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Natural Polyreactive Secretory Immunoglobulin A Autoantibodies as a Possible Barrier to Infection in Humans

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Secretory immunoglobulin A (S-IgA) was investigated in human secretions for the presence of natural antibodies (Abs) acting as the first "immune barrier" to infection before induction or boosting of specific responses. These molecules could be the secretory counterpart of the natural Abs in serum that were previously shown by our laboratory to be polyreactive to autoantigens. Significant levels of S-IgA Abs to human actin, myosin, tubulin, and spectrin were detected in 10 saliva and 8 colostrum samples from normal subjects. Computer-assisted analysis of immunoblots of extracts from human muscles showed these Abs to react with a large number of autoantigens. Their polyreactivity was confirmed by cross-inhibition and by immunoblotting studies of affinity-purified natural Abs, assayed against a large variety of surface or secreted antigens from *Streptococcus pyogenes*. The thiocyanate elution method showed that functional affinities of some natural Abs can be of the same order of magnitude as those of tetanus vaccine antitoxins. Moreover, nonimmune binding of these natural Abs to the gut protein Fv (Fv-fragment binding protein) can enhance their effector functions. This demonstrates that human secretions contain polyreactive auto-Abs which can also react with pathogens. These secretory Abs of "skeleton key" specificities are possibly produced by a primordial B-1-cell-associated immune system and can be involved in a plurispecific mucosal protection against pathogens, irrespective of the conventional immune response.

Immune protection against microorganisms in the digestive tract is primarily due to secretory immunoglobulin A (S-IgA) (12, 40, 53). It has been shown that S-IgA acts as the first "immune barrier" to infection in the lumen. The pathogens are maintained in the lumen by "immune exclusion," including agglutination and/or shielding of microbial adhesins (51). The antigens (Ags) can also be cleared from the lamina propria (29) and intracellular viruses can be neutralized (38) by binding to polymeric IgA before and during transcytosis towards the lumen, respectively. Binding to S-IgA favors adherence to mucus and clearance in the mucus stream, mainly when leading to immune agglutination. The polyvalency of S-IgA increases its functional affinity toward Ags, and shielding by the secretory component (SC) protects the Fcα domains from human digestive proteases (34). In addition, a gut-associated Ig-binding protein, described by one of us (10) and called protein Fv (pFv), forms large endoluminal complexes with S-IgA and $F(ab')_{2\alpha}$ fragments (9). These complexes maintain and potentiate the agglutination properties of the secretory antibodies (Abs) and also increase apparent cross-reactivity due to the multispecificities of the aggregates.

The mucosal immune system is clearly delineated from the systemic immune system (18, 39). Despite some regional differences, the overall functions and specificities seem to be shared by most exocrine immune effector sites. Salivary and mammary glands are of particular interest because they release S-IgA molecules, which provide protection against pathogens at remote sites, namely, in the gut and in the infant digestive tract, respectively.

The role of luminal S-IgA as a first barrier to infection requires the presence of an Ab activity before the first encounter with the pathogen. However, the primary response peaks after 3 weeks and can decrease to a low and often barely detectable level by 3 months (18, 44). The role of "antiseptic paint" by conventional S-IgA might thus be restricted to the cases of repeated antigenic challenges during chronic infection, an asymptomatic carrier state, endemic contamination, and vaccination with a live or long-lasting Ag. However, the presence of natural Abs before the first encounter with the pathogen could complement the role of monospecific S-IgA.

"Natural" auto-Abs were found long ago (5, 41) in the serum of both humans and animals and are considered part of the "nonspecific" defenses of the organism, as referred to in recent reviews (1, 19). Our laboratory has reappraised both the specificity and the biological significance of these molecules by showing them to recognize self- and non-self Ags and to be polyreactive, i.e., to react individually with unrelated epitopes (20, 23). The preimmune nature of these polyreactive auto-Abs is currently accepted for several reasons. Their polyreactivity and their frequent germ line configuration suggest a lack of Ag-induced selection. Their emergence is independent of known or intentional immunizations, and their production has been observed in B lymphocytes from newborn (21), nude (4), germ-free (4, 7), and Ag-free (7) mice.

In this paper, we report the presence of polyclonal natural auto-Abs of the S-IgA isotype in human saliva and colostrum. We demonstrate that these Abs are polyreactive and recognize a large number of bacterial Ags, strongly suggesting a basic protective role of the preimmune S-IgA against microorganisms.

