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Antibody Response to Individual Rubella Virus Proteins in Congenital and Other Rubella Virus Infections

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Serum samples from patients with various forms of rubella virus infection were tested for antibodies to each of three viral structural proteins by radioimmunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In most sera antibody to E1 protein was the predominant species. Sera from patients with congenital rubella syndrome, however, contained significantly more E2 antibody relative to E1 antibody than did sera from other rubella patients.

Rubella virus virions contain two envelope glycoproteins (E1 and E2) and a nonglycosylated capsid (C) protein (10, 12). Because the antibody to E1 protein has hemagglutination-inhibiting activity (12; Y. Umino, T. A. Sato, S. Katow, T. Matsuno, and A. Sugiura, Arch. Virol., in press), E1 protein is thought to be involved in hemagglutination. The role of E2 protein, on the other hand, has yet to be determined.

Most rubella virus infections leave a long-lasting immunity which is believed to be mediated by circulating antibody. Little information is available, however, about the amount of antibody to each of the viral structural proteins which is formed after infection or vaccination. Some rubella virus infections are known to develop into chronic infections such as congenital rubella syndrome (CRS), arthritis (15), and progressive rubella panencephalitis (PRP) (11).

It is of interest to know whether the antibody response to individual proteins of rubella virus virions differs in various forms of rubella infection. Antibodies against different measles virus virion proteins have been differentiated and quantitated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of immunoprecipitated labeled antigens in various forms of measles infection (3–5, 9, 13). We conducted a similar study with various forms of rubella infection and found that the antibody response to E1 protein was predominant in most rubella infections but that antibodies to E2 protein were relatively more abundant in CRS than in other forms of rubella infection.

BHK-21 cells infected with the M33 strain of rubella virus were labeled with either 30 μCi of [^{3}H]glucosamine (26.8 Ci/mmol) per ml or 15 μCi each of [^{3}H]leucine (157 Ci/mmol), [^{3}H]tryosine (40 Ci/mmol), and [^{3}H]valine (36 Ci/mmol) per ml. Labeled virions were purified by a method described previously (6). Either labeled virions or labeled infected cells were lysed in RIPA buffer (8) without aprotinin (Trasylol; Bayer, Leverkusen, Federal Republic of Germany) and used as antigens. Mercaptoethanol (1%) was added to RIPA buffer as well as to phosphate-buffered saline for washing immunoprecipitates to dissociate heterodimers of E1 and E2 (12). Serum (25 μl) diluted 1:16 was added to 50 μl of antigen (10^5 cpm of disrupted virions or 2×10^6 cpm of infected cell lysate). The serum-antigen mixture was allowed to stand overnight at 4°C. A 25-μl portion of a 50% suspension of protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals AB, Uppsala, Sweden) in 0.01 M phosphate-buffered saline (pH 7.2) was added. The immune complexes adsorbed to the beads after 2 h at 4°C were pelleted at 100 × *g* for 30 s, washed four times with RIPA buffer and once with phosphate-buffered saline, suspended in 0.0625 M Tris-hydrochloride (pH 6.8)–3% sodium dodecyl sulfate–5% 2-mercaptoethanol–10% glycerol–0.005% bromophenol blue, and boiled for 2 min. Electrophoresis was carried out in a 10% polyacrylamide slab gel by the method of Laemmli (7). Gels were dried and fluorographed on Sakura type A films (2).

Serum samples were tested at a dilution of 1:16 because higher concentrations of most sera precipitated less antigen. The inhibitory effect on nondiluted serum on the immunoprecipitation of rubella virus antigens was confirmed by the finding that an antibody-positive serum was more reactive when diluted 1:16 in phosphate-buffered saline than when diluted in an antibody-negative serum (data not shown).

