INFECTION AND IMMUNITY, Mar. 1991, p. 1126–1130
0019-9567/91/031126-05$02.00/0

Vol. 59, No. 3

Subclass and Molecular Form of Immunoglobulin A Antibodies to *Actinobacillus actinomycetemcomitans* in Juvenile Periodontitis

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Received 17 August 1990/Accepted 19 December 1990

Patients with juvenile periodontitis frequently have elevated levels of serum immunoglobulin A (IgA) antibodies to antigens of *Actinobacillus actinomycetemcomitans*. IgA occurs in two subclasses, IgA1 and IgA2, and in monomeric and polymeric forms. Because IgA1 is susceptible to cleavage by IgA1 proteases produced by microorganisms found at mucosal sites and in the gingival crevice, we wished to determine the IgA subclass distribution of antibodies to antigens of *A. actinomycetemcomitans*. The molecular form was examined because it may indicate the origin of the IgA and because the form differs in acute and chronic infections. There is also evidence that monomeric and polymeric IgA have different biological functions. Serum was taken from patients with juvenile periodontitis before and at intervals during and after initiation of therapy. IgA subclass distribution was determined against a sonic extract of *A. actinomycetemcomitans* ATCC 29524 (serotype b) by using monoclonal anti-subclass reagents in an enzyme-linked immunosorbent assay. To determine the molecular form of the antibodies, sera were separated by high-performance liquid chromatography on a size-exclusion column. Fractions were assayed for antibody activity by the enzyme-linked immunosorbent assay, as described above. The results of the subclass analysis of the sera indicated that while both IgA1 and IgA2 antibodies to *A. actinomycetemcomitans* sonic extract are often found before, during, and after treatment, IgA1 antibodies dominated the response. There was a predominance of monomeric IgA1 antibodies to *A. actinomycetemcomitans* sonic extract in most samples before, during, and after treatment. The monomeric form is consistent with what is seen in other chronic infections. The predominance of IgA1 antibodies implies that any protective effects of the IgA response to *A. actinomycetemcomitans* could be compromised by microbial IgA1 proteases.

Immunoglobulin A (IgA) occurs in humans in two subclasses, IgA1 and IgA2, and in monomeric and polymeric forms (14–16). The proportion of IgA1 varies from 80 to 90% in serum to about 60% in secretions. Serum IgA is characteristically monomeric (ca. 90%), while 95% or more of IgA in secretions is polymeric (7). The subclass and molecular form of IgA can be important parameters in immune responses. The subclass with which an individual responds can be important because the IgA1 subclass is susceptible to cleavage by microbial IgA1 proteases; this cleavage susceptibility may compromise host immunity (10). The molecular form of IgA may also be important, because some evidence indicates a difference in biological activity of monomeric and polymeric forms (20, 33). We have previously investigated the IgA subclass distribution of naturally occurring antibodies to microbial antigens and the responses to influenza virus which indicate a subclass-restricted response (3–6, 17). We and others have demonstrated that while most serum IgA is monomeric, a polymeric IgA antibody response may be elicited by systemic immunization (2, 3, 5, 6, 11, 12). There is also evidence that polymeric IgA is a characteristic of acute infections or acute immune responses, whereas monomeric IgA predominates in chronic immune responses (1, 18, 19). In order to further examine the parameters which influence the subclass distribution and molecular form of IgA responses, we examined the antibodies occurring in a chronic bacterial infection, namely juvenile periodontitis.

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MATERIALS AND METHODS

Clinical samples. Serum was obtained from patients diagnosed as having juvenile periodontitis according to established criteria (24). These subjects had serum IgG and IgA1 antibody levels to *Actinobacillus actinomycetemcomitans* ATCC 29524 (serotype b) greater than 3 standard deviations above the mean levels for a group of 20 normal control subjects (data not shown). Samples were taken at various intervals before and after initiation of treatment and stored at −70°C until analyzed. Treatment included surgical debridement and tetracycline therapy (250 mg, four times per day for 3 weeks).

