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Immunoglobulin Class and Subclass Distribution of Antibodies Reactive with the Immunodominant Antigen of *Actinobacillus actinomycetemcomitans* Serotype b

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The aims of this study were to determine the immunodominant antigens of *Actinobacillus actinomycetemcomitans* serotype b (Aab) for the different immunoglobulin (Ig) classes and subclasses and to determine the relative levels of these different Igs in serum. Seropositive early-onset periodontitis patients were sampled, and the Ig classes IgG, IgA, and IgM and subclasses IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2 were studied. Reactivity with Aab antigens was assessed by using the Western blot (immunoblot) in limiting dilution analysis and radioimmunoassay with sera from 13 early-onset periodontitis subjects. A smeared antigen in the upper portion of the immunoblots, typical of high-molecular-weight LPS, was immunodominant for IgG, IgA, IgM, IgG1, IgG2, IgG3, IgA1, and IgA2. This smeared antigen was present in every patient for all of these Igs at the endpoint. A few additional antigens were also present at the endpoint in some patients, but none were present in more than half of the subjects. The distribution of antibody titers by Ig classes reactive with the Aab immunodominant antigen was IgG > IgA > IgM. The distribution of antibody titers by IgG subclass was IgG2 > IgG1 ≈ IgG3. Further quantitation by radioimmunoassay revealed that the mean concentration of IgG2 (65.7 μg/ml) was significantly greater than that of IgG1 (8.8 μg/ml). The IgA subclass distribution was IgA1 ≫ IgA2, with IgA1 apparently being second only to IgG2. Therefore, the Aab antigen eliciting the highest antibody level in virtually all Ig classes and subclasses appeared to be lipopolysaccharide, and IgG2 was markedly elevated over all other serum Ig classes or subclasses reactive with Aab.

Actinobacillus actinomycetemcomitans serotype b (Aab) is frequently isolated from lesions of patients with juvenile periodontitis (1, 10, 14, 29). This organism is thought to be a major etiologic agent of this form of periodontal disease (22), and it elicits remarkably high serum antibody titers (6, 11, 13, 16, 24, 26). However, Aab does not represent an unusual proportion of the cultivable subgingival flora in these patients (14). This suggests that antigens of Aab are potent immunogens. Previous work on the antigens responsible for the high titers in seropositive patients revealed that the immunodominant antigen for immunoglobulin G (IgG) was the serotype-specific carbohydrate (3, 4). Sims et al. (20) reported that hot phenol lipopolysaccharide (LPS) extracts contain the immunodominant antigen, and Wilson and Schifferle (28) found that the serotype-specific antigen is high-molecular-weight LPS.

It is known that the human Ig classes IgG and IgA are often elevated and the IgG2 subclass predominates in response to certain polysaccharide antigens (8, 19, 21), including LPS from *Porphyromonas gingivalis* (15, 17, 18). It is also known that IgA2 can be associated with anti-LPS responses to the O portion of the molecule (2) and IgA1 can be associated with responses to the lipid of LPS (2). Interestingly, IgA1 is known to be elevated against antigens in a sonic extract of Aab (2). We therefore hypothesized that the human immune response against the Aab immunodominant LPS antigen would be dominated by Ig classes IgG and IgA and subclasses IgG2 and IgA1. Further, IgG1 and IgG3 are typically produced in response to protein antigens (18). Consequently, we hypothesized that the immunodominant antigens for these Igs would be proteins. To test these hypotheses, we used the Western blot (immunoblot) technique with limiting dilution analysis. The antigen(s) reactive with antibody at the final positive serum dilution were defined as the immunodominant antigen(s). Analysis using these Western blots is similar to radioimmunoassay (RIA) in that the inverse of the serum dilution at the endpoint may be used to indicate the titer for a given Ig. Many of these antibody titers were confirmed and further defined by RIA.

The data indicated that the carbohydrate smeared antigen was immunodominant not only for IgG2 and IgA1 but also for IgG1, IgG3, IgA2, and IgM. This smeared antigen was present at the endpoint for all of these Igs in every patient. Certain bands were also present at the endpoint in some patients, but none were present in more than half of the subjects. Furthermore, the antibody titers by Ig class were IgG > IgA > IgM, and the antibody titers by IgG subclass were IgG2 > IgG1 ≈ IgG3. Using RIA, we found 65.7 μg of IgG2 per ml and 8.8 μg of IgG1 reactive with Aab. The IgA subclass distribution was IgA1 ≫ IgA2, with the IgA1 titer apparently being second only to IgG2. In short, the Aab antigen eliciting the highest antibody level in most Ig classes and subclasses appeared to be LPS, and the predominant Igs reactive with Aab were IgG2 and IgA1, which are associated with anti-LPS responses in other systems (2, 18).