MATERIALS AND METHODS

Specimens. Resting mixed saliva was collected from 10 normal subjects by spitting for 10 min before mealtime; it was centrifuged immediately for 5 min at 10,000 × *g* to remove cells. No mechanical or chemical stimulation was used. Paired autologous serum and saliva samples were also collected from two additional subjects. Eight individual colostrum samples collected on the first postpartum day and a purified S-IgA preparation from a pool of 10 specimens (27)

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were a kind gift of S. Iscaki, Institut Pasteur. All the specimens were cooled immediately after centrifugation and stored for less than 1 year at −30°C until use.

Detection of Ab activities by ELISA. Natural Abs were detected with plates precoated with 100 μl of Ags per well. The following human Ags were prepared by classical methods: muscle actin (10 μg/ml) (50), muscle myosin (5 μg/ml) (56), erythrocyte spectrin (5 μg/ml) (54), and brain tubulin (3 μg/ml) (48). The purity of the auto-Ags was controlled by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Corresponding Ags of bovine or rabbit origin were used as controls. The plates were blocked with phosphate-buffered saline (PBS) 0.5% (wt/vol) gelatin–0.1% (vol/vol) Tween 20 for 1 h at 37°C. Abs were incubated for 1 h at 37°C and then overnight at 4°C and detected with peroxidase-labeled sheep Abs to the human SC or to the human α chain. The labeled Abs were prepared and their specificity was controlled in our laboratory (27). The peroxidase activity was revealed with *o*-phenylenediamine and read at 492 nm. Ab activity was correlated with the concentration of S-IgA measured by indirect enzyme-linked immunosorbent assay (ELISA), using plates precoated with a sheep Ab to the α chain and a peroxidase-labeled sheep Ab to $F(ab')_2$ as a second Ab. Dilutions of the reference S-IgA (480 μg/ml) were used for quantitation. The specific activity of Abs was expressed as the reciprocal amount of S-IgA giving an optical density at 492 nm (OD_{492}) of 0.5 during the assay. The specific activity ratio was established by comparison with the specific activity of the reference S-IgA measured simultaneously in the same plate: specific activity ratio = specific activity of the sample/specific activity of the reference S-IgA. Abs to the human SC and to Ig fragments were raised, purified, and labeled in this laboratory. The percentage of IgA2 was estimated in S-IgA Abs to actin and to myosin or in total S-IgA by comparing the ELISA results for samples treated or not treated with IgA1 protease (Boehringer, Mannheim, Germany) (26). Briefly, dilutions of six specimens were incubated with plates coated either with actin or myosin or with Abs to $F(ab')_2$. The bound S-IgA were then assayed after incubation at 37°C for 2 h with the enzyme (0.4 μg/ml) in 0.1 M Tris-HCl buffer (pH 7.5). Uncleaved S-IgA2 were detected with the labeled Abs to SC and the amounts were compared with a control curve for the same molecule incubated without IgA1 protease. In other experiments, natural Abs were compared in the same specimen with Abs to the cell wall carbohydrates (CHO) of a dental caries-associated bacterium, *Streptococcus sobrinus*, which are present in most normal subjects (27). These Abs were investigated by ELISA, as described previously (27). In other experiments, the polyreactivity of the natural auto-Abs was investigated by competitive inhibition of Abs to human actin with dilutions of human tubulin in the soluble phase. The results were expressed as percent inhibition, as described previously (23). In the latter experiments, saliva samples were selected since their auto-Abs eluted in the dimeric S-IgA fractions, as demonstrated by gel permeation through a high-performance liquid chromatography Superose 12 column of (Pharmacia).

Preparative gel permeation. The three major classes of serum Igs were fractionated with respect to the molecular mass by using a set of three 2.5- by 100-cm columns of Sephacryl S-300 (Pharmacia, Uppsala, Sweden), mounted in series, and equilibrated with a PBS-azide solution. Proteins were collected as 10-ml fractions and assayed by a classical capture ELISA with plates precoated overnight at 4°C with sheep Abs to the γ chain, α chain, or μ chain. Saturation was carried out with PBS containing 3% (wt/vol) bovine skim milk. After incubation with diluted fractions for 1 h at 37°C and washing, the captured molecules were detected with a peroxidase-labeled Ab to $(Fab')_2$. Both labeled and unlabeled Abs were prepared and their specificity was controlled in this laboratory (27). Contaminant IgG was depleted from the IgA-containing fractions by incubation with protein G-Sepharose (Pharmacia), and the depletion was controlled by ELISA. IgG from unfractionated saliva and colostrum samples were similarly absorbed with insoluble protein G and controlled.