Growth of strains and antigen preparation. *A. actinomycetemcomitans* strains were grown overnight at 37°C in thioglycolate medium supplemented with 8.4 g of $NaHCO_3$ per liter, which had been filter sterilized as an 8.4% solution and added after autoclaving. Cells were harvested by centrifugation, washed three times with 0.01 M phosphate-buffered saline (PBS), pH 7.4, and suspended in 0.5% Formalin overnight. Cells were washed again and disrupted by sonication (model 300 sonicator; Artek Systems Corp., Farmingdale, N.Y.) in the presence of glass beads by using 10 30-s bursts at 90% power with a 2-min cooling period between bursts. The extract was separated from the glass beads and centrifuged at 20,000 × *g* for 30 min.

Measurement of IgA subclasses. Specific IgA1 and IgA2 antibodies to *A. actinomycetemcomitans* were measured by an enzyme-linked immunosorbent assay (ELISA), which was a biotin-avidin modification of the solid-phase radioimmunoassay described previously (6). Briefly, polystyrene plates (Flow Laboratories, McLean, Va.) were coated with 10 μg of sonic extract of *A. actinomycetemcomitans* ATCC 29524 (serotype b) per ml in PBS overnight at 4°C. Plates

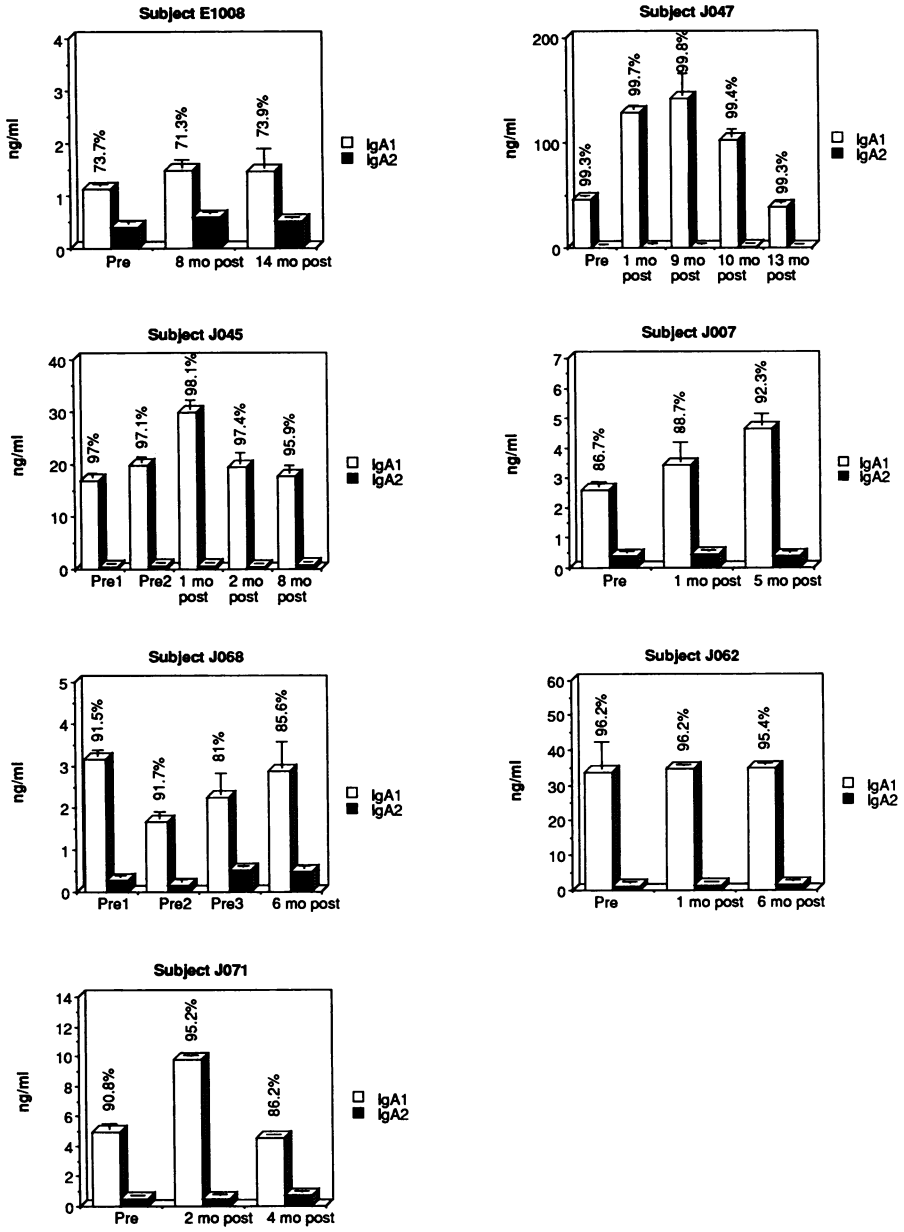

FIG. 1. Subclass distribution of IgA antibodies to *A. actinomycetemcomitans* ATCC 29524 (serotype b) sonic extract at various times before (Pre) and after (post) initiation of therapy. Open bars represent IgA1, and solid bars represent IgA2. The percentage of IgA1 antibody for each time point is indicated above the open bar.

