 in activity, did compete to some extent with all of the E1-specific MAbs. MAb 2-13, which exhibited high-level HI activity but no NT activity, competed only with itself. MAb 2-12, which showed activities similar to MAb 2-13, competed with 2-13 (data not shown); however, testing for the reciprocal competition was not done. MAb 2-34, which did not exhibit HI or NT activity, did not compete with any of the other MAbs (data not shown). These data indicate that at least three epitopes exist on the E1 polypeptide which bind antibodies with different functional HI and NT activities.

DISCUSSION

The envelope of RV contains spike proteins composed of two closely associated glycopolypeptides, E1 and E2, which are involved in virus-host interactions. However, the specific role of each of these envelope glycopolypeptides in the host immune response to RV have not been fully characterized. In this study, epitopes that are involved in hemagglutination and NT were identified and characterized by reactions with MAbs. These data can be compared with previous data obtained with polyspecific immune sera to gain further insight into the structure and functions of the envelope glycoproteins.

Similar to other investigators, we found that MAbs exhibiting HI activity were specific for the E1 glycopolypeptide, which confirms its role in hemagglutination. A MAb specific for the E2 protein did not exhibit HI activity, although low dilutions of this antibody appeared to cause a partial inhibition of hemagglutination. This may have been due to nonspecific inhibitors of the HA in the ascites fluid, although the ascites had been pretreated with $CaCl_2$ and dextran sulfate and negative ascites controls did not exhibit this activity.

TABLE 2. Competition assays

Biotinylated MAb	% Competition with nonlabeled (competing) MAb[a]:							Polypeptide specificity	Activity	
	2-6	2-42	3-8	2-13	3-7	1-6	2-59		HI[b]	NT[c]
2-6	66	42	66	11	7	35	7	E1	+	+
2-42	85	63	79	0	20	40	3	E1	+	+
3-8	77	78	71	0	11	25	0	E1	+	+
2-13	0	0	0	81	6	48	16	E1	+	−
3-7	0	13	21	6	59	16	0	E1	+/−	+
1-6	46	47	41	36	31	69	8	E1	+/−	+
2-59	5	12	18	0	6	0	90	C	−	−

[a] Percent competition was calculated as described in the text.
[b] +, High-level activity; +/−, low-level activity; −, no activity.
[c] +, Activity; −, no activity.

Another possibility is that this partial HI activity was a result of nonspecific inhibition of the HA by steric hindrance caused by the binding of antibody to a site on the E2 glycoprotein in close proximity to the HA site. Similar findings were reported for Sindbis virus (18), in which partial HI activity was observed by using a MAb directed against the E2 protein. This was attributed to the close proximity of the E2 molecule to the Sindbis virus HA, located on the E1 protein. It appears unlikely that the E2 polypeptide of RV has any direct participation in binding to the erythrocytes; however it does appear to be closely associated with the E1 polypeptide. Biochemical studies of disrupted RV showed that E1:E1 homodimers and E1:E2 heterodimers are present in detergent-disrupted virus (8, 26). Waxham and Wolinsky (27) postulated that the E1 and E2 glycopolypeptides exist as repeating hexamers, similar to the Sindbis virus glycopolypeptides (17). However, data concerning the stoichiometric and spatial relationships of the RV glycopolypeptides in the envelope are inconsistent (12, 15, 24), and the roles of the homodimers and the heterodimers in the formation of the envelope spikes are not known. External labeling of intact virions with boro[^{3}H]hydride produced radioactive E1, E2a, and E2b glycopolypeptides which indicates that all three molecules are accessible on the envelope (12). Trypsin digestion of intact virions showed that the E1 glycopolypeptide is most susceptible to degradation (8), which is consistent with a more prominent display of the E1 protein on the envelope. However, it was not determined whether the higher resistance of the E2 glycopolypeptide to digestion was a result of heavy glycosylation, a lack of cleavage sites, or a lack of access. Immunoprecipitations were done with E1- or E2-specific MAbs and with intact or disrupted virus to determine which epitopes were accessible on the surface of the virion. When they were reacted with nondisrupted virus two E1-specific MAbs each exhibiting neutralizing activity, precipitated all three structural polypeptides. This is consistent with the exposure of these NT epitopes on the surface of the virion. Waxham and Wolinsky (27) reported the isolation of a MAb specific for the E2 glycopolypeptide which was unable to precipitate E2 from detergent extracts of whole virus or to interact with intact virions. However, our MAb precipitated the E2 glycopolypeptide from detergent extracts of virus, precipitated nondisrupted virus, and exhibited NT activity. Thus, at least one epitope on the E2 glycopolypeptide must be accessible on the external surface of the virion, and this epitope is involved in NT. It appears from our data that the E1 and E2 glycopolypeptides may be closely associated and that both are exposed on the virion surface. Thus, the E1:E2 heterodimer may be an important configuration of the glycoproteins in the envelope.

An HI antibody titer following RV infection persists for many years and is used to monitor immunity to rubella. However, cases have been reported in which HI-positive individuals were reinfected with the virus, resulting in a congenital rubella syndrome (10, 14). Trudel reported the isolation of a MAb with low NT activity but no HI activity (22). These data imply that different epitopes elicit these antibody functions, but they do not indicate the polypeptide location(s) of the NT epitopes or whether HI antibody could exhibit an NT function. Recently, Waxham and Wolinsky (28) constructed MAbs specific for the E1 polypeptide that exhibited (i) HI and NT activities, (ii) HI activity only, (iii) NT activity only, or (iv) neither HI nor NT activity. These MAbs were used to define six nonoverlapping antigenic sites on the E1 polypeptide and to show that multiple epitopes are involved in HI and NT activities. Mapping with the MAbs constructed in our laboratory showed that both the E1 and E2 glycopolypeptides contained epitopes which were involved in neutralizing viral infectivity. These data are consistent with the findings of Pettersson et al. (16) that antiserum to E1 was able to reduce viral infectivity in a plaque reduction assay. It is also consistent with earlier reports by Ho-Terry and Cohen who suggested that the E2 glycopolypeptide contains NT antigens (8).

MAbs specific for the E1 polypeptide were isolated which had high- or low-level HI activity and had NT activity. Competition studies indicated that these MAbs reacted with two distinct epitopes. It is possible that MAbs 1-6 and 3-7 react with different NT epitopes; however, the competition data are not convincing. A third epitope identified on the E1 protein by competition studies reacted with a MAb which had high-level HI activity but no NT activity. This shows that at least two distinct epitopes involved in high-level HI activity are located on the E1 polypeptide. One of the epitopes which binds high-level HI antibody is involved in NT, whereas the other is not.

Further studies will be conducted to elucidate the mechanism by which antibody to the E1 or E2 glycopolypeptide neutralizes viral infectivity. This information should provide insight into the functions of each glycopolypeptide in the pathogenesis of rubella. Similarly, it must be determined whether the in vitro NT activity of these MAbs correlates with protective immunity in vivo. The identification of the RV antigen(s) which evokes a protective immune response may lead to the development of an engineered subunit vaccine which does not have the risks associated with the live attenuated vaccine.

ACKNOWLEDGMENT

This study was supported in part by Public Health Service grant AI 11255 from the National Institute of Allergy and Infectious Diseases.

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