Western blot analysis. Human muscle lysate was prepared and subjected to SDS-PAGE (10% polyacrylamide) under reducing conditions, as previously described (3). Human serum proteins were used as a standard for molecular mass calibration. After electrophoresis, proteins were transferred onto nitrocellulose (pore size, 0.45 μm; Schleicher and Schüll, Dassel, Germany) by a semidry isotachophoretic procedure. The sheets were dried at room temperature and kept dry until use. They were then cut into vertical strips, which were individually placed into incubation tray wells. Saturation took place by successive incubations at room temperature with 0.3% (vol/vol) Nonidet P-40 in PBS for 30 min and then with 0.03% (wt/vol) gelatin in PBS for 1 h, under constant shaking. After an additional wash with 0.1% (vol/vol) Tween 20 in PBS, the strips were incubated overnight with 700 μl of IgA (80 μg/ml for total S-IgA or 80 ng/ml for affinity-purified natural Abs) in the presence of 0.2% (wt/vol) sodium azide and 0.03% (wt/vol) gelatin. Gelatin alone was used in control strips instead of IgA. After being washed, the strips were incubated for 18 h at room temperature with 1 μg of peroxidase-labeled sheep Ab to the human α chain per ml in PBS-Tween 20 containing 0.03% (wt/vol) gelatin. Positive controls were incubated with monoreactive monoclonal murine Abs to human actin or myosin and revealed with a peroxidase-labeled Abs to murine Igs. After an additional wash in PBS-Tween, peroxidase activity was revealed with diaminobenzidine-HCl (Sigma Fast) enhanced with 0.03% (wt/vol) nickel chloride. In each experiment, serum proteins were left to migrate in separate wells of the same gel, blotted, and then revealed by staining with India ink.

Computer-assisted analysis of the Ab activity spectrum. The bands revealed by peroxidase on immunoblots were integrated as ODs with a densitometer (Masterscan; Scanalytics, Billerica, Mass.) in a reflective mode, using a high resolution charge-coupled device camera system (Masterscan) connected to a PC-compatible computer. The RFLPscan program (Masterscan) was used to manipulate the camera data. The integration was carried out under visual control on a width of 10 pixels and was corrected by subtraction of the integrated OD values of the adjacent control strip. A calibration curve was calculated by reference to standards stained with India ink, allowing us to determine the molecular mass for each detected band. Subtraction of the pattern of controls incubated with the sole second Ab led to an Ab activity spectrum represented as a curve of OD values (in arbitrary units) versus calibrated molecular mass (in kilodaltons).

Ab denaturation. Reduction by dithiothreitol was carried out at room temperature for 1 h in the presence of 4 M guanidine HCl–0.5 M Tris buffer (pH 8) to dissociate the H and L chairs and to cleave the intrachain disulfide bridges. The reduction was followed by alkylation in the presence of excess iodoacetamide (0.1 M) for 1 h at 4°C. The reagents were removed by extensive dialysis against PBS. Due to a low and inappropriate reassociation with irrelevant chains from the Ig background, no significant polyclonal Ab activity can be recovered under these conditions.

Microbial Ags. Cell wall CHO from the cariogenic strain *S. sobrinus* BC 93087 were purified by nitrous extraction (27). *S. pyogenes* was grown in a dialysate of Todd-Hewitt broth to avoid contamination of the secreted bacterial components by unrelated bovine proteins from the culture medium. The dialysate was prepared as follows. Dehydrated Todd-Hewitt broth (3 g) (Difco Laboratories, Detroit, Mich.) was suspended in 10 ml of distilled water. The solubilized medium was placed in a dialysis tubing (13-kDa cutoff) and dialyzed against two changes of 50 ml of PBS. The dialysate was sterilized by autoclaving at 115°C for 15 min and then diluted to 2% (vol/vol) in sterile distilled water. The medium was inoculated with the reference strain *S. pyogenes* ATCC 12344 at 37°C, and bacterial growth was monitored by measuring the OD_{650}. Bacterial cells and their supernatant were harvested during the exponential phase of growth and separated by centrifugation at 10,000 × *g* for 15 min. The cells were washed with PBS and then treated with the SDS–2-mercaptoethanol solution which serves as a sample buffer during SDS-PAGE. After removal of the insoluble material by centrifugation, the supernatant was concentrated 10-fold by air drying and applied to the gel. The growth medium was cleared of most low-molecular-mass molecules of the Todd-Hewitt dialysate by successive concentrations and dilutions in an ultrafiltration cell through a UM10 membrane (Amicon, Danvers, Mass.), leading to a 500-fold final concentration. The concentrate was then diluted with an equal volume of the SDS buffer.