were blocked for 30 min with 1% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) and washed with PBS containing 0.05% Tween 20. Samples were diluted in PBS-bovine serum albumin, applied to plates, and allowed to incubate overnight at 4°C. After being washed, biotinylated monoclonal anti-human IgA1 and IgA2 antibodies (provided by Jiri Radl and J. J. Haaijman, Institute for Experimental Gerontology, Rijswijk, The Netherlands) were applied to the plates and incubated overnight at 4°C. The plates were washed and incubated with 0.5 μg of avidin peroxidase per ml (Sigma) followed by development with *o*-phenylenediamine substrate. The assays were standardized by using purified IgA1(λ) and IgA2(λ) myeloma proteins (provided by Jiri Mestecky, University of Alabama at Birmingham) on wells coated with anti-human λ chain (Tago Inc., Burlingame, Calif.) (6). Standard curves were constructed by using the logit-log method of interpolation (21).

Assay of monomeric and polymeric IgA antibodies. The distribution of monomeric and polymeric IgA antibodies to *A. actinomycetemcomitans* in serum samples was determined by fractionation using high-performance liquid chromatography (HPLC) (6). Ten-microliter samples were fractionated on a Bio-Sil TSK-250 size-exclusion column (300 by 7.5 mm; Bio-Rad Laboratories, Richmond, Calif.). The column was eluted with 0.05 M sodium sulfate in 0.02 M sodium phosphate pH 6.8, at a flow rate of 1 ml/min. Five-drop fractions (ca. 142 μl) were collected into tubes containing 50 μl of PBS-bovine serum albumin. Samples