MATERIALS AND METHODS

Human subjects. Subjects included 13 clinically characterized early-onset periodontitis (EOP) patients (seven blacks, five whites, and one white-Asian). Six subjects had the localized or juvenile form of the disease (JP) and 7 had the more generalized or severe form of the disease (SP). We

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selected subjects representing both sexes, black and white races, and SP and JP, reasoning that any indication of differences in responses between these groups could be followed up during the study. These patients were clinically characterized as follows: (i) for JP, subjects of 30 years or less with a localized pattern of severe periodontal destruction limited to first-molar or incisor teeth and up to two additional teeth; (ii) for SP, subjects 35 years of age or less with a generalized pattern of severe destruction, with attachment loss of at least 5 mm on eight or more teeth, at least three of which were not first molars or incisors. All subjects had a serotype-specific response to Aab defined by using the 3 *A. actinomycetemcomitans* serotypes (a, b, and c) in the previously described competition assay (4). These sera were collected from the patients at the time of diagnosis and before treatment. Six healthy nonperiodontitis subjects (four whites and two blacks) were included for comparison. Periodontally healthy subjects were between ages 20 and 30 and had no probable depth in excess of 3 mm and an absence of periodontal attachment loss.

Bacteria and antigen preparation. Aab (VPI strain #13127) was grown in mass culture by W. E. C. Moore and L. V. H. Moore of Virginia Polytechnic Institute. The bacteria were centrifuged, frozen, shipped to the Medical College of Virginia/Virginia Commonwealth University, sonicated, and used as the antigen for Western blot analyses and RIA. Protein concentrations in the sonicates were determined by the method of Lowry et al. (12).

Western blot-limiting dilution analysis. The method for limiting dilution analysis using Western blots has been previously described (3). Briefly, bacterial sonicate was diluted to 10 μg/50 μl in sample buffer (0.0626 M Tris, 10% glycerol, 2.3% sodium dodecyl sulfate [SDS] [pH 6.8]) and then boiled for 90 s. The running buffer was 0.025 M Tris containing 0.192 M glycine and 0.1% SDS. Linear gradient (5 to 20%) polyacrylamide gels were prepared with an SE 600 vertical gel apparatus with a model 500/200 power supply (Bio-Rad Laboratories, Richmond, Calif.). Following electrophoresis, the antigens were blotted to nitrocellulose in a transblot cell (Bio-Rad Laboratories) for 5 h at 210 mA. The blots were incubated in equal volumes of distilled water containing 10% nonfat dry milk and phosphate-buffered saline (PBS) (pH 7.2) for 1 h to block nonspecific binding (9). The blots were then cut into individual lanes to allow treatment of replicate lanes with different dilutions of serum (diluted in equal volumes of distilled water containing 10% nonfat dry milk and PBS, pH 7.2). Each lane was incubated in 4 ml of diluent containing 10^{-3} ml of serum at the lowest dilution and 10^{-6} ml of serum at the highest dilution for 6 h. This was then followed by four 10-min washes with TTBS (Tween 20 plus Tris-buffered saline). The lanes were then exposed to a 1/1,000 dilution of alkaline phosphatase-labeled goat anti-human IgG, IgA, IgM, and IgE antibodies (see "Standardization of antibody detection systems" for descriptions of antibodies) for 2 h, and this was followed by four more washes with TTBS to determine Ig class reactivity. The blots were developed with the 5-bromo-4-chloro-3-indolylphosphate–Nitro Blue Tetrazolium phosphatase substrate system. Controls included omission of Aab antigen, human serum, subclass reagent, and the enzyme-labeled tertiary antibody.

To detect the IgG and IgA subclass antibodies, the lanes were incubated with mouse anti-human IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2 subclass monoclonal antibodies (see "Standardization of antibody detection systems" for descriptions of antibodies) for 2 h. This was followed by four more washes with TTBS. Then, the lanes were exposed to a 1/1,000 dilution of alkaline phosphatase-labeled goat anti-mouse IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) for 2 h, and this was followed by four more washes with TTBS. The blots were developed with the 5-bromo-4-chloro-3-indolylphosphate–Nitro Blue Tetrazolium phosphatase substrate system (Kirkegaard & Perry Laboratories, Inc.).

Densitometry. The quantitation of labeling on antibody-positive Western blots was done with a Shimadzu CS-9000 scanning densitometer with the wavelength of the light source set at 620 nm. The Western blot was placed in the sample compartment, and the stage was manually moved until the beam rested on the center of the labeled area in the lane. The size of the beam used was 0.4 by 0.2 mm (width by height). The photo mode was set to read reflected light. The x and y positions were recorded for locating the scanning starting from the bottom to the top of each lane. After scanning, the area and the peak of the bands were calculated and units representing the area and peak were recorded in arbitrary densitometry units. The endpoint was defined as the inverse of the smallest fraction of a milliliter of serum producing a total optical density reading greater than 2 standard deviations above the mean for 13 control lanes (1 control lane without any serum was setup for each patient).

