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Selective Reactivity of Antibodies to Human Immunoglobulins G, M, and A with Rubella Virus Proteins

PAUL PARTANEN,[1,2]* HELENA SEPPÄNEN,[2] JUKKA SUNI,[2,3] AND ANTTI VAHERI[2]

Labsystems Research Laboratories, SF-00810 Helsinki,[1] Department of Virology, University of Helsinki, SF-00290 Helsinki,[2] and Virus Laboratory, Aurora Hospital, SF-00250 Helsinki,[3] Finland

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Proteins of purified rubella virus were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and immunoblotted with human sera and immunoglobulin class heavy-chain-specific peroxidase conjugates. The levels of rubella antibodies in these sera were predetermined by the radial hemolysis test, the density gradient centrifugation method for immunoglobulin M (IgM) antibodies, and IgG-, IgM-, and IgA-specific enzyme immunoassays. In immunoblotting, rubella-specific IgG antibodies reacted with both envelope glycoproteins (E1 and E2) and the capsid protein (C). In contrast, rubella IgM antibodies reacted predominantly with E1, whereas the specific reactivity of IgA antibodies was directed mainly to the capsid protein. Purified IgM rheumatoid factor added to IgG-positive, IgM-negative serum did not give false-positive reactivity in the immunoblotting test as it did in solid-phase enzyme immunoassays. The immunoglobulin class-specific reactivities with the different viral proteins are expected to have diagnostic applications.

Rubella virus has been found to contain three structural polypeptides, two externally located glycoproteins E1 (M_r 58,000) and E2 (M_r 42,000 to 47,000) and the nucleocapsid protein C (M_r 33,000) (4, 12). E2 occurs in virions in two different forms, E2a (M_r 47,000) and E2b (M_r 42,000), which apparently differ only in glycosylation. Monoclonal antibodies reactive with E1 inhibit the hemagglutinin function of the virus, suggesting that E1 carries, in part at least, that function (15).

It is well known that human rubella virus infection induces specific immunoglobulin G (IgG), IgM, and IgA antibodies which all have pathogenetic significance and diagnostic value (3, 14). In this report we show that all three structural proteins, E1, E2 (a and b), and C, elicit antibodies in natural human rubella infection, but the different proteins raise distinct immunoglobulin class-specific responses.

MATERIALS AND METHODS

Virus. The Therien strain of rubella virus grown in B-Vero roller cell cultures was purified as described before (4) and was used as antigen in both enzyme immunoassays (EIA) and immunoblotting.

EIA. The procedure (13) used in solid-phase EIA was briefly as follows. Purified rubella virus, 0.5 μg/ml, was used to coat polystyrene cuvettes (FP-9; Labsystems, Helsinki, Finland). The sera were used at a 1:100 dilution, and binding of the antibodies was determined by using alkaline phosphatase-labeled swine antibodies to human IgG, IgM, or IgA heavy chains (Orion Diagnostica, Helsinki, Finland). *Para*-Nitrophenylphosphate was used as the substrate, and the results were measured with a photometer (FP-901 chemistry analyzer; Labsystems) based on vertical light path (9) at 405-nm wavelength. The EIA results were expressed as EIA units (EIU), using a known positive reference serum containing 100 EIU (see serum 1, Table 1) of rubella IgG antibodies. This reference serum gave in the radial hemolysis test (11) a 10-mm disk of hemolysis and contained no rubella IgM antibodies as determined by both EIA and the density gradient centrifugation method (14). A rubella convalescent serum (see no. 5, Table 1), devoid of rheumatoid factor (RF) determined by solid-phase EIA using immobilized human IgG and heavy-chain-specific anti-IgM conjugate as described previously (7), was used as the reference serum (100 EIU) for both IgM and IgA.

Immunoblotting. Our modification of the immunoblotting technique (10) has been described in detail previously (5). For immunoblotting, 4-μg samples of purified rubella virus were subjected per track to sodium dodecyl sulfate-polyacrylamide (8%) slab gel electrophoresis in reducing conditions, and the polypeptides were then transferred to a nitrocellulose sheet. Before immunoblotting, 10% newborn calf serum and 0.2% Triton X-100 were used to saturate nonspecific binding sites. The sera were tested at 1:100 (IgG) or 1:50 (IgM and IgA). The peroxidase conjugates specific for rabbit anti-human IgG, IgM, and IgA heavy chains (δ, μ, and α chains, respectively) (Dako, Copenhagen, Denmark) were used.

