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Immunoglobulin A Subclass Distribution of Naturally Occurring Salivary Antibodies to Microbial Antigens

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The distribution of immunoglobulin A1 (IgA1) and IgA2 antibodies to various microbial antigens was determined in human parotid saliva by using monoclonal antibodies to the IgA subclasses in a solid-phase radioimmunoassay. In 12 subjects examined, antibodies to *Streptococcus mutans* glucosyl transferase, protein antigen I/II, and cell wall carbohydrate, as well as dextrans B1355 fraction S and B512 and phosphorylcholine, occurred predominantly in the IgA1 subclass. In contrast, antibodies to lipoteichoic acid from *Streptococcus pyogenes* and whole lipopolysaccharides from *Bacteroides gingivalis*, *Bacteroides fragilis*, and *Escherichia coli* were predominantly of the IgA2 subclass. The data indicate that in most individuals naturally occurring antibodies to a given antigen are predominantly associated with one of the two subclasses of IgA.

Naturally occurring antibodies to *Streptococcus mutans* have been detected in the saliva of children and adults by a variety of techniques (1–4, 10, 15). These antibodies are present in the immunoglobulin A (IgA) isotype—the predominant immunoglobulin class in human external secretions (2). Human IgA consists of two subclasses, IgA1 and IgA2, which differ in the primary structure, carbohydrate composition, antigenic determinants (for a review see J. Mestecky and M. Kilian, Methods Enzymol., in press), and sensitivity to the proteolysis by IgA1 proteases produced by a variety of microbial species that inhabit the mucosal surfaces (13, 14). Although comparable amounts of total IgA1 and IgA2 molecules are found in several external secretions, including saliva (7), the distribution of specific IgA1 or IgA2 antibodies, or both, to various antigens of oral bacteria has not been explored. Analogous studies concerning the distribution of specific antibodies among the four subclasses of human IgG revealed that certain antigens induce antibodies that are associated predominantly with a certain subclass (21, 24). For example, human IgG antibodies to polysaccharides such as dextrans, levans, and teichoic acid are found solely in the IgG2 subclass, whereas antibodies to diphtheria vaccine are present in IgG1 and IgG3 but not in IgG2 or IgG4 subclasses (for a review see reference 24). Since IgA1 cleavage by bacterial proteases may interfere with IgA effector functions (12, 25), we examined the distribution of specific IgA1 and IgA2 antibodies to several distinct groups of antigens produced by bacteria living on mucosal surfaces.

Unstimulated parotid saliva was collected for ca. 10 min from healthy adult laboratory personnel (age, 26 to 43 years; both sexes) by using a plastic intraoral collection cup placed over each parotid duct (23). Samples were clarified by centrifugation and stored at −20°C for later analysis. Glucosyl tranferase (GTF) from *S. mutans* 6715 and cell wall carbohydrate from *S. mutans* GS-5 (provided by R. Gregory, University of Alabama at Birmingham, Birmingham, Ala.) were purified as previously described (11). Protein antigen I/II (a gift from M. W. Russell, University of Alabama at Birmingham) was purified from Guy's strain of *S. mutans* as described by Russell et al. (27). Dextrans from *Leuconostoc mesenteroides* type B1355 fraction S (obtained from M. E. Slodki, Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.) and type B512 (Sigma Chemical Co., St. Louis, Mo.), as well as *S. mutans* carbohydrate, were coupled to poly-L-lysine type IV (Sigma) by the procedure of Gray (9). Phosphorylcholine coupled to human serum albumin was a gift from D. Briles, University of Alabama at Birmingham. Lipoteichoic acid (LTA) was purified from *Streptococcus pyogenes* by the method of Moskowitz (20) and was a gift from I. Morisaki (Osaka University School of Dentistry, Osaka, Japan.). Phenol-extracted lipopolysaccharide (LPS) from *Bacteroides gingivalis* and *Bacteroides fragilis* and both phenol and butanol extracts of *Escherichia coli* K235 were prepared as described (18, 19). The LPS preparations were a gift from M. J. Wannemuehler, University of Alabama at Birmingham.

IgA1 and IgA2 antibodies in parotid saliva were determined by using a solid-phase radioimmunoassay. Monoclonal mouse IgG1κ anti-human IgA1 and IgA2 antibodies (generously provided by J. Radl and J. J. Haaijman, Institute of Experimental Gerontology, Rijswijk, The Netherlands) were purified on protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) (8) and labeled with ^{125}I by using the lactoperoxidase method (17). These antibodies have been shown to be specific for human IgA1 and IgA2, respectively, regardless of light-chain type or IgA2 allotype as previously described (6).

