Virus-Specific Polymeric Immunoglobulin A Antibodies in Serum from Patients with Rubella, Measles, Varicella, and Herpes Zoster Virus Infections

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More than 85% of the immunoglobulin A (IgA) antibodies in normal adult serum are monomeric (m-IgA). By contrast, virus-specific IgA is mainly polymeric (p-IgA) in sera from patients with rubella, measles, and varicella. Specific m-IgA antibodies only reach quantitative significance in late convalescence. In patients with herpes zoster, on the other hand, a varying response was observed: in three of six sera, specific IgA was absent or at a very low titer, whereas in the remaining three cases, a high titer of both p-IgA and m-IgA was noted. These results suggest that in the initial response to rubella, measles, and varicella-zoster viruses, specific IgA first appears as p-IgA and only later becomes, or is replaced by, m-IgA.

The specific antibodies observed in the sera of patients with rubella belong to the immunoglobulin M (IgM), IgG, and IgA classes. IgM can be detected for a short time only, whereas IgA antibodies persist for 2 months to several years and thus are of no assistance in the detection of recent infections (7, 8). In varicella-zoster virus infections, too, specific IgA antibodies are not a reliable marker of active infection (6, 14, 16).

In normal adult serum, most IgA antibodies are monomers (m-IgA), and only 10 to 15% are present as polymers (p-IgA) (15). Both forms are present in the sera of patients with rubella (9); p-IgA persists for a short time and can be used in the diagnosis of recent infection, whereas m-IgA remains for much longer and is of no diagnostic importance.

In this paper we describe the characteristics of virus-specific IgA antibodies studied in sera from patients with rubella, measles, varicella, and herpes zoster at various times after the onset of symptoms.

MATERIALS AND METHODS

Sera. A total of 36 serum samples were titrated from 25 patients (8 suffering or recovering from rubella, 5 with measles, 6 with varicella, and 6 with herpes zoster). In some cases, repeated withdrawals at various times from the onset of symptoms were made.

Serum fractionation. All sera were fractionated by chromatography on Bio-Gel A-5m (200/400 mesh; Bio-Rad Laboratories, Richmond, Calif.) on K16/100 columns (1.6 by 100 cm; Pharmacia, Uppsala, Sweden). Two columns packed with 83 and 85.8 cm of gel, respectively, were used. They were equilibrated with 25 mM HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)-0.14 M NaCl-1 mM CaCl2-3 mM NaN3 (pH 7.3) and calibrated with standard polymers (p-IgA, Pharmacia). All fractionations were carried out by loading the columns with 2 ml of a 1:2 dilution of serum in HEPES buffer at 2 cm/h. After the optical density was read in a flow cell at 280 nm, the eluate was collected in 2.5-ml fractions.

Serum reduction and alkylation. Reduction was performed by incubating the serum with dithiothreitol (final concentration, 0.005 M) for 1 h at 37°C (13). Spontaneous reoxidation

FIG. 1. Fractionation on a Bio-Gel A-5m column (1.6 by 83 cm) of a serum sample from a patient with rubella 8 days after the onset of rash. The elution positions of thyroglobulin (thyr), ferritin (ferr), aldolase (ald), and ovalbumin (ovalb) are indicated by arrows. (A) ELISA determination of rubella virus-specific antibodies. Before titration, the fractions were diluted 1:6 for IgM, 1:11 for IgA, and 1:66 for IgG. (B) RID determination of immunoglobulin (Ig) concentrations. OD, Optical density.
of the disulfide bonds was then prevented by the addition of iodoacetamide (final concentration, 0.05 M) and incubation for 1 h at room temperature. Before fractionation on the column, the serum was brought to a final dilution of 1:2 and centrifuged at 12,000 × g for 15 min.

**RID.** Single radial immunodiffusion (RID) was done by the method of Mancini et al. (11) with LC-Partigen plates (Behringwerke, Marburg-Lahn, Federal Republic of Germany). A calibration curve was made in each plate.

**ELISA.** For the enzyme-linked immunosorbent assay (ELISA), plates coated with rubella, measles, and varicella-zoster virus antigens (Enzygnost; Behringwerke) were used; also used were alkaline phosphatase-conjugated anti-human immunoglobulin sera (anti-IgM and anti-IgG [Behringwerke] and anti-IgA [Orion Diagnostica, Helsinki, Finland]). The fractions represented an approximately 1:10 to 1:15 dilution of the initial serum. They were further diluted before titration (see figure legends). Readings were taken with a Titertek Multiskan photometer (Flow Laboratories, Inc., Milan, Italy).

**RESULTS**

**Rubella.** To separate and titrate specific p-IgA and m-IgA antibodies, 16 sera from eight patients were fractionated by gel filtration. RID was used to determine the total concen-
tration of IgM, IgA, and IgG antibodies in each fraction. The titers of IgM, IgA, and IgG specific for the rubella virus were determined by the ELISA.

The results observed 8 days after the appearance of rash are shown in Fig. 1. Comparison of the total immunoglobulin distribution (Fig. 1B) with that of the rubella virus-specific immunoglobulin (Fig. 1A) showed that the two IgM and IgG elution peaks coincide. Elution of the IgA antibodies, on the other hand, began immediately after that of the IgM with the heavier p-IgA and continued to come close to the IgG peak, where most of the IgA (m-IgA) was eluted (Fig. 1B). In contrast, most of the specific IgA antibodies eluted in a peak that occurred well before that of the m-IgA and was composed of p-IgA (probably dimers and trimers) with only a small amount of m-IgA (Fig. 1A).