RF. IgM RF, a kind gift from O. Wager (7), was purified from the cryoglobulinemic serum of a patient with Waldenström's macroglobulinemia. The RF preparation reacted with human IgG but not with rabbit IgG as shown by the latex and the Waaler-Rose tests, respectively, and contained no rubella antibodies. The preparation was used at a final concentration of 5 μg/ml in the EIA and in immunoblotting experiments.

RESULTS

Immunoglobulin class-specific reactivity. After transfer to nitrocellulose the preparation of purified virus showed in protein staining the two glycoproteins E1 (M_r 58,000) and E2 (M_r 42,000 to 47,000) and the capsid protein C (M_r 33,000) as well as a polypeptide comigrating with albumin, apparently as a contaminant. IgG antibodies of sera with high titers of rubella antibodies (determined by the radial hemolysis and IgG EIA tests) reacted strongly with E1 but also with E2 and C (Fig. 1). In addition, several sera (for instance, no. 1 and 2 in Fig. 1) reacted with a high-molecular-weight polypeptide, which probably represents E1 dimer. Sera negative in

* Corresponding author.

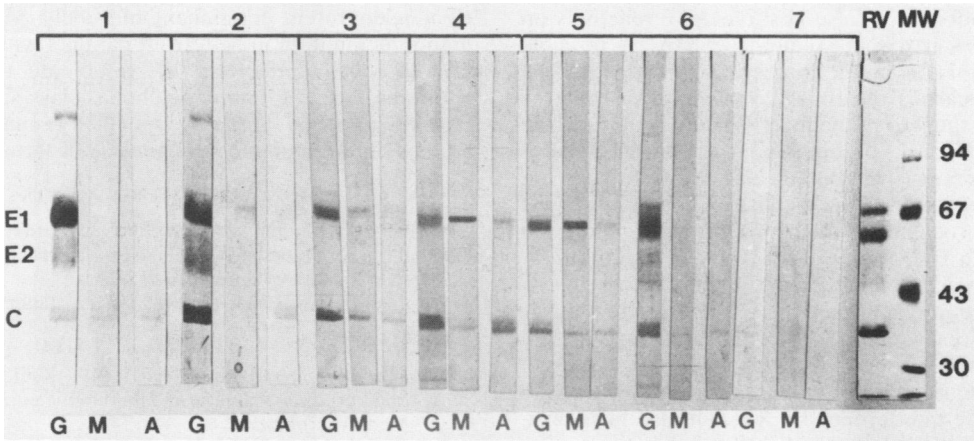

FIG. 1. Immunoblotting of rubella virus structural proteins with seven individual human sera. Each serum (no. 1 to 7) was studied by IgG, IgM, and IgA antibodies, using the immunoperoxidase procedure. Track RV shows protein staining of the purified virus preparation. Track MW shows the molecular weights of the marker proteins ($\times 10^3$). E1, E2, and C indicate the three structural proteins of rubella virus. Other serological findings on these seven sera are shown in Table 1.

the radial hemolysis and EIA tests (no. 7 in Fig. 1) gave no specific reaction; however, very faint reactivity was seen in the position of the C protein (Fig. 1). This low level of reactivity was also obtained with the conjugate control. IgM antibodies reacted predominantly with E1 and only weakly with the C protein. Numerous seropositive rubella sera were tested with the immunoblotting procedure. The six positive sera were chosen as they represent typical findings. Table 1 summarizes the other serological findings of these sera.

Interference by RF. RF (IgM anti-IgG), known to be common in rubella convalescent sera (7), was found to cause a strong false-positive IgM reaction in solid-phase EIA when added to IgG antibody-positive sera (Table 2), in agreement with previous observations (7). This effect was also seen when RF was added to sera positive for both IgG and IgM rubella antibodies as an additional increment in the absorbance value (Table 2). The decrease in absorbance at 405 nm for IgG rubella antibodies after addition of RF (serum no. 2, Table 2) is also in agreement with previous observations (6). To study the role of RF, we added the purified RF preparation to the same sera before immunoblotting. Unexpectedly, the added RF did not mediate any false-positive IgM reactivity in the immunoblotting test (Fig. 2).

