Oligomeric Immunoglobulin A Antibody Response to Rubella Virus Infection

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Pooled sera from rubella patients in the early convalescent stage, containing a high titer of hemagglutination-inhibiting (HI) antibody, were treated with protein A-conjugated gel to reduce immunoglobulin G (IgG) antibody and then centrifuged in sucrose gradients. This treatment resulted in the detection of an HI activity peak sedimenting at a rate intermediate between 7S and 19S. In contrast to the 19S antibody, the HI activity of this peak was not abolished by 2-mercaptoethanol, but sedimented at 7S after this treatment. The activity was considered to consist of IgA oligomers, since it was removed by anti-IgA immunosorbent. The appearance of the oligomeric IgA antibody after the infection was then studied using serum samples collected sequentially from five rubella patients. Shortly after the onset of the disease, the HI activity appeared at high titer and thereafter gradually decreased in titer until it could no longer be detected in the sera. The time of its disappearance varied with each patient.

While studying the sedimentation patterns in sucrose gradients of hemagglutination-inhibiting (HI) antibodies in the sera of rubella patients, we noticed that the leading edge of the 7S antibody peak extended far down the gradient or sometimes had a "shoulder," particularly with early convalescent sera, but that the 7S peak was sharp if the sera were treated with 2-mercaptoethanol (2ME) before centrifugation. This suggested that, in addition to the 19S antibody, the sera contained HI antibody which normally sedimented faster than 7S. Although Ankerst et al. (2) have treated sera with Formalinized *Staphylococcus aureus* (protein A) to detect any rubella-specific immunoglobulin M (IgM) antibody by absorbing IgG from sera, we find in addition that the protein A treatment results in an HI peak which clearly sediments between 7S and 19S in sucrose gradients. This paper reports on the nature of the immunoglobulin species in this intermediate peak and also the duration of its occurrence in sera following infections with rubella virus.

MATERIALS AND METHODS

Serum specimens. Two kinds of serum samples were analyzed: one was a pool of sera collected from the early convalescent phase of patients with rubella, having HI antibody titers of 1:512 or greater; the second was a series of individual serum samples sequentially collected from five rubella patients, all of whom were medical personnel, male, and 25 or 26 years old.

Protein A treatment of sera. Sera were first

treated with heparin-MnCl₂ (5) to remove nonspecific hemagglutination inhibitors, and then with protein A to reduce the concentration of IgG, as follows: 0.15 ml of sera was mixed with 0.3 ml of Veronal-buffered saline (pH 7.3), 0.15 ml of a 1:1 mixture of heparin (5,000 U/ml) and 1 M MnCl₂, and 0.1 ml of a 50% goose erythrocyte suspension, and then incubated at 4°C for 1 h with occasional shaking. After centrifugation at 900 × g for 20 min, the supernatant was diluted fivefold with Veronal-buffered saline and passed through a column (5 by 30 mm) containing protein A-Sepharose CL-4B (Pharmacia). The eluate was concentrated to 0.3 ml using a single hollow fiber (MDA Scientific, Park Ridge, Ill.) (17). As controls, sera were treated in the same way as above except that the protein A treatment was omitted.

Sucrose gradient centrifugation. Following the above treatment, the serum samples were mixed with 0.05 ml of 10-mg/ml beef liver catalase (Sigma; sedimentation rate 11.4S) and centrifuged in 12.5 to 37% (wt/vol) sucrose gradients in Veronal-buffered saline in a Spinco SW50.1 rotor at 35,000 rpm for 16 h at 4°C (16). After centrifugation, the gradient was overlaid with water containing phenol red, the tube bottom was pierced by a needle, the water was pumped onto the top at a constant speed, and constant volume fractions were collected. All gradients yielded the same number of fractions.

To determine the location of the peak of catalase activity, 10-μl samples from each fraction were mixed with 1 ml of 2% hydrogen peroxide and 0.1% sodium dodecyl sulfate in phosphate-buffered saline (pH 7.3). The fractions giving maximum froth production were considered to contain the 11S catalase marker.