Standardization of antibody detection systems. Differences in anti-Aab titer between various isotypes and subclasses could relate not only to the amounts of the different antibodies in serum but to differences in the detection systems as well. To control for this we utilized purified human Igs (IgG1, catalog no. AG502; IgG2, catalog no. AG504; IgG3, catalog no. AG506; IgG4, catalog no. AG508, IgA1, catalog no. AG500; and IgA2, catalog no. AG501 [Chemicon International, Inc., Temecula, Calif.]; and IgM, catalog no. 401108 [Calbiochem, Inc., San Diego, Calif.]) and applied decreasing amounts to nitrocellulose strips. This was then followed by four 10-min washes with TTBS exactly as we do the Western blots. As a control, to be sure that the same amount of each Ig was present at each dilution, we used amido black staining followed by densitometry. This allowed us to standardize the detection system so that the same amount of each Ig class or subclass was present at each Ig dilution over a wide range of dilutions. The anti-human Ig reagents were anti-IgG1 (catalog no. 411451; clone no. HP6069; mouse IgG1; affinity, 8.8×10^{-7} to 15×10^{-7} liter/M), anti-IgG2 (catalog no. 411461; clone no. HP6002; mouse IgG1; affinity, 6.6×10^{-7} to 13×10^{-7} liter/M), anti-IgG3 (catalog no. 411482; clone no. HP6047; mouse IgG1; affinity, 36×10^{-7} to 78×10^{-7} liter/M), and anti-IgG4 (catalog no. 411492; clone no. HP6025; mouse IgG; affinity, 9.4×10^{-7} to 20×10^{-7} liter/M) (all from Calbiochem, Inc.) and anti-IgA1 (catalog no. MAB007; clone no. NIF2; mouse IgG1) and anti-IgA2 (catalog no. MAB008; clone no. 2E2; mouse IgG1) (from Chemicon International Inc.). Anti-IgG, -IgA, and -IgM were obtained from Kirkegaard & Perry Laboratories, Inc.

The relationships between the detection systems for IgG1 to IgG4 are illustrated in Fig. 1. Note that the detection systems for IgG1 and IgG2 are about twofold less sensitive in detecting these Igs than are those for IgG3 and IgG4. As listed above, the monoclonal antibodies reactive with IgG1 and IgG2 have lower affinity than the monoclonal antibody for IgG3 and IgG4 (2a). The reduced ability to detect IgG1 and IgG2 may relate to the lower affinity of these reagents. The anti-IgA1 and anti-IgA2 were comparable in their ability to detect Ig and similar to anti-IgG3 and -IgG4 in sensitivity.

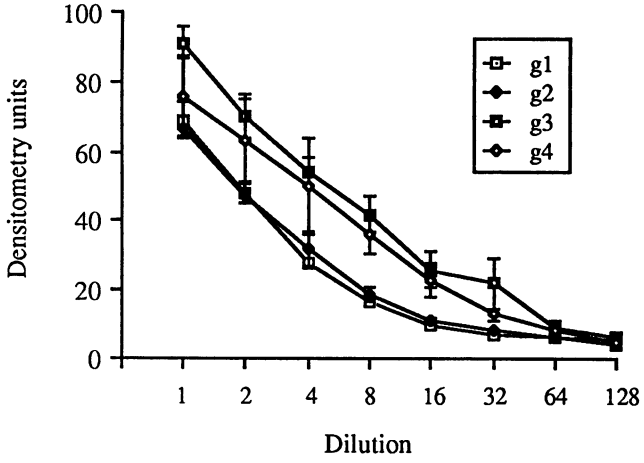

FIG. 1. Comparison of the various IgG subclass detection systems. Purified human IgG subclass Igs were applied to nitrocellulose membrane strips over a wide range of dilutions. Amido black staining indicated that the same amount of each Ig subclass was present on the membrane at each dilution. The IgG subclass-specific monoclonal antibodies were applied, and the strips were then exposed to the 1/1,000 dilution of alkaline phosphatase-labeled goat anti-mouse IgG and developed with the 5-bromo-4-chloro-3-indolylphosphate–Nitro Blue Tetrazolium phosphatase substrate system, as were the Western blots. Finally, the strips were run through a densitometer, and the level of labeling at the various dilutions was recorded in arbitrary densitometry units. Note that the detection systems for IgG3 and IgG4 are about twofold more sensitive than those for IgG1 and IgG2.

The detection system for IgM was about sixfold more sensitive than the IgG detection system and about fourfold more sensitive than the IgA detection system. Since the anti-Aab IgG response was largely IgG2 at the endpoint and the IgA response was IgA1 at the endpoint, we used IgG2 and IgA1 as the antigen in this standardization.