Polystyrene wells (Immulon I; Dynatech Laboratories, Inc., Alexandria, Va.) were coated overnight with 125 μl of antigen (1 μg/ml) in 0.125 M borate saline (pH 8.2) (10 μg/ml for the dextrans and cell wall carbohydrate). This and all subsequent operations were carried out at room temperature. The wells were emptied and blocked for 1 h by incubation with 1% bovine serum albumin in 0.01 M phosphate-buffered saline (pH 7.2) (PBS-BSA). After being washed three times with PBS-BSA, samples of saliva (100 μl; appropriately diluted in PBS-BSA) were applied to both coated and uncoated blocked wells and incubated overnight. After washing, 100 μl of ^{125}I-anti-IgA1 or ^{125}I-anti-IgA2 (1.5×10^6 cpm/ml) in PBS-BSA was added and incubated overnight. The strips of wells were washed three times with PBS-BSA and counted for bound ^{125}I. Nonspecific counts

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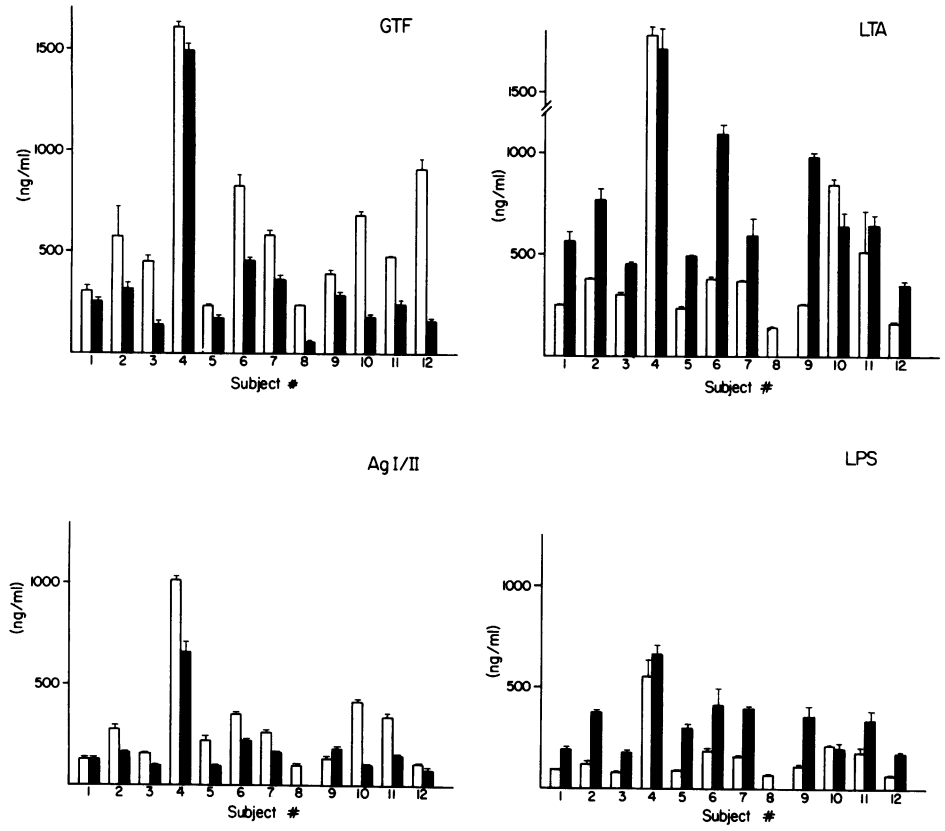

FIG. 1. Subclass distribution of IgA antibodies in parotid saliva from 12 subjects to representative microbial antigens: glucosyl transferase, (GTF), lipoteichoic acid (LTA), protein antigen I/II (Ag I/II), and *B. gingivalis* lipopolysaccharide (LPS). Symbols: □, IgA1; ■, IgA2. Vertical lines at the top of the bars represent the standard deviation of assay replicates. Results are presented as nanograms of antibody per milliliter relative to IgA1 or IgA2 standards (see the text).

bound to uncoated wells were subtracted from the counts bound to coated wells for each sample.

The assay was standardized in parallel in a manner which simulated antigen binding. Wells were coated as described above with rabbit anti-human lambda light chain (produced in this laboratory) (10 μg/ml) and then incubated with serial twofold dilutions of purified human IgA1λ or IgA2λ myeloma proteins from this laboratory. The standard wells were developed with the appropriate labeled anti-IgA subclass hybridoma antibody. The results of unknowns are expressed as nanograms per milliliter relative to these standards by using the logit-log method of interpolation (26). The

TABLE 1. Ratio of IgA1 to IgA2 antibodies to microbial antigens in parotid saliva[a]