Purification of natural Abs. To delineate polyreactive Abs from the monoreactive Ag-induced molecules, natural Abs were purified by affinity absorption on human actin or on 2,4-dinitrophenol (DNP)–lysine and were assayed against the streptococcal Ags. This method was valid because, to our knowledge, no cross-reactivity has been described between human actin and these foreign Ags, and it would be poorly immunogenic in humans. A 30-ml volume of saliva S01 or 3 ml of colostrum (specimen C40) or of the purified S-IgA preparation was incubated for 1 h at room temperature with 1 ml of Ags insolubilized on cyanogen bromide-Sepharose 4B (Pharmacia). After being washed, the actin-bound Abs were eluted with 0.2 M glycine-HCl (pH 2.6) and the pH was immediately neutralized with 1 M K_2HPO_4. The Abs to DNP were specifically eluted with 0.2 M DNP-glycine and then dialyzed against PBS and concentrated by a low-pressure system.

Affinity. The functional affinity of native salivary S-IgA to human actin was compared with that of its serum IgA counterpart and with tetanus antitoxin curves of serum IgG and IgA from the same individual by the method of Pullen et al. (46). Briefly, Ag-coated plates were incubated with a selected dilution of Abs giving about 1 OD_{492} unit in control experiments. The bound Abs were then dissociated with increasing molarities of a mild nondenaturing (22) chaotropic agent, sodium thiocyanate, for 30 min at room temperature. After being washed, the remaining Abs were detected with peroxidase-labeled Abs to the α or the γ chain. In these experiments, weak Ag-Ab interactions reversibly dissociate at low molarities whereas the strong interactions resist dissociation (22). This simple method was chosen because it allows a functional comparison between the heterogeneous polymeric polyreactive population and hyperimmune monomeric Abs. It has provided unquestionable results in previous studies (8, 27, 47) and has been shown to correlate well with the intrinsic affinity (35). Although the dissociation effect of salts can vary according to the nature of the nonconvalent binding, this method was valid here because both Ags were proteins and because the Abs were polyclonal, thus leading to a large variety of interactions. The coating and incubation conditions were those described above (Abs to actin) or in reference 47 (tetanus antitoxins). IgG antitoxin from subject 1 was chosen as a reference of high-affinity Ab because it had already been investigated by the Biacore method, giving a K_a value of as much as 5.6×10^{10} M^{-1} (47).

Protein Fv binding. Human pFv was purified from the stools of a normal subject by gel permeation of a water extract treated with dithiothreitol in the presence of 6 M guanidine HCl and then dialyzed against PBS-deoxycholate, as already described (9). For the pFv binding assay, dilutions of the reference S-IgA were incubated with actin- or spectrin-coated plates for 1 h at 37°C and then overnight at 4°C. After being washed, the plates were incubated for 1 h at 37°C with a constant dilution of pFv for a nonimmune binding to the captured auto-

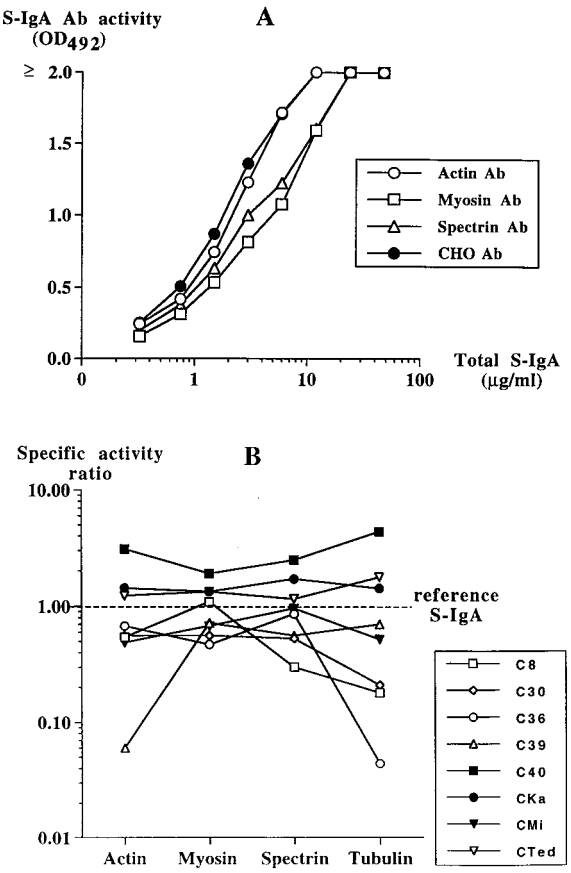

FIG. 1. Ab activity of colostral S-IgA to human autoAgs (actin, myosin, spectrin, and tubulin) and to the cell wall CHO of the common oral microorganism *S. sobrinus*. (A) Dose-dependent binding of the reference S-IgA purified from a pool of normal colostrum specimens. The curve of auto-Abs to actin is almost identical to that of the Ag-induced Abs to CHO. (B) Individual specific activity ratios toward the reference S-IgA. In spite of individual variations, all specificities were detected in every subject, and the median value was close to 1 for both auto-Abs and Ag-induced Abs. The bound Abs were detected with labeled Abs to the SC chain (A) or to the α chain (B). The symbols represent mean values of duplicate measurements from one of two similar experiments (A) or means from two different experiments in duplicate at five different concentrations of samples (B).