We studied serum samples from four subjects in late convalescence, five patients with CRS, two patients with suspected PRP (1), one patient recovering from reinfection, and a pig hyperimmunized with the M33 strain. Electrophoretic patterns of labeled rubella virus virions are shown in Fig. 1, lane 1. E1 was more intensely labeled with the radioactive amino acid mixture than were E2 and C, whereas more radioactive glucosamine was incorporated into E2 than into E1, reflecting the higher carbohydrate content of the former (10). Electrophoresis of viral proteins immunoprecipitated from disrupted virions by representative sera is also shown in Fig. 1. Proportions of the three viral proteins precipitated varied from one sample to the other. In general, ratios of E2 and C to E1 were lower than in nonprecipitated virions, indicating that antibody to E1 was the predominant species in most sera. However, the E2/E1 ratio was higher in immunoprecipitates with the serum from a patient with CRS (Fig. 1, lane 3) than in the labeled virions. Figure 2 shows the densitometric tracings of fluorograms of labeled virions (Fig. 1A and B, lanes 1) and immunoprecipitates (Fig. 1A and B, lanes 5) made by a DM-K densitometer (ATAGO, Tokyo, Japan) at a wavelength of 625 nm. The radioactivity of each protein band was quantitated by measuring the area under the respective peak. Table 1 shows a comparison of the amounts of E1 and E2 in [^{3}H]glucosamine-labeled rubella virus virions and in those precipitated by various sera. The ratio of E2 to E1 was higher only in the immunoprecipitates with CRS sera than in the labeled virions, and the difference in the E2/E1 ratio between CRS and late-convalescence sera was highly significant ($P < 0.001$ by Student's *t* test).

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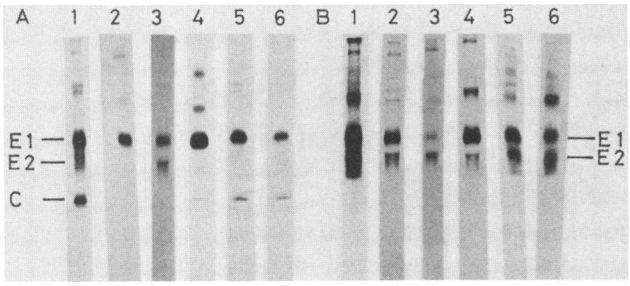

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of labeled rubella virus polypeptides immunoprecipitated with various sera. Virions labeled with either ^{3}H-amino acids (A) or [^{3}H]glucosamine (B) and disrupted with RIPA buffer were used as antigens. All sera were examined at a 1:16 dilution. Lanes: 1, virions alone; 2, late-convalescence serum; 3, serum from a patient with CRS; 4, serum from a patient with suspected PRP (1); 5, convalescence serum from reinfection; and 6, pig hyperimmune serum against rubella virus M33.

The serum samples listed in Table 1 plus some additional samples taken during early and late convalescence, after reinfection, and after vaccination with the To-336 strain were also tested for immunoprecipitation with labeled infected cell lysates as antigens. Although quantitation of antibody was not done because of unknown proportions of E1, E2, and C in infected cell lysates as opposed to purified virions, immunoprecipitation patterns were essentially similar to those obtained with purified virions as antigens; namely, antibody to E1 was predominant in most sera except in those from CRS patients. Levels of antibodies to E1 and E2 in some sera were also determined by the dilution endpoint method on a fourfold serial dilution of serum (Table 2). The results confirmed the relative abundance of E2 antibody in CRS sera, as compared with late-convalescence sera.

What caused the observed difference between the antibody response in CRS and in other rubella virus infections? The E2 protein of rubella viruses which caused the CRS could have been antigenically closer to the E2 protein of the M33 strain than to that of other rubella viruses. This possibility is unlikely, however, because the CRS patients included in this study were born after 2 different rubella epidemics more than a decade apart. Alternatively, E2 may be less immunodominant than E1, and the antibody response to E2 beyond a certain limit may require repeated antigenic stimuli provided by virus persisting in the body. A relatively elevated E2/E1 ratio in hyperimmune porcine serum suggests this possibility. Finally, elevated E2/E1 ratios could be caused not only by increased antibody reponses to E2 but also by decreased antibody responses to E1. Serum hemagglutination inhibition antibody titers were, indeed, relatively low in some CRS patients, and it is conceivable that the antibody response to E1 was somehow suppressed in CRS, e.g., as a result of partial tolerance established during fetal infection.