Subject or predominant subclass[b]	*S. mutans*			Dextran		PC-HSA	LTA	*B. gingivalis* LPS	*B. fragilis* LPS	*E. coli*	
	GTF	Ag I/II	CHO	B1355S	B512					phLPS	buLPS
1	1.19	1.00	1.26	1.07	1.26	0.58	0.45	0.47	0.30	0.52	0.30
2	1.73	1.75	0.34	0.90	0.79	0.58	0.49	0.32	0.35	0.41	0.42
3	3.21	1.68	2.36	0.16	2.30	1.06	0.67	0.46	0.46	0.69	0.57
4	1.08	1.54	(A1)[c]	4.46	0.98	1.77	1.04	0.84	0.73	1.29	1.20
5	1.38	2.44	5.79	4.72	2.01	1.70	0.47	0.30	0.43	0.42	0.49
6	1.80	1.59	1.87	2.08	1.20	1.39	0.34	0.44	0.28	0.43	0.43
7	1.61	1.62	1.42	1.21	0.85	1.35	0.62	0.40	0.40	0.41	0.76
8	3.92	(A1)	1.75	(A1)	(A1)	0.62	(A1)	(A1)	0.71	1.25	1.15
9	1.39	0.75	0.92	0.98	0.62	1.18	0.26	0.32	0.37	0.30	0.29
10	3.78	4.15	2.64	1.92	2.10	3.39	1.31	1.08	1.42	1.81	1.51
11	1.98	2.27	3.34	2.06	1.46	2.26	0.80	0.53	0.49	0.55	0.55
12	5.69	1.34	1.60	2.67	1.53	1.24	0.46	0.38	0.35	0.46	0.38
A1	12	11	10	9	8	9	3	2	1	3	3
A2	0	1	2	3	4	3	9	10	11	9	9

[a] GTF, Glucosyl transferase; Ag I/II, protein antigen I/II; CHO, carbohydrate; PC-HSA, phosphorylcholine coupled to human serum albumin; phLPS, phenol-water-extracted LPS; buLPS, butanol-extracted LPS.

[b] AI, Number of subjects with an IgA1/IgA2 antibody ratio of ≥1.0; A2, number of subjects with an IgA1/IgA2 antibody ratio of <1.0.

[c] (A1), Only IgA1 was detected.

lower limit for reliable detection of IgA1 or IgA2 antibodies was ca. 1 ng/ml.

Examination of the IgA1 and IgA2 antibodies in parotid saliva samples from 12 subjects revealed that, with a few exceptions, most individuals responded predominantly in one subclass to a given antigen. Figure 1 shows the quantitative results for four representative antigens. The results expressed in terms of IgA1/IgA2 ratios for all antigens are summarized in Table 1. Nearly all subjects had a predominance of IgA1 antibodies to the protein antigens from *S. mutans*, GTF (12 of 12) and surface antigen I/II (11 of 12). Similarly, IgA1 antibody responses were observed for *S. mutans* GS-5 cell wall carbohydrate (10 of 12), *L. mesenteroides* dextrans B1355 fraction S (9 of 12) and B512 (8 of 12), and phosphorylcholine (9 of 12). In contrast, most individuals had a predominance of IgA2 antibodies to LTA (9 of 12) and the phenol and butanol extracts of LPS (9 of 12 to 11 of 12). Preliminary evidence obtained by using LPS mutants suggests that most of the salivary IgA2 antibody against LPS is directed towards the lipid A portion of the molecule (T. A. Brown, manuscript in preparation).

Certain individuals deviated from this pattern. For example, subject 10 showed a predominance of IgA1 antibodies to all antigens examined. Subjects 4 and 8 had predominantly IgA1 to the *E. coli* LPS preparations and to LTA. Likewise, some subjects (e.g., subjects 2 and 9) had predominantly IgA2 antibodies to several of the antigens which typically evoked an IgA1 response. It is not known whether these differences represented genetic variations in the immune response or whether they reflected past experience with identical or cross-reacting antigens. Furthermore, IgA subclass ratios may change during an active immune response. For one antigen system we have evidence that infection with live influenza A virus stimulated a predominantly IgA1 response against the hemagglutinin, detectable in nasal washes. Even though IgA2 antibodies were found before infection, they did not increase during the course of the infection. (T. A. Brown, B. R. Murphy, J. Radl, J. J. Haaijman, and J. Mestecky, J. Clin. Microbiol., in press). The subclass ratios measured in the present study with healthy volunteers presumably represent the "resting" state and could possibly change during the course of an infection or active immunization with those antigens.

It is clear from Table 1 that the subclass distribution of IgA antibodies in saliva was not necessarily related to the distribution of total IgA1 and IgA2, since the same saliva sample from an individual could display totally different subclass distributions of antibodies to different antigens. A recent report indicates that the IgA subclass distribution of antibodies to phosphorylcholine, β-lactoglobulin, and tetanus toxoid in serum is similar to the subclass distribution of total serum IgA (5). This may reflect the different origins of serum and secretory IgA and the distinction between the systemic and secretory immune systems.

The subclass distribution of IgA antibodies may be important with respect to their effectiveness at mucosal surfaces, since IgA1 may be cleaved by microbial IgA1 proteases. Although there is conflicting evidence as to whether Fabα fragments from cleaved IgA1 retain their ability to bind antigen (16, 22), cleavage of the molecule would result in a loss of the ability to agglutinate particulate antigens, as well as a loss of any effector functions associated with the Fcα portion of the molecule. If Fabα fragments can bind antigen, they could mask surface determinants of microbial cells and alter the hydrophobicity or charge of the organism, or both, and thus modulate adherence to mucosal surfaces (12). Knowledge of the IgA subclass association of specific antibodies to various antigens of mucosal pathogens may explain the failure of the protective effect of IgA1 antibodies (12, 25) and should be considered in designing the antigenic composition of potential vaccines for immunization against mucosally encountered pathogens.

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