TABLE 1. Rubella antibody determinations by radial hemolysis and EIA tests[a]

Serum no.[a]	Radial hemolysis (mm)	EIA (EIU)[b]		
		IgG	IgM	IgA
1	10	100	<30	27
2	12	178	<30	12
3	9	47	156	145
4	11	55	99	>200
5	10	37	100	100
6	12	176	43	43
7	<5	<10	<30	13

[a] These sera were the ones used in the immunoblotting assays in Fig. 1. Sera (no. 1 to 7) were tested for their RF activity and only one serum (no. 6) had high reactivity. This serum had no IgM-specific reactivity in immunoblotting.

[b] The results of the EIA tests are expressed in EIU using the formula: EIU (sample) = [A (sample) − A (b)]/[A (ref) − A (b)] × 100, in which A (sample) is absorbance reading of the sample, A (b) is that of the reagent blank, and A (ref) is that of the positive reference serum.

DISCUSSION

The present study on the reactivity of immunoglobulin class-specific antibodies with the individual viral proteins is based on the specificity and sensitivity of the immunoblotting procedure and the availability of heavy chain-specific anti-immunoglobulin conjugates. The major finding was that IgM and IgA antibodies, unlike IgG, showed selective reactivity. We cannot exclude the possibility that the differences are, in part at least, due to selective denaturation of antigenic determinants during the sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting procedures; it should be noted, however, that IgG antibodies reacted with all three structural proteins, E1, E2, and C. IgM antibodies reacted mainly with E1, which according to trypsin digestion experiments (2) and use of E1-specific monoclonal antibodies (15) carries the receptors for attachment to erythrocytes. The present studies do not distinguish whether the IgM antibody reactivity with E1 is directed to carbohydrate versus polypeptide determinants. According to recent immunoblotting analyses of the *Toxoplasma gondii* trophozoite, human IgM antibodies have a highly selective reactivity with those toxoplasma polypeptides to which the receptor function has been tentatively assigned (5, 6, 8).

The observation that rubella IgG antibody-positive sera, many of which contain RF detectable by EIA (7), were IgM negative in the immunoblotting test already suggested that RF did not interfere in this test. Nevertheless, an unexpected finding was that RF even when added in high con-

TABLE 2. Effect of added RF in IgG and IgM rubella EIA[a]

Serum[b]	Absorbance value (at 405 nm) in EIA			
	No RF added		RF added	
	IgG	IgM	IgG	IgM
1 (RH, <5 mm; IgM−)	0.022	0.129	0.050	0.159
2 (RH, 12 mm; IgM−)	1.728	0.293	1.483	1.421
3 (RH, 11 mm; IgM+)	0.766	1.610	0.810	>2.00

[a] The sera were tested at a 1:100 dilution before and after the addition of 5 μg of purified RF per ml.

[b] The reactivities of the sera in the radial hemolysis (RH) test and in the rubella IgM determination by the density gradient centrifugation method are indicated in parentheses.

centrations did not cause false-positive IgM reactivity in immunoblotting, as it did in solid-phase EIA. A possible explanation for this apparent discrepancy is that in the immunoblotting analysis the rubella virus antigens may be denatured so that they do not induce RF-binding sites in the rubella-specific IgG-antigen complex. An alternative possibility, among others, is that the low affinity of RF does not lead to immune reactivity in the incubation conditions of the immunoblotting procedure, which are somewhat different from those used in EIA.

The selective reactivity of IgM antibodies also suggests that the respective polypeptides, the E1 glycoprotein in the case of rubella virus, when purified, could be used in IgM antibody assays under conditions corresponding to those of the present immunoblotting procedure, possibly thus eliminating not only the interference by RF, but also false-positive reactions with impurities present in viral antigen preparations. The preferential reactivity of IgA antibodies with the capsid protein, though unexpected, is in agreement with the findings of Ho-Terry and Cohen (1). By analogy with influenza, in which recent infection is characterized by an antibody response to the internal ribonucleoprotein component, they detected IgA and IgG antibodies to the isolated ribonucleoprotein component of rubella virus by radioimmunoprecipitation in sera from patients with recent but not remote rubella infection. We anticipate that the present approach, use of immunoglobulin class-specific immunoblotting to study antibody specificity, may contribute to studies on the immunopathogenesis of virus infections.