RIA. Details of the solid-phase RIA technique have been described elsewhere (3, 25). Briefly, 200 μl of sonicated bacteria (200 μg/ml in carbonate buffer, pH 9.6) was placed in each well of plastic assay strips (Immulon 2; Dynatech Laboratories, Inc., Alexandria, Va.). The strips were incubated at 4°C for 16 h and then washed in tap water 20 times. Sera were serially diluted (in the range of 1:50 to 1:1,024,000) in diluent (equal volumes of distilled water containing 10% nonfat dry milk and PBS, pH 7.2). Each dilution of serum was added to two antigen-coated wells and two uncoated wells. The final quantity of serum in the wells varied from 1/250 ml in the first well to 1/512,000 ml in the final well. The uncoated wells served as background controls. After incubation and washing as described above, 200 μl of 1-μg/ml mouse anti-human IgG subclass monoclonal antibody was added to the wells and incubated for 2 h. After 20 washes, 200 μl of iodinated (specific activity, 25 μCi/μg) goat anti-mouse IgG in diluent was added per well and incubated for 2 h. After washing, radioactivity was measured to an accuracy of 10% (LKB Instruments Inc., Rockville, Md.; 1282 Compugamma Counter). The difference in counts per minute between coated and uncoated wells was the basic data unit. A plot of this difference versus serum dilution was then prepared. A cutoff (4,000-cpm difference) was selected as the standard for this assay. This 4,000-cpm cutoff was in the linear section of the curve well above the inflection point where the titration curve begins to asymptotically approach zero as the serum dilution increases (previously illustrated [25]). The RIA titer was defined as the inverse of the final fraction of a milliliter of serum that was just sufficient to reach the 4,000-cpm cutoff or endpoint.

To further define the concentration of IgG subclass antibodies reactive with Aab, we used a reference serum kindly provided by M. Wilson at State University of New York, Buffalo. Wilson used a pool of patent serum and quantitated the level of Aab-specific IgG1 and IgG2 in this pool reactive with Aab LPS using a chimeric human-mouse antibody as a reference standard as previously reported (27). We used Wilson's Aab-reactive standard serum to establish a standard curve in our RIA. This standard curve was used to calculate micrograms of anti-Aab per milliliter in our patients' sera.

RESULTS

Ig classes reactive with immunodominant antigen(s) of Aab. Thirteen serum specimens representing black and white subjects of both sexes that were known to have a serotype b-specific antibody response were selected (4). Western blot lanes were incubated with decreasing amounts of patient serum in a limiting dilution analysis. This allowed us to observe the antigens at the endpoint for each Ig class. An example of this analysis for one patient is shown in Fig. 2. As replicate lanes were labeled with less serum (10^{-3} to 10^{-6} ml), most discrete bands were eliminated, leaving only the immunodominant smeared antigen in the upper portion of the blot at the endpoint for IgG. IgA and IgM reacted with both the smeared antigen and a few low-molecular-weight bands at the endpoint. A summary of results for all 13 patients is shown in Table 1. Note that 100% of the immunoblots had the smeared antigen at the endpoint. Bands representing other antigens were also present at the endpoint, but none were present in more than half of the patients. The fact that the smeared antigen was immunodominant for IgG confirms previous reports (3, 4), and the present work extends this pattern to include IgA and IgM as well.

Densitometry was done on each lane to allow for an objective measure of the endpoint for the determination of antibody titer. The inverse of the dilution in the final lane with a densitometry reading 2 standard deviations above that of the background control lanes was considered to be the titer. The titer for IgG in Fig. 2 was 10^5, which was 10-fold greater than that for IgA (10^4) and 100-fold greater than that for IgM (10^3). There was no detectable serum IgE antibody at 10^{-3} ml of serum. The antibody class responses for the 13 subjects are recorded in Table 2. In this analysis, the IgG titer was consistently as high as or higher than that for IgA or IgM, and this was true regardless of sex and race. Furthermore, the IgG detection system was less sensitive than the IgA system, and the IgA system was less sensitive than the IgM system. These differences in the detection systems were less than 10-fold but do further reinforce the apparent relationship of IgG > IgA > IgM for the immunodominant Aab antigen.