The results observed with four sera collected from a single patient at various times after the onset of rash are shown in Fig. 2. During the acute stage, the specific IgA consisted almost entirely of p-IgA (dimers, trimers, and possibly tetramers) (Fig. 2A), whereas m-IgAs were only clearly demonstrable during late convalescence, when the p-IgA peak became smaller and remained primarily composed of dimers (Fig. 2B). In sera taken after 134 days (Fig. 2C) and 342 days (Fig. 2D), the IgA titer was very low, with a predominance of monomers. This patient displayed an unusually protracted IgM response, with titers that were high after 40 days and still detectable after 134 days. This persistence may be ascribable to complications (arthritis) occurring about 2 weeks after the appearance of rash; in complicated rubella infections, a prolonged IgM response has been reported on several occasions (12).

To dissociate the IgM and p-IgA into monomers, a sample of the serum shown in Fig. 2B was fractionated after reduction and alkylation. The results obtained with ELISA titration of specific antirubella antibodies in the fractionated serum are shown in Fig. 3. IgM which dissociated into monomers was probably eluted with the IgG (molecular weight, 160,000) but could not be detected since it had lost its immunological activity. The p-IgA peak was still detectable although greatly reduced, whereas the m-IgA peak was enhanced. The IgG was virtually unchanged (Fig. 3).

The other fractionated sera displayed similar m-IgA and p-IgA distribution of specific antirubella antibodies (results not shown).

**Measles.** Virus-specific IgAs were studied in five sera collected from patients or convalescents 3 to 13 days after the onset of symptoms. In the five sera, nearly all of the specific IgAs were p-IgA (data not shown).
Varicella and herpes zoster. The pattern of specific IgAs in varicella-zoster virus infections was studied in six patients with varicella and six with herpes zoster. Titers of total and specific immunoglobulin taken from a patient with varicella 12 days after onset are shown in Fig. 4. The distribution pattern was virtually that shown in Fig. 1, and nearly all the specific IgA antibodies were p-IgA. Two sera from another patient 4 and 17 days after onset are compared in Fig. 5. In the acute stage (Fig. 5A), p-IgAs greatly predominated, whereas in convalescence, their peak was thinner and a small m-IgA peak appeared (Fig. 5B). It should be noted that IgM and IgG titers in this patient were very similar in both the day 4 and day 17 sera. IgM titers usually decline rapidly, whereas IgG titers reach their peak later. When titers of antivaricella antibodies are taken by methods such as radioimmunoprecipitation, however, IgM and IgG high titers may be both reached in the first 4 to 5 days (1). The pattern of specific IgAs in the other four patients with varicella was virtually the same (data not shown).

The pattern in patients with herpes zoster, on the other hand, varied from one patient to the next and was different from that of the varicella group. In two sera, the only specific immunoglobulins demonstrable were IgG (data not shown).

In a third, these immunoglobulins were accompanied by traces of IgM and IgA (Fig. 6A). In the remaining three, there was a high IgA titer. In contrast with results from the varicella group, however, m-IgA equalled or slightly exceeded p-IgA (Fig. 6B, C, and D). In one of these sera, there was also a high IgM titer (Fig. 6B), whereas in the other two, IgM was absent or at a very low titer (Fig. 6C and D).

**DISCUSSION**

Fractionation of serum by gel filtration gives good separation of p-IgA from m-IgA (5), whereas their titration by RID and ELISA results in rather wide margins of error. RID underestimates polymers more markedly the greater their aggregation. In particular, it underestimates dimeric IgA by a factor of about 1.55 and trimeric IgA by a factor of 1.85 (3). In the same way, ELISA may overestimate specific p-IgA. In fact, when used to assay a single dilution with the expression of the titer in terms of optical density, it evaluates the avidity of antibodies rather than their concentration (10), and it may be that p-IgA is more avid than m-IgA. These limitations must be kept in mind when seeking an exact evaluation of m-IgA and p-IgA concentrations and titers, but...
they do not preclude a comparison between sera fractionated and titrated by the same method.

Virus-specific p-IgA in the sera of patients with rubella has been previously reported (2, 9). Our results, however, make it clear that it is almost or entirely predominant in the acute stage (Fig. 1B and 2A), whereas m-IgA does not become quantitatively significant until late convalescence (Fig. 2B, C, and D).

In acute measles and acute varicella (Fig. 4 and 5), too, the specific IgA consists almost solely of p-IgA. In herpes zoster, on the other hand, both p-IgA and m-IgA are present (Fig. 6). Moreover, p-IgA antibodies from the sera of patients with acute rubella, measles or varicella are both dimeric and polymeric (Fig. 2A and 5A), whereas in convalescent sera and in herpes zoster they are primarily dimeric (Fig. 2B, 5B, and 6).

Our results thus offer strong support to the view that in some infections, specific IgA in sera first appears in the polymeric form and only later becomes, or is replaced by, m-IgA. In herpes zoster, the early appearance of specific m-IgA may be linked to the fact that this disease develops in the immunologically experienced individual. Another suggestion is that the high percentage of p-IgA observed in the sera of small children (36%, compared with 13% in the normal adult serum and in 14% in umbilical cord serum) (4) is a reflection of the frequency of primary infections in such subjects.

Rubella, measles, and varicella infections are alike in their initial invasion of the primary airways and subsequent generalization. Our data, of course, offer no indication of whether this common feature, especially insofar as involvement of the mucosa is concerned, has any relation to the appearance of specific p-IgA.

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