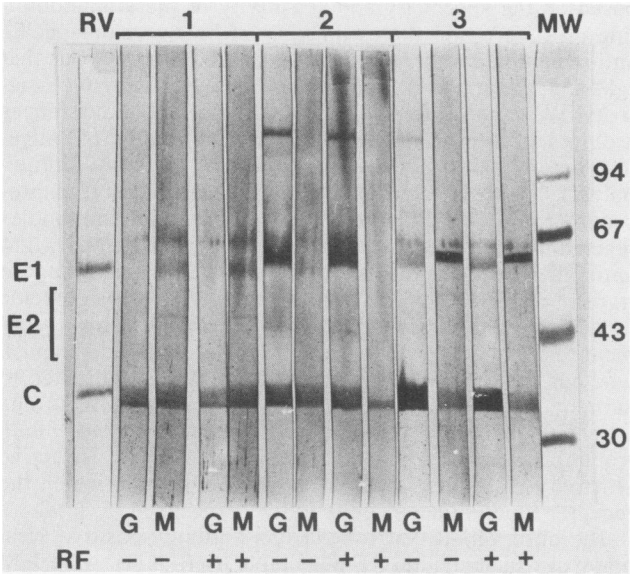

FIG. 2. Effect of added RF in immunoblotting of three sera. The three sera, no. 1 (IgM−, IgG−), no. 2 (IgM−, IgG+), and no. 3 (IgM+, IgG+), are the same as those in Table 2. For other explanations, see legend to Fig. 1. In the rubella virus preparation used here E2 forms a broad and diffuse band. Note the reactivity of IgG antibodies with the high-molecular-weight polypeptide, corresponding to the E1 dimer position. Also note the difference in the reactivity of IgG versus IgM antibodies with the E1 monomer.

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LITERATURE CITED

1. **Ho-Terry, L., and A. Cohen.** 1979. Radioimmunoassay for antibodies to rubella virus and its ribonucleoprotein component. J. Med. Microbiol. **12**:441–448.
2. **Ho-Terry, L., and A. Cohen.** 1980. Degradation of rubella virus envelope components. Arch. Virol. **65**:1–13.
3. **Ogra, P. L., D. Kerr-Grant, F. Umana, J. Dzierba, and D. Weintraub.** 1971. Antibody response in serum and nasopharynx after naturally acquired virus. N. Engl. J. Med. **285**:1333–1339.
4. **Oker-Blom, C., N. Kalkkinen, L. Kääriäinen, and R. F. Pettersson.** 1983. Rubella virus contains one capsid protein and three envelope glycoproteins, E1, E2a, and E2b. J. Virol. **46**:964–973.
5. **Partanen, P., H. J. Turunen, R. Paasivuo, E. Forsblom, J. Suni, and P. O. Leinkki.** 1983. Identification of antigenic components of Toxoplasma gondii by an immunoblotting technique. FEBS Lett. **158**:252–254.
6. **Partanen, P., H. J. Turunen, R. T. A. Paasivuo, and P. O. Leinikki.** 1984. Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulins G, M, and A antibodies at different stages of infection. J. Clin. Microbiol. **20**:133–135.
7. **Salonen, E.-M., A. Vaheri, J. Suni, and O. Wager.** 1980. Rheumatoid factor in acute viral infections: interference with IgM, IgG and IgA antibody determinations in enzyme-immunoassay. J. Infect. Dis. **142**:250–255.
8. **Sharma, S. D., J. Mullenax, F. G. Araujo, H. A. Ehrlich, and J. S. Remington.** 1983. Western blot analysis of the antigens of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. J. Immunol. **131**:977–983.
9. **Suovaniemi, O.** 1984. The vertical measurement principle in photometry. Int. Biotech. Lab. May/June, p. 43–45.
10. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
11. **Väänänen, P., and A. Vaheri.** 1979. Haemolysis-in-gel test in immunity surveys and diagnosis of rubella. J. Med. Virol. **3**: 245–252.
12. **Vaheri, A., and T. Hovi.** 1972. Structural proteins and subunits of rubella virus. J. Virol. **9**:10–16.
13. **Vaheri, A., and E.-M. Salonen.** 1980. Evaluation of solid-phase enzyme-immunoassay procedure in immunity surveys and diagnosis of rubella. J. Med. Virol. **5**:171–181.
14. **Vesikari, T., and A. Vaheri.** 1968. Rubella; a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. Br. Med. J. **1**:221–223.
15. **Waxham, M. N., and J. S. Wolinsky.** 1983. Immunochemical identification of rubella hemagglutinin. Virology **126**:194–203.