Abs. The bound multivalent pFv molecules were then revealed with peroxidase-labeled nonimmune $F(ab')_2$ fragments, as described previously (10). To investigate an increased auto-Ab activity in the presence of pFv, a dilution of the reference S-IgA was incubated for 1 h at 37°C with two dilutions of pFv. The mixture was then assayed for Ab binding to human actin-coated plates, and the bound Abs were revealed by the peroxidase-labeled Ab to the α chain. Tubes incubated in the absence of either pFv or S-IgA served as reference and negative controls, respectively.

RESULTS

Presence of auto-Abs in colostrum and saliva. When labeled Abs to the α chain were used for detection, dilutions of the purified colostral S-IgA showed dose-dependent binding to the four unrelated auto-Ags actin, myosin, spectrin, and tubulin. These auto-Abs were shown to be true S-IgA molecules by using the Abs to SC for detection (Fig. 1A). Interestingly, the simultaneous assay in the same specimen of conventional Abs to CHO from caries-associated *S. sobrinus* led to a curve almost identical to that obtained with Abs to human actin. Investigation of individual colostrum samples showed the presence of these Abs in all eight specimens assayed. The titers

TABLE 1. Detection of S-IgA natural Abs to auto-Ags and of spontaneous Ag-induced Abs to the cell wall CHO of *S. sobrinus* in saliva

Specimen[a]	S-IgA concn (μg/ml)	S-IgA Ab titer[b] for:			
		Actin	Myosin	Spectrin	CHO
S01	74	80	60	40	100
S02	90	120	80	60	240
S8	93	160	200	160	320
S2	44	120	30	20	60
S11	69	80	30	20	80
S13	73	160	50	30	160
S50	121	80	80	60	160
SX4	72	60	80	40	240
SX3	31	30	20	20	60
SX1	33	320	80	40	640
Median	73	100	70	40	160
Interquartiles	69–90	80–160	30–80	20–60	80–240
S-IgA (reference)	480	640	320	320	640

[a] S01 to SX1, saliva from 10 normal subjects; S-IgA (reference), reference s-IgA purified from a pool of colostrum samples from normal individuals.
[b] Reciprocal dilution giving OD_{492} of 0.5 with Ab to SC.

varied according to both the Ag and the concentration of S-IgA (median, 5.25 μg/ml; interquartiles, 1.4 to 11.6 μg/ml). When expressed as specific activity ratios by comparison with the reference S-IgA, the median values derived from Fig. 1B were 0.6 (actin), 0.9 (myosin), 0.9 (spectrin), and 0.6 (tubulin). Similar results were obtained with saliva samples, as shown in Table 1. The median specific activity ratios drawn from Table 1 were close to 1: 0.9 for actin, 1 for myosin, and 0.8 for spectrin. Abs to CHO, usually considered to be induced by repeated encounters with *S. sobrinus*, did not show significantly higher results, with a median ratio of 1.5. Comparison of the reactivity to auto-Ags with that to homologous animal Ags showed similar levels, indicating that the autoreactivity was not a side effect of a strong response against related food Ags. Moreover, it is likely that most of these Abs to food Ags are natural auto-Abs to highly conserved structures. Investigation of the S-IgA2/total S-IgA ratio in six specimens confirmed that this ratio was in the normal range in colostrum or in saliva: 35.9% in the reference colostral S-IgA, 27% in colostrum 1, 20% in colostrum 2, 24.4% in colostrum 3, 20% in the saliva from subject 1, and 21.8% in the saliva from subject 2. Similar percentages were observed in five of the six cases when the S-IgA Abs to actin and to myosin in the same specimens were investigated: 28.4 and 26.3% (reference S-IgA), 17.9 and 33.3% (colostrum 1), 14.5 and 31% (colostrum 3), 33.3 and 15% (saliva from subject 1), and 27.8 and 30.3% (saliva from subject 2). In contrast these percentages were much lower in colostrum 2, with values of only 4% (actin) and 3.7% (myosin), as is often the case with Abs to protein Ags (27, 53).