Prominent antibody responses to E1, E2, and C proteins have been demonstrated by radioimmunoprecipitation in a patient with PRP (14). These may have resulted from a sustained antigenic stimulus caused by an ongoing infection.

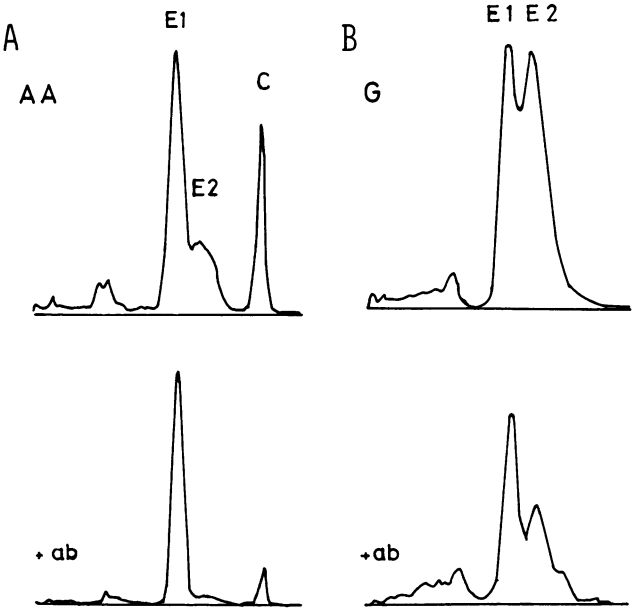

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of labeled rubella virus polypeptides immunoprecipitated with convalescence serum from reinfection. Virions labeled with either ^{3}H-amino acids (A) or [^{3}H]glucosamine (B) were electrophoresed from left to right. Virions alone (top; see Fig. 1A and B, lanes 1) or virions immunoprecipitated with convalescence serum from reinfection (bottom; see Fig. 1A and B, lanes 5) are shown. Grain absorbance of the fluorogram was traced by a densitometer at a wavelength of 625 nm. AA, Amino acids; G, glucosamine; ab, antibody.

TABLE 1. Rubella virus polypeptides immunoprecipitated with various sera

Serum	Subject	Age (yr)	Hemagglutination inhibition titer	Amt[a] of: E1	Amt[a] of: E2	E2/E1 ratio[b]	Avg E1/E2 ratio
None (virions alone)				475	675	1.420	
Late convalescence	1[c]	26	256	59	33	0.559	0.444
	2	32	256	13	4	0.308	
	3	30	128	11	6	0.545	
	4	29	128	11	4	0.363	
CRS	1	0	128	15	18	1.200	
	2	1	64	12	9	0.713	
	3[c]	1	128	15	36	2.400	3.363[d]
	4	13	64	3	17	5,000	
	5	13	32	4	30	7.500	
Suspected PRP	1[c]	23	512	233	33	0.142	0.365
	2	17	256	68	40	0.588	
Reinfection	1[c]	23	512	120	74	0.617	
Hyperimmune porcine	1[c]		4,096	85	103	1.212	

[a] Values are areas under peaks of corresponding virus polypeptide densitometric tracings (see Fig. 2) in arbitrary units.

[b] The E2/E1 ratio was calculated with [^{3}H]glucosamine-labeled virions as antigens. The data are from one experiment in which all sera were tested against the same antigen.

[c] Sera shown in Fig. 1.

[d] Level of significance in Student's t test in comparison with late-convalescence sera, $P < 0.001$.

TABLE 2. Antibody titers against E1 and E2

Serum	Subject	Antibody titer[a] against:		E2 titer/ E1 titer ratio
		E1	E2	
Late convalescence	1	1,024	16	1/64
	4	64	16	1/4
CRS	4	64	64	1
	5	64	64	1

[a] Reciprocal of the dilution endpoint which produced visible bands when lysates of [^{3}H]glucosamine-labeled infected cells were used as antigens.

Analysis of antibodies by radioimmunoprecipitation may well serve to elucidate the interaction of rubella virus with the host.

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