IgG subclasses reactive with immunodominant antigen(s) of Aab. In the next set of experiments, limiting dilution using Western blots was done to determine the IgG subclass distribution for antibody reactive with Aab. An example is shown in Fig. 3. Similar to the previous analysis, Western blot lanes were incubated with decreasing amounts of patient serum (10^{-3} to 10^{-6} ml) and then incubated with subclass-specific monoclonal antibodies followed by alkaline phos-

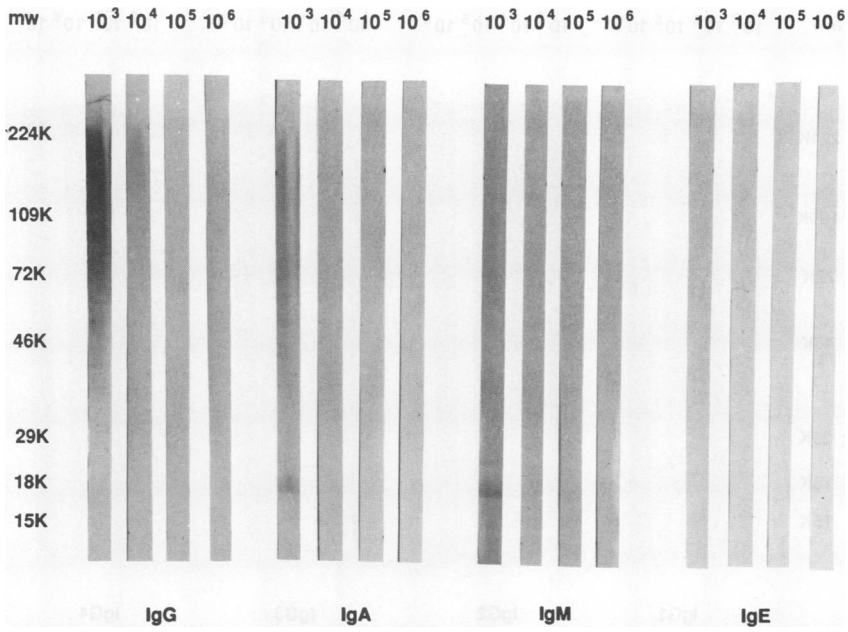

FIG. 2. Ig classes reactive with antigens of Aab by limiting dilution analysis. These Western blots prepared with a single patient's serum show the distribution of IgG, IgA, IgM, and IgE antibodies reactive with the immunodominant antigen of Aab (serum levels from 10^{-3} to 10^{-6} ml represent a titer of 10^3 to 10^6). mw, molecular weight; K, 10^3.

phatase-conjugated goat anti-mouse antiserum. As hypothesized, we noted that the immunodominant antigen for IgG2 was the smeared antigen in the upper portion of the immunoblot. In addition, the immunodominant antigen for IgG1 and IgG3 was also the same smeared antigen. In contrast, the reactivity at the endpoint for IgG4 was with discrete low-molecular-weight bands. A summary of results for all 13 patients is shown in Table 1. Note that 100% of the patient sera reacted with the smeared antigen in the upper portion of the immunoblots for IgG1 to IgG3. Some bands were also present at the endpoint, but none were present in more than half of the patients. It appears that the smeared antigen is immunodominant for all IgG subclasses except IgG4. However, we only found IgG4 at 10^{-3} ml of serum in two subjects, so it is possible that IgG4 reactive with the smeared antigen exists. A series of serum samples from six normal controls was also analyzed for comparison. As in the patient sera, a variety of low-molecular-weight bands was found at low serum dilutions. These bands were subject specific and were not found in the sera of a large percentage of healthy subjects. However, unlike the patient sera, the sera from healthy controls did not react with the smeared antigen.

Densitometry was done on each lane to determine the endpoint for the antibody titer. For the patient serum sample

TABLE 1. Prevalence of individual bands on Western blots for Aab in EOP patients

Band (kDa)[a]	% of blots with:							
	IgG	IgA	IgM	IgG1	IgG2	IgG3	IgA1	IgA2
Smear	100	100	100	100	100	100	100	100
166		7						
116			7					
109	7			7	7	7		
105						7		
96	7	7			7	7	7	
88		15					15	
78			7	7				
72	15		7		15	7		
66		15					15	
56	7				7			
46		15					15	
42	7			7	7	7		
39			7	7				
34		15					15	
29	7	23	7	7	7	30	23	
23			7			23		
18		30	15			15	15	15
17		7	7				7	
15	23	23	7	46	23	23	23	

[a] Bands appearing on Western blots at the endpoint for at least 1 of the 13 patients.

TABLE 2. Ig class distribution derived by endpoint analysis

Patient no.	Race[a]	Sex[b]	Endpoint titer of:		
			IgG	IgA	IgM
1	B	M	10^5	10^4	10^3
2	B	M	10^4	10^4	10^3
3	B	M	10^4	10^3	10^3
4	B	M	10^5	10^4	10^3
5	B	F	10^6	10^3	10^3
6	B	F	10^4	10^4	10^4
7	B	F	10^4	10^3	10^4
8	W	M	10^6	10^4	10^3
9	W	M	10^5	10^4	10^4
10	W	F	10^5	10^4	10^4
11	W	F	10^6	10^4	10^4
12	W	F	10^4	10^4	10^3
13	W/A	F	10^5	10^3	10^4

[a] B, black; W, white; W/A, patient has one white parent and one Asian parent.