To determine which fractions contained 19S or 7S material, rabbit hemolysin was centrifuged in one of the gradients, and the hemolytic activity of each frac-

tion was determined as described elsewhere (10).

Sephadex G-200 gel filtration. Heparin-MnCl₂-treated serum (0.8 ml; dilution 1:4) was applied to a column (1.5 by 80 cm) of Sephadex G-200 (Pharmacia) which had been equilibrated with Veronal-buffered saline. The void volume fractions were pooled and concentrated to 0.3 ml by the single hollow fiber technique.

Rubella HI test. HI antibody titration was carried out in microtiter plates using goose erythrocytes. Higher HI antibody titers were obtained by incubating the gradient fractions with the antigen overnight at 4°C (1, 15).

2ME treatment. Antibody samples were treated with 2ME at a final concentration of 0.1 M at 37°C for 1 h; 0.025 ml from each fraction and 0.025 ml of 0.2 M 2ME were mixed in a microplate well, and the plate was sealed with pressure-sensitive film (Falcon) and incubated in a water bath. The HI titers of each fraction were then assayed in the same microplate.

Immunoglobulin class determination of antibody fractions: The absorbance of HI activity by anti-γ or -α heavy-chain-specific immunosorbents was measured as follows: 1 ml of anti-human IgG or -IgA serum (lots no. 2622M and 2919B, respectively; Behringwerke, Marburg, W. Germany) was mixed with 0.8 ml of saturated ammonium sulfate; the precipitate was dissolved, dialyzed against borate buffer (pH 8.0), and conjugated with 0.5 g of CNBr-activated Sepharose 4B (Pharmacia) according to the manufacturer's directions. A 0.15-ml sample of the immunosorbent gel suspended in 0.1% bovine serum albumin-phosphate-buffered saline was mixed with an equal volume of antibody fractions from the sucrose gradients and incubated for 2 h at room temperature with occasional mixing. The HI activity of the supernatant was titrated after overnight incubation of the antigen-antibody mixture.

RESULTS

Sedimentation patterns of rubella HI antibodies in the pooled convalescent sera from rubella patients. The pooled convalescent sera were divided into three portions after heparin-MnCl₂ treatment. One aliquot was treated with protein A, the second with 2ME, and the third was an untreated control. These samples were centrifuged in sucrose gradients at the same time. Figure 1 shows the distribution of HI activity for both treatments and the control. When the IgG concentration in the serum was reduced by protein A treatment, an HI peak sedimenting at about 10S was clearly disclosed (Fig. 1c). If the serum was pretreated with 2ME, the 19S peak disappeared and all the activity resided in a narrow band at the 7S position (Fig. 1b), suggesting that the 10S HI activity also disappeared after 2ME treatment.

Nature of the 10S HI activity. It was suspected that the 10S activity consisted of IgA oligomers, since it was not absorbed by protein A (Fig. 1c). To confirm this, we tested to see

FIG. 1. Sedimentation patterns of HI antibody activity in the rubella convalescent serum pool. High-titer convalescent sera were treated first with heparin-MnCl₂ to remove nonspecific hemagglutination inhibitors, and then with 2ME or protein A. The samples were centrifuged in sucrose gradients. The gradients were fractionated as described in the text. As sedimentation velocity markers, rabbit hemolysin (7S, 19S) and beef liver catalase (11.4S) were used. In the HI antibody titration of the gradient fractions, the antigen-antibody mixtures were incubated overnight at 4°C.

whether or not the HI activity was removed by anti-IgA immunosorbent.