Polyreactivity of the auto-Abs. As previously reported with serum Igs, incubation of salivary Abs with human tubulin was found to inhibit the binding of salivary Abs to human actin (Fig. 2). This cross-inhibition was dose dependent and similar to the homologous inhibition by soluble actin. The results of other experiments showing polyreactivity of Abs to actin or to DNP versus microbial Ags will be presented below.

Auto-Ab activity spectrum of salivary IgA and of serum IgA. By using the one-step three-column gel permeation technique, it was possible to separate the three major classes of serum Igs.

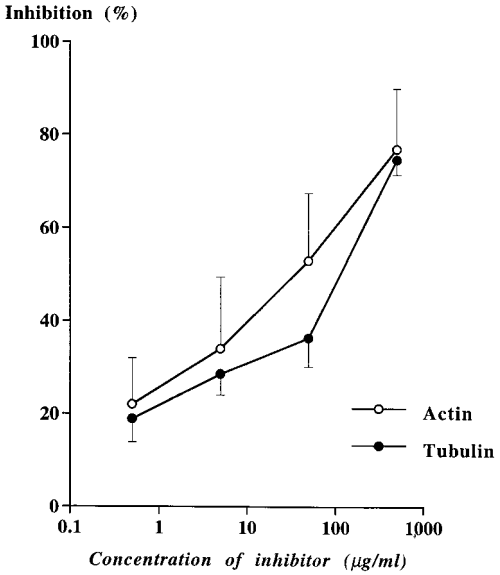

FIG. 2. Inhibition of salivary S-IgA Abs to human actin by soluble actin and by an unrelated auto-Ag (human tubulin) showing that these polyclonal auto-Abs are polyreactive. Symbols and vertical bars represent the mean and standard deviations obtained with specimens from three different subjects.

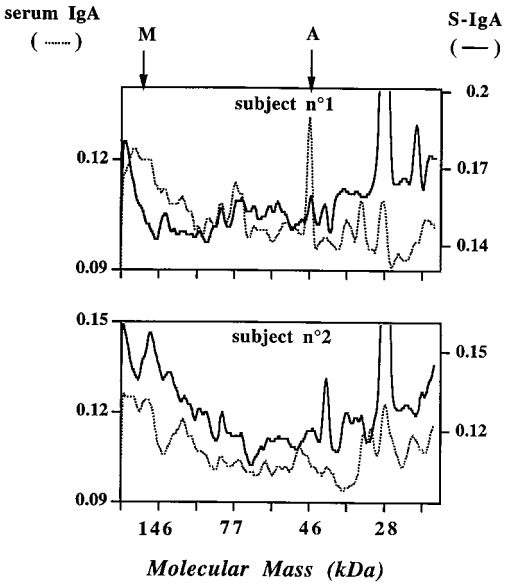

FIG. 3. Reactivity of paired salivary and serum IgA with a large number of muscle auto-Ags, as demonstrated by computer-assisted Western blot analysis. The IgA was from two normal subjects. A lysate of human muscle served as the Ag. The ordinate corresponds to the intensity of bands detected by the Abs, and the abscissa corresponds to the apparent molecular mass of the Ags, calculated from the reciprocal of the electrophoretic migration distances. Many bands were detected, in contrast to the one-peak spectra of control monoreactive murine monoclonal Abs to human actin (arrow A, 42 kDa), or myosin (arrow M, 200 kDa). The slight intraindividual variations between S-IgA and autologous serum IgA confirm that the secretory and systemic immune systems are largely independent. The interindividual variations are in agreement with the strain-associated spectrum already observed in mouse serum Igs.

A large IgG contaminant of the γ3 subclass was cleared from the IgA peak by absorption with protein G. This purification allowed a comparison of the specificity of serum IgA with that of salivary S-IgA collected from the same subject in the absence of interfering IgG auto-Abs. The ratio of purified serum IgA specific activity to saliva IgA specific activity was very low for the Abs to actin, despite the lower sensitivity of the detection of S-IgA with anti-α-chain Abs, compared with monomeric IgA. This ratio was only 0.12 for subject 1 and 0.1 for subject 2, showing a much higher specific activity in the secretion than in the corresponding serum. Incubation of both IgA fractions with the Western blot strips of human muscle allowed simultaneous analysis of the reactivity of IgA to dozens of different auto-Ags. Many peaks were observed with polyclonal serum IgA and S-IgA (Fig. 3), whereas only one was detected with the murine monoreactive monoclonal Abs to human muscle components. The specificity of this technique was evidenced by subtracting the patterns of controls incubated with the sole second Ab and by comparing the results of different specimens. The subtracted nonspecific background of the controls was very low, and comparison with the India ink staining showed no correlation between the Ab spectrum to auto-Ags and the total protein pattern. The Ab activity spectra of S-IgA and of serum IgA showed sligth intraindividual variations in addition to noticeable differences between individuals. A loss of reactivity after denaturation by dissociation of the H and L chains confirmed that the binding was due not to interactions with the carbohydrate chains of S-IgA but to an Ag-Ab reaction. The possibility that some peaks are due to Abs against bacterial Ags cross-reacting with muscular molecules has not been ruled out but seems unlikely because these Ags are infrequent and poorly immunogenic under homologous conditions.