[b] M, male; F, female.

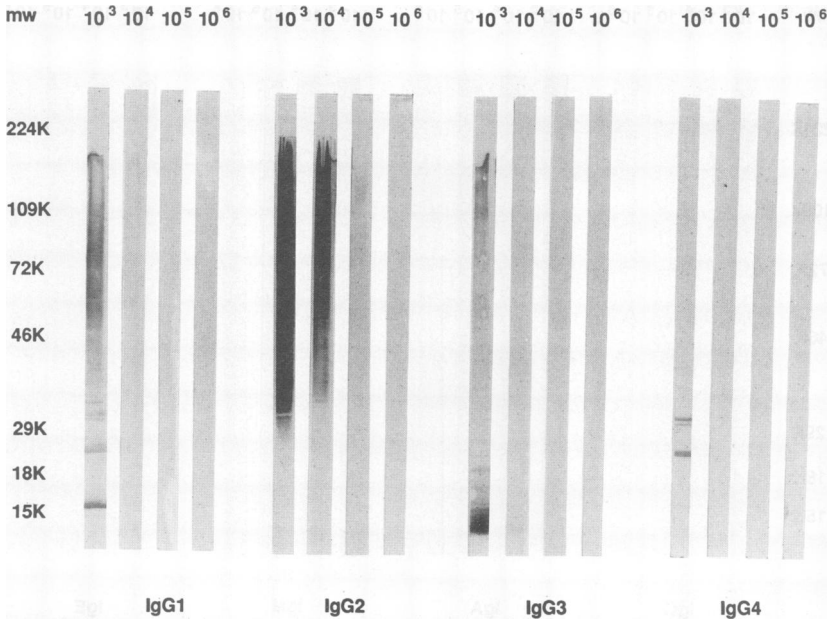

FIG. 3. IgG subclasses reactive with antigens of Aab by limiting dilution analysis. Western blots with 10^{-3} to 10^{-6} ml of this patient's serum show the distribution of IgG1, IgG2, IgG3, and IgG4 antibodies reactive with the immunodominant antigen of Aab. mw, molecular weight; K, 10^3.

in Fig. 3, the IgG2 titer was 10^5, which was 10-fold greater than those of IgG1 (10^4) and IgG3 (10^4). The results for all 13 subjects for the endpoint are summarized in Table 3. In this analysis the IgG2 titer was as high as or higher than the titer for any other subclass. Furthermore, the detection system for IgG1 and IgG2 was about twofold less sensitive than that for IgG3 and IgG4, so the dominance of IgG2 over IgG3 is even a bit greater than these data indicate. IgG4 did not have reactivity with the immunodominant smeared antigen by using 10^{-3} ml of the serum, and one patient (patient 13) did not have detectable IgG1 or IgG3 by using this amount of serum.

A weakness in the above analysis was that only large differences between subclass antibody titers would be apparent. It is clear from the Western blot analysis that the immunodominant antigen (the antigen being measured at the endpoint) is the smeared antigen in the upper portion of the immunoblots (i.e., LPS) for IgG1, IgG2, and IgG3. We therefore assessed the antibody titers for IgG1 to IgG3 in the 13 patients by using an RIA with twofold dilutions which yielded more-quantitative data. We reasoned that the titer at the RIA endpoint would be primarily attributable to anti-LPS (Fig. 4). The results confirm the titers from the Western blot analysis, with the median IgG2 titer about 10 times greater than that for IgG1 or IgG3. To further quantitate the RIA data, we utilized a standardized serum sample from M. Wilson (27) which contained known concentrations of Aab LPS-specific IgG1 and IgG2. We used this as an internal reference standard in our RIA and found that our Aab-

TABLE 3. IgG subclass distribution derived by endpoint analysis

Patient no.[a]	Endpoint titer of:		
	IgG2	IgG3	IgG1
1	10^5	10^3	10^4
2	10^4	10^3	10^3
3	10^4	10^4	10^4
4	10^5	10^3	10^4
5	10^6	10^5	10^5
6	10^4	10^4	10^3
7	10^4	10^3	10^4
8	10^6	10^5	10^4
9	10^5	10^3	10^3
10	10^5	10^5	10^4
11	10^6	10^3	10^4
12	10^4	10^3	10^4
13	10^5		

[a] For the race and sex of each patient, see Table 2.