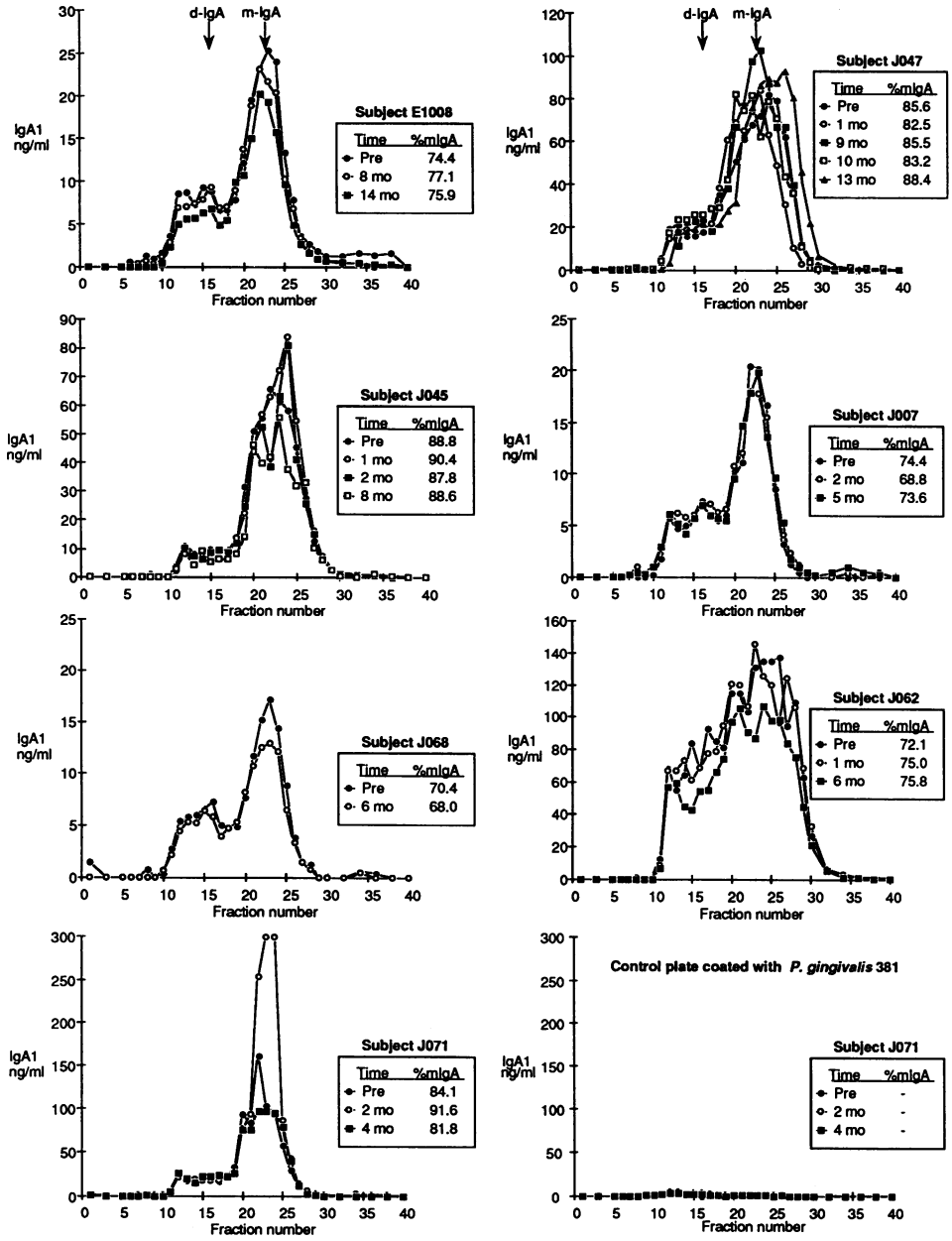

FIG. 2. Distribution of IgA1 antibodies to *A. actinomycetemcomitans* ATCC 29524 (serotype b) sonic extract (or *P. gingivalis* 381 sonic extract, bottom right panel) prior to and after initiation of therapy. Samples were separated by HPLC, and fractions were assayed for IgA1 antibodies by ELISA. The elution positions of authentic monomeric and dimeric myeloma IgA1 and IgA2 standards (determined separately) are indicated by the arrows. The percentage of monomeric IgA (%mIgA) for each time point is indicated within the legend.

were applied to ELISA plates coated with sonic extract and were assayed for antibody activity as described above.

RESULTS

Subclass distribution of IgA antibodies to *A. actinomycetemcomitans*. The subclass distribution of IgA antibodies in serum from juvenile periodontitis patients was determined against sonic extracts of *A. actinomycetemcomitans* ATCC 29524 (serotype b). With respect to the IgA isotype, all of the subjects included in this study had a predominance of antibody to serotype b strains of *A. actinomycetemcomitans*, as determined by preliminary screening against all three serotypes (data not shown). Figure 1 shows the results of the subclass analysis. Even though there was some IgA2 antibody present in most samples, by far the predominant antibody subclass was IgA1. Frequently, the proportions of IgA1 were higher than the 80 to 90% normally found in serum. In some cases, a transient increase was seen after initiation of treatment, presumably due to antigenic stimulation from mechanical debridement as has been described previously (9). However, in these cases the increase was in IgA1 alone (e.g., Fig. 1, subject J045) or in IgA1 predominantly with a small rise in IgA2 (e.g., Fig. 1, subject J007).

Molecular form of IgA antibodies. Ten-microliter serum samples were separated by size-exclusion HPLC, and fractions were assayed for IgA1 antibodies to *A. actinomycetemcomitans* by ELISA as described above. IgA2 antibody levels were not determined because the levels were too low to be detected after HPLC. Figure 2 shows the serum profiles for the subjects shown in Fig. 1. Arrows indicate the elution positions of authentic monomeric and dimeric IgA myeloma standards. The void volume occurred at approximately fractions 9 and 10. The typical pattern that was seen with the serum samples was a relatively stable distribution consisting of predominantly monomeric IgA antibodies. In most cases, monomer levels were significantly higher than polymer levels; however, one subject, J062, showed a nearly equal distribution. While the distributions were generally stable with time, one subject (J071) showed an increase 2 months after initiation of therapy. Even in this case, the increase appeared to be confined to the monomeric fraction. Little activity was detected against sonic extract of another gram-negative organism, *Porphyromonas gingivalis* 381, a suspected etiological agent in some forms of adult periodontitis (Fig. 2, bottom right panel).