Binding of auto-Abs to bacterial Ags. The investigation of natural Abs to pathogens was based on their polyreactivity. S-IgA was incubated with insoluble human actin and with DNP. The absorbed Abs were then eluted and assayed with surface (Fig. 4) or secreted (Fig. 5) molecules from *S. pyogenes*. A low percentage of S-IgA was harvested during each experiment, but the eluted molecules were active at a low concentration, establishing that polyreactive Abs can react with bacterial Ags. Both similarities and variations of the Ab spectrum were observed according to the Ag (actin or DNP) used for affinity purification.

High functional affinity of some natural Abs. The dissociation curve of S-IgA Abs to actin from subject 1 was of the same order of magnitude as that of the corresponding serum IgA Abs (Fig. 6A), whereas the two curves were slightly different but had similar slopes in the paired Abs from subject 2 (Fig. 6B). As expected from the literature, the specific activities of IgA antitoxins were 47-fold (subject 1) and 112-fold (subject 2) lower than that of corresponding serum IgG. However, the dissociation curves were similar (subject 2) or even identical (subject 1). Comparison between natural and vaccine Ab curves showed that the former Abs started to dissociate at low molarities but were still partly or largely resistant to high molarities of sodium thiocyanate. In contrast, the hyperimmune Abs exhibited a conventional curve, starting with plateau values for low molarities followed by a decrease for high molarities (Fig. 6).

Increase of the activity of natural S-IgA Abs by pFv binding. Reactivity of pFv with captured auto-Abs to human actin and human spectrin (Fig. 7A) confirmed that this V_H3-specific super-Ag (49) not only binds S-IgA, as already reported (10), but also binds the subpopulation of secretory auto-Abs. These results led us to investigate the effect of pFv binding on the apparent auto-Ab activity of S-IgA. A clear-cut dose-dependent increase of the S-IgA binding to human actin was ob-

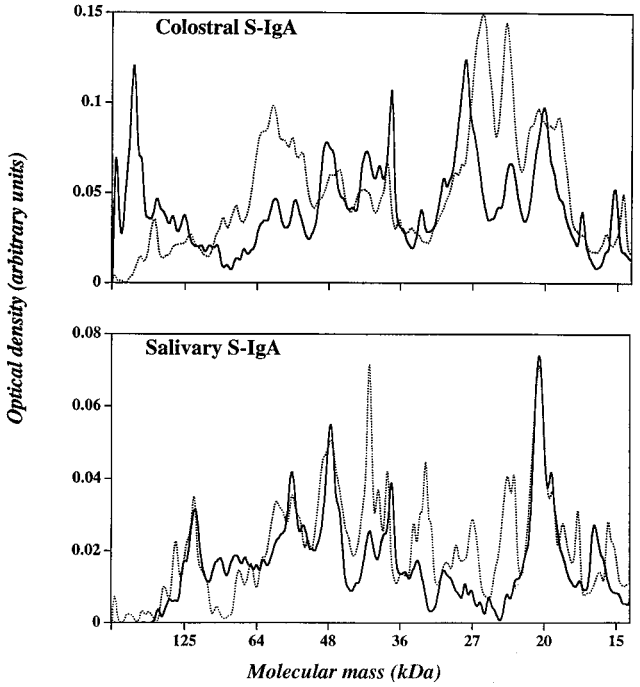

FIG. 4. Reactivity of colostrum C40 and saliva S01 natural Abs with a large number of surface molecules from *S. pyogenes*, as demonstrated by computer-assisted Western blot analysis. The Abs were affinity purified with unrelated Ags: actin (heavy lines) or DNP (dotted lines). Therefore, the bands were detected only by polyreactive, i.e., natural, Abs. Both differences and similarities were observed depending on the specimen and the Ag used for affinity purification.