The 10S material obtained from gradients after repeated centrifugation of the protein A-treated serum pool was mixed with anti-IgA or -IgG gels or with phosphate-buffered saline, and HI titers of the supernatant were assayed. As shown in Table 1, the HI activity was absorbed by anti-IgA but not by anti-IgG gels. As a control, the 7S fraction of the untreated control (Fig. 1a) was diluted 1:16 and mixed with the immunosorbents. In this case, effectively all of the activity was removed after mixing with anti-IgG gel (Table 1), indicating that the majority of the 7S activity was due to IgG. We therefore suspected that the 10S material observed after treating sera with protein A might consist of IgA

oligomers (dimers and trimers).

2ME treatment of the 10S activity. The data indicated that 2ME treatment of the isolated 10S material slightly reduced the HI titers, but only by a single dilution. However, Fig. 1b suggested that the 2ME treatment of the serum before centrifugation did not result in any HI activity in the 10S fraction, so this point was investigated further.

The serum pool treated with heparin-MnCl₂ was chromatographed on Sephadex G-200, and the void volume fraction was centrifuged in sucrose gradients. Figure 2a shows that the void volume fraction contained both 19S and 10S

TABLE 1. Removal of HI activity by immunoglobulin class-specific immunosorbents

Antibody fractions	HI titers after absorption by		
	PBS	Anti-IgA	Anti-IgG
Intermediate peak (about 10S) ^a	8	<1	8
7S peak ^b	8	8	<1

^a Obtained after protein A treatment of the convalescent serum pool.

^b Obtained without protein A treatment, and appropriately diluted.

FIG. 2. Effect of 2ME treatment on the HI activity of the 10S material. The pooled convalescent sera after heparin-MnCl₂ treatment were chromatographed on Sephadex G-200. The void volume fraction, containing IgM and IgA oligomers, was concentrated before centrifugation in sucrose gradients. (a) Control; (b) 2ME treatment of the sample before centrifugation. (.....) HI titers of each fraction treated with 2ME after centrifugation.

material. When each sucrose gradient fraction was treated with 2ME, no HI activity was detected in the 19S fraction, while the activity of the 10S fraction was only reduced slightly (Fig. 2a). If the void volume sample was treated with 2ME before centrifugation, all HI activity sedimented at 7S (Fig. 2b). Thus, the 7S activity is probably derived from the 10S rather than the 19S material, since the latter was 2ME sensitive. This provides additional support for the hypothesis that the 10S material consisted of oligomeric antibodies.

Time course of the appearance of the 10S activity following rubella infections. The chronology of the appearance of the 10S activity was studied using serum specimens collected sequentially from five rubella patients. Figure 3 shows the sedimentation patterns after protein A treatment as well as the patterns of control sera from patients no. 1 and 2. In patient no. 1, the 10S-sedimenting material appeared in the protein A-treated serum 8 days after the onset of rash (Fig. 3b, patient I). Two months later, it had disappeared, but a low level of HI activity was detected at the 7S position (Fig. 3d). A sample taken 15 months later still showed no HI activity in the protein A-treated serum, whereas high titers were obtained for the 7S fraction in the control serum (Fig. 3e). In patient no. 2, 10S activity of high titer was detected in the protein A-treated sample from day 4 (Fig. 3a, patient II), but 6 months later only low HI activity was detected at the 10S position (Fig. 3b). After 15 months, low HI antibody activity was detected only at the 7S position (Fig. 3c).

Figure 4 shows the sedimentation patterns of sera from patients no. 3, 4, and 5 after protein A treatment. The appearance of the antibody that was observed is similar to that for patients no. 1 and 2, i.e., the rapid appearance but gradual waning of the 10S activity, eventually showing low HI activity contained solely in 7S material of protein A-treated sera.

The use of immunosorbents confirmed that the 10S material in the sera of all five patients was probably IgA. Furthermore, the 7S material in the protein A-treated sample taken from patient no. 3, 14 months after the onset of disease (Fig. 4d), was also found to be mainly IgA by the same criterion. The HI activity of the 7S material in the protein A-treated sera of patients no. 2 (Fig. 3c), 4 (Fig. 4d), and 5 (Fig. 4c) was too low to be tested for the immunoglobulin class.