DISCUSSION

The purpose of this study was to examine the subclass response and molecular form of IgA antibodies to a microbial antigen prior to and after initiation of treatment. The predominant response to *A. actinomycetemcomitans* in all subjects involved in IgA1 subclass. Even in cases in which increases occurred in the IgA1 subclass, very little increase was seen in the IgA2 subclass. Similar restrictions in the subclass of the IgA response have been seen with other antigens. For example, the IgA response to influenza A virus hemagglutinin in serum has been shown to occur almost exclusively in the IgA1 subclass (3, 5, 6). Likewise, immunization with tetanus toxoid produces predominantly IgA1 antibodies, whereas immunization with polysaccharide antigens induces primarily an IgA2 response in serum (22). In the influenza virus studies, in which a single dose of antigen was given, responses had dropped off by 6 months. In the present study, IgA levels remained elevated for as long as 14 months, probably due to the more intense stimulation with antigen. It is not known to which component of *A. actinomycetemcomitans* the IgA response is directed. We have previously shown that naturally occurring antibodies to lipopolysaccharides (LPS) from smooth strains of salmonellae are found in both the IgA1 and IgA2 subclasses (17). In contrast, the distribution of antibodies in the same serum samples to polysaccharide-deficient Rb and Re mutant LPS is almost exclusively IgA1, implying that the IgA2 antibody titers seen against smooth LPS may be directed to the O polysaccharide antigen, while IgA1 antibodies may be primarily directed towards the lipid A portion (17). Interestingly, the ratio of IgA1 to IgA2 in secretions such as saliva to the same antigens can be quite different. Thus, IgA2 antibodies in saliva from the same subjects predominate against both smooth and rough forms of LPS. These differences may reflect the different origins of systemic and secretory IgA (13).

With regard to the molecular form of IgA, there was a predominance of monomeric IgA to *A. actinomycetemcomitans* in all the subjects examined. This finding contrasts with previous studies of volunteers receiving systemic immunizations with influenza vaccines, in which we found that the initial response was in the polymeric IgA fraction in serum (3, 5, 6). Normal serum IgA is 90% monomeric, and thus it was surprising to find polymeric IgA in the latter instance. It has been speculated that even with systemic immunization, one may stimulate mucosally primed IgA precursor cells, which have been shown to be more predisposed to production of polymeric IgA (13). These results were later confirmed in studies with other viral and microbial antigens (1, 2, 11, 12, 18, 22). Monomeric IgA antibodies, however, have been found to predominate in chronic diseases such as hepatitis (1). A shift from initial polymeric IgA antibody to monomeric IgA antibody has been reported during the course of certain viral infections (18, 19). Interestingly, in one case (Fig. 2, subject J071) in which we saw a marked increase in levels during treatment, the increase was confined to the monomeric fraction. Apparently, during a chronic response further antigenic stimulation still results in a monomeric response. The presence of monomeric IgA antibodies to *A. actinomycetemcomitans* in subjects with juvenile periodontitis may thus reflect the consequences of a chronic stimulation with an antigen that is known to induce very high immunoglobulin levels in these subjects (8); however, the mechanisms involved in the polymer-to-monomer shift are unknown.

The parameters of subclass and molecular form of the IgA immune response may have some important biological consequences. The polymeric state of IgA may affect its biological activity since it appears that viral neutralization by monomeric and polymeric IgA occurs by different mechanisms (20, 23). It is well known that IgA1 antibodies but not IgA2 antibodies are susceptible to cleavage by microbial IgA1 proteases; this cleavage susceptibility, may compromise host immunity by a variety of mechanisms (10). Understanding the mechanisms of stimulation of IgA1 and IgA2 responses may aid in developing strategies to avoid or overcome such microbial interference.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service grants DE07322, DE06436, and DE07908 and by Research Career Development Award DE00236 to T.A.B.

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