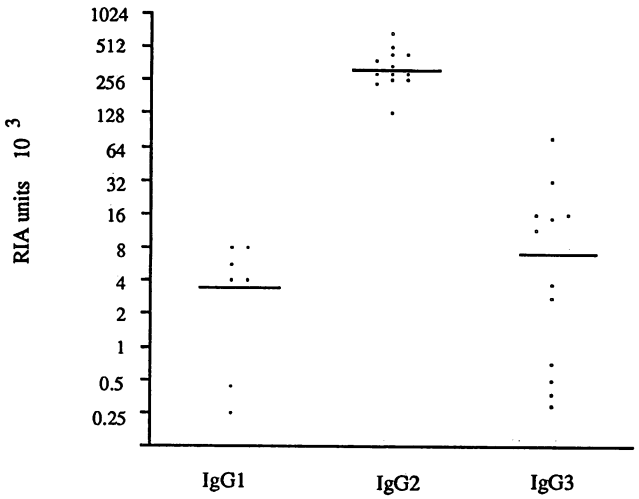

FIG. 4. Serum anti-Aab RIA titers for IgG subclasses IgG1, IgG2, and IgG3. Bars indicate the median antibody titer in RIA units. Each dot represents one patient, but some patients did not have an IgG1 level above the 4,000-cpm cutoff.

TABLE 4. IgA subclass distribution derived by endpoint analysis

Patient no.[a]	Endpoint titer of:	
	IgA1	IgA2
1	10^4	10^1
2	10^4	10^2
3	10^3	10^2
4	10^4	10^2
5	10^3	10^1
6	10^5	10^3
7	10^3	10^1
8	10^4	10^1
9	10^4	10^3
10	10^4	10^2
11	10^4	10^1
12	10^4	10^1
13	10^3	10^1

[a] For the race and sex of each patient, see Table 2.

specific IgG1 in the patient sera shown in Fig. 4 averaged 8.8 + 2.5 μg/ml (mean + standard error; data ranged from 0.5 to 16.6 μg/ml) and that our Aab IgG2 averaged 65.7 ± 6.9 μg/ml (mean + standard error; data ranged from 26.1 to 127 μg/ml). These data indicated a seven- to eightfold-higher level of IgG2 ($P < 0.001$) and further confirmed the marked differences seen in the Western blot analysis. However, this is a minimum difference, because IgG1 data for six patients were not represented in Fig. 4 or in the 8.8-μg/ml average because titers were low and were not reliably measured. If estimates for the IgG1 levels for the six missing patients (which ranged from 0.2 to 0.01 μg/ml) were included, the mean IgG1 level would become 4.8 μg/ml, so the IgG2 concentration would be 13- to 14-fold higher than that of IgG1.

Distribution of IgA subclass to the Aab immunodominant antigen. The subclass distribution of IgA antibodies was determined with the same methodology used for IgG subclasses. An example of limiting dilution analysis for IgA subclasses is shown in Fig. 5. Again the immunodominant antigen for both IgA1 and IgA2 was the smeared antigen in the upper portion of the immunoblots. This is summarized for the 13 subjects in Table 1, where all the subjects had the smeared antigen at the endpoint. In addition, a few additional antigen bands were present at the endpoint.

The Aab titer for IgA1 for the patient in Fig. 5 was 10^4 and was 1,000-fold greater than that for IgA2 (10^1). The antibody titers for IgA1 and IgA2 for all the subjects are summarized in Table 4. Note that the IgA1 level was 10 to 1,000 times greater than the IgA2 level for all subjects and that only 2 of the 13 subjects had a detectable level of IgA2 by using 10^{-3} ml of serum. IgA1 was apparently the second most common anti-Aab Ig, with a median RIA titer of 25,000 U compared with 4,000 U for IgG1 and 8,000 U for IgG3.

DISCUSSION

Carbohydrate antigens, including LPS, are known to elicit antibody responses that have unusually high quantities of IgG2 (8, 19, 21). On the basis of our data indicating that the immunodominant antigen of Aab was the serotype-specific carbohydrate (3, 20) and that this carbohydrate is a high-molecular-weight form of LPS (20, 28), we hypothesized that IgG and IgA classes reactive with Aab would predominate in serum and that IgG2 reactive with Aab would be extraordinarily high in Aab-seropositive EOP patients. The results reported here clearly indicate that IgG2 is the predominant Ig reactive with the Aab immunodominant antigen and that the Ig class response is IgG > IgA > IgM. Though we did not detect IgE using 10^{-3} ml of serum, we do not mean to suggest that IgE is not produced. Indeed, others have reported IgE reactive with Aab (23). Our results simply indicated that the serum IgE concentration reactive with Aab was much lower than that of IgG, IgA, and IgM. Given the known association of IgG1 and IgG3 with protein antigens (18), we were surprised that the immunodominant antigen for IgG1, IgG3, and IgM was also the LPS smeared antigen in the upper portion of the immunoblots. In fact, the only apparent exception to this pattern of LPS immunodominance was with IgG4, for which bands were found at the final positive dilution.