DISCUSSION

The HI activity of 10S material contained in sera sampled from rubella convalescent patients

FIG. 3. Time course of the appearance of the 10S material after the onset of rubella infection. Serum specimens sequentially collected from patients no. 1 (I) and 2 (II), with or without protein A treatment, were centrifuged in sucrose gradients. (a) Control; (b) protein A treatment. D and M, Days and months after the onset of rash. Figures in parentheses indicate HI antibody titers of whole sera, obtained by conventional 1-h incubation of the antigen-antibody reaction mixture.

is probably due to IgA oligomers (dimers and trimers), since it was not affected by protein A but was removed by anti-IgA immunosorbent. This material appeared, at high titer, immediately following the infection and gradually disappeared. Therefore, in addition to IgM antibody, the detection of IgA oligomers in sera might be useful for diagnosing recent rubella infections. Bürgin-Wolff et al. (4) were the first to report the detection of rubella IgA HI antibody of larger molecular size than IgG by agarose gel chromatography. Their finding is confirmed by our study using protein A treatment and sucrose gradient centrifugation.

The 7S material, of low HI titer, present in protein A-treated sera long after infection might be IgA monomers or IgG subclass 3 antibody, or possibly both (IgG3 has been reported not to react with protein A [11]). We found that the 7S material in protein A-treated serum of patient no. 3 (Fig. 4d) was mainly IgA. Low HI activity at the top fractions in the serum of patient no. 2 (Fig. 3b and c) might be nonspecific hemagglu-

tination inhibitors that were not completely removed by heparin-MnCl₂ treatment, since it was not removed by either anti-IgA or -IgG immunosorbents.

There have been several studies on serum IgA antibody responses to rubella virus infections by the immunofluorescence technique (3, 6, 8, 9). However, that technique does not differentiate IgA monomers from oligomers. Hornsleth et al. (9) concluded that the detection of IgA antibody could not be considered as decisive evidence of a recent rubella infection. However, they may have been detecting IgA monomers of low HI titer, which persisted in the serum for a long time period, in addition to early-appearing IgA oligomers, because their procedure utilized a diethylaminoethyl-Sephadex ion-exchange step; this could have had the effect of eliminating any competition between IgG and IgA antibodies in serum samples taken long after the onset of infection. On the other hand, Craddock-Watson et al. (6) claimed that the presence of IgA antibody did indicate a recent infection. They might

FIG. 4. Time course of the appearance of the 10S material after rubella infection. All serum specimens from patients no. 3 to 5 (III, IV, and V) were treated with protein A and centrifuged in sucrose gradients.

have detected only early-appearing, high-titer IgA oligomers, and, in fact, their data showed that the IgA antibody peak sedimented in sucrose gradients between those of IgG and IgM antibodies (6).

Rubella IgA antibody response was also studied by Ogra et al. (14) using the radioimmuno-diffusion technique (12, 13). They reported that the serum IgA antibody response only occasionally appeared and then only in samples taken long after the onset of infection. The radioimmuno-diffusion test they employed might not have been sensitive enough to detect such IgA oligomers. In carrying out that test, the first step was to allow IgA molecules in the serum to form a precipitation line in the agar plate by reaction with anti-IgA antibodies. A majority of the IgA oligomers are unlikely to participate in this reaction because they would diffuse more slowly than the monomers.

In our study it is interesting that the HI activity of the IgA oligomers was not greatly decreased by 2ME treatment, which contrasts strongly to the reaction of IgM antibody. Handsher and Fogel (7) encountered some rubella convalescent sera whose HI titers, after the

staphylococcal absorption treatment, were not greatly reduced by 2ME treatment. Possibly, in such sera, the HI titers of IgA oligomers could have been higher than the IgM titers, although the authors did not refer to the IgA antibody.

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