Our findings contrast with recent results reported by Ebersole et al. (5). They found that the major IgG subclasses reactive with Aab were IgG1 and IgG3 and concluded that the major Aab antigens were likely proteins. We do not understand why our results differ. However, our claim that the immunodominant antigen is the serotype-specific carbohydrate has been confirmed and extended by Wilson and Schifferle (28) and Sims et al. (20), who have indicated that the immunodominant carbohydrate is a high-molecular-weight form of LPS. Since submission of this work, an independent study of IgG subclass concentrations in JP patients with elevated antibody reactive with Aab has been published by Wilson and Hamilton (27). They reported that in patients with elevated IgG, the concentration of IgG2 (136.5 μg/ml) was strikingly greater than that of IgG1 (7.8 μg/ml). The results reported here confirm the IgG1 and IgG2 relationship reported in Wilson and Hamilton's study. Our IgG2 level of 65.7 μg/ml appears a bit lower than their 136.5-μg/ml level (values ranged from 3.5 to 1,116.8 μg/ml). We do not have patient samples in our serum bank with titers in the 1,000-μg/ml range. Our average value of 65.7 is very compatible with our previous data obtained with purified IgG from Aab high-responder patients. In the particular assay analyzed, we found that an RIA unit contained about 250 pg of IgG and that high-responder patients had about 500,000 RIA units of Aab-specific IgG, or about 125 μg/ml (25). In addition, our work confirms that of Brown et al. (2) indicating that IgA1 is the predominant IgA subclass and extends this work to indicate that IgA1 is reacting with the immunodominant serotype-specific carbohydrate antigen of Aab.

Igs of the IgG2 and IgA1 subclasses were the most common serum antibodies reactive with Aab in seropositive people. Serum antibody titers reactive with Aab are known to be very high in EOP patients (6, 11, 13, 16, 24, 26). However, IgG2 and IgA1 may not be the best Igs to protect a host infected with Aab. Igs of the IgG2 subclass fix complement poorly and do not interact with Fc receptors as well as other IgG subclasses. High titers of IgG2 might bind the surface epitopes of LPS and prevent binding of other subclasses that would fix complement and facilitate opsonization and clearance of the organism. It is possible that sera with the highest titers of IgG1 or IgG3 reactive with Aab have more opsonic capacity than sera with a much higher titer of IgG2. Furthermore, IgA1 but not IgA2 is susceptible to microbial IgA proteases. Brown et al. (2) discuss this relationship in detail and point out that any protective effect of IgA1 could be reduced by the presence of IgA proteases in the area.

There is a known effect of race on the antibody response to Aab (3, 7). Gunsolley et al. (7) reported that black EOP subjects were seropositive more frequently than white EOP subjects and that black EOP subjects generally had the less

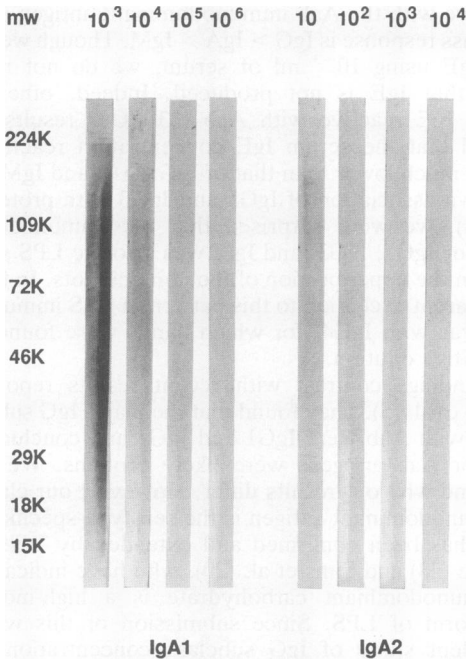

FIG. 5. IgA subclasses reactive with antigens of Aab by limiting dilution analysis. These Western blots of this patient's serum show the distribution of IgA1 and IgA2 antibodies reactive with the immunodominant antigen of Aab. The serum levels IgA1 were from 10^{-3} to 10^{-6} ml, and for IgA2 they were from 10^{-1} to 10^{-4} ml. mw, molecular weight; K, 10^3.

severe localized form of EOP. Furthermore, black patients had the highest antibody titers reactive with the immunodominant antigen of Aab (3). However, there was no race effect apparent in the present study of Ig class and subclass reactive with Aab. Both black and white patients had similar Ig class and subclass reactivity with the immunodominant antigen of Aab (Tables 2 through 4). It appears that as a group, black subjects respond more frequently than white subjects but the distribution of Ig class and subclass reactive with the immunodominant antigen of Aab in seropositive subjects is about the same regardless of race.

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