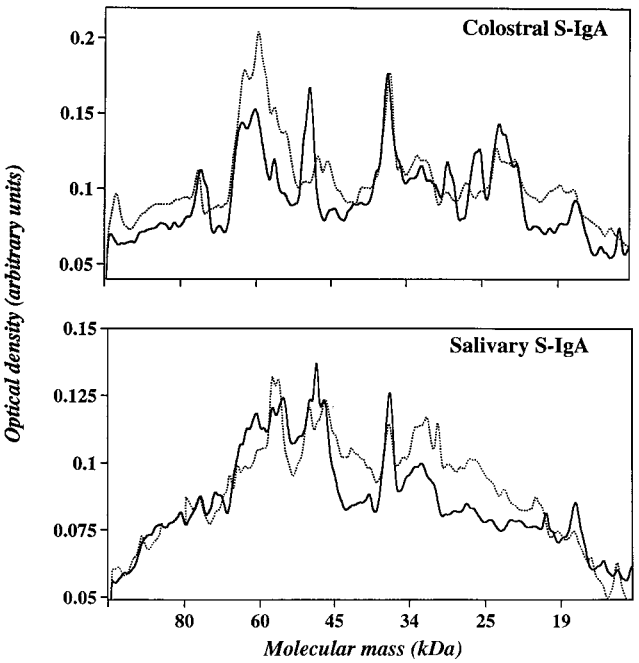

FIG. 5. Reactivity of colostrum and saliva natural Abs with a large number of secreted molecules from *S. pyogenes* as demonstrated by computer-assisted Western blot analysis. As in Fig. 4, the Abs were affinity purified with actin (heavy lines) or with DNP (dotted lines), and the bands were detected only by natural Abs. Both differences and similarities were also observed depending on the specimen and the Ag used for affinity purification. Some peaks were shared with the Fig. 4 patterns.

served after incubation of the polyclonal S-IgA with pFv (Fig. 7B).

DISCUSSION

Investigations of the presence of natural polyreactive Abs in two major secretions were undertaken to understand the role of S-IgA as a first barrier to infection during the preimmune period. We describe the finding of natural auto-Abs in both colostrum and saliva and demonstrate their polyreactivity. Comparisons with the corresponding IgA in serum show a much higher specific activity of S-IgA and confirm that their Ab repertoires are slightly different. Furthermore, the reactivity of these auto-Abs with a large number of bacterial Ags strongly suggests their involvement in protection against pathogens. This role is favored by the high functional affinity of some secretory natural Abs and by their binding to pFv, a gut-associated molecule which increases the efficiency of S-IgA.

The presence of auto-Abs is classically associated with autoimmune diseases. However, the description of auto-Ab specificities in hybridoma molecules from neonatal mice (21) and studies of polyclonal serum Igs from disease-free subjects (23) have demonstrated that these specificities are present at significant levels in the normal repertoire. The S-IgA Abs detected here correspond to the same criteria as those found in the serum: they are directed against auto-Ags in the absence of any apparent disease (Fig. 1; Table 1), and they are polyreactive, as shown both by competitive inhibition (Fig. 2) and by affinity purification with unrelated Ags. Moreover, the specific activity of these secretory molecules is much higher than that of their serum counterpart. How these Abs can bind unrelated epitopes, instead of exhibiting the conventional monoreactivity, remains largely undetermined despite some studies suggesting a role for the CDR3 framework region of the H chain (16, 36).

Computer-assisted analysis of the reactivity of natural Abs to organ extracts has been developed for simultaneous investigation of the Ab reactivity to a large number of auto-Ags. A method similar to ours has been used for analysis of the Ig repertoire (24). Comparison of the Ab activity spectra of S-IgA from different subjects (Fig. 3) is in agreement with the individual patterns already observed with serum IgG and IgM (3). These interindividual variations could be genetically determined, as suggested by the differences observed between lines of mice (3, 4). The variations can also originate from an allelic diversity, since many auto-Abs are encoded by unmutated (2) or poorly mutated (37) germ line Ig V genes. They can also be associated with interference with unrelated immune system experiences leading to negative (31, 42) or positive (28, 37) stimulations of different multireactive B-cell clones. The analysis of S-IgA and of serum IgA has provided an unique opportunity to compare the Ab spectra of Igs sharing the same α isotype but generated either in the secretory or in the systemic immune system (Fig. 3). The association of both differences and similarities between the Ab spectra of serum IgA and of S-IgA provides evidence of the duality of these immune systems, in agreement with the classical cellular studies (18, 39).

The auto-Ab specificity detected in S-IgA natural Abs is of major interest because the digestive lumen contains auto-Ags from the epithelial and glandular cells, as well as from various blood cells such as lymphocytes, polymorphonuclear leukocytes, and erythrocytes. The number of exfoliated intestinal cells is specially large, because their half-life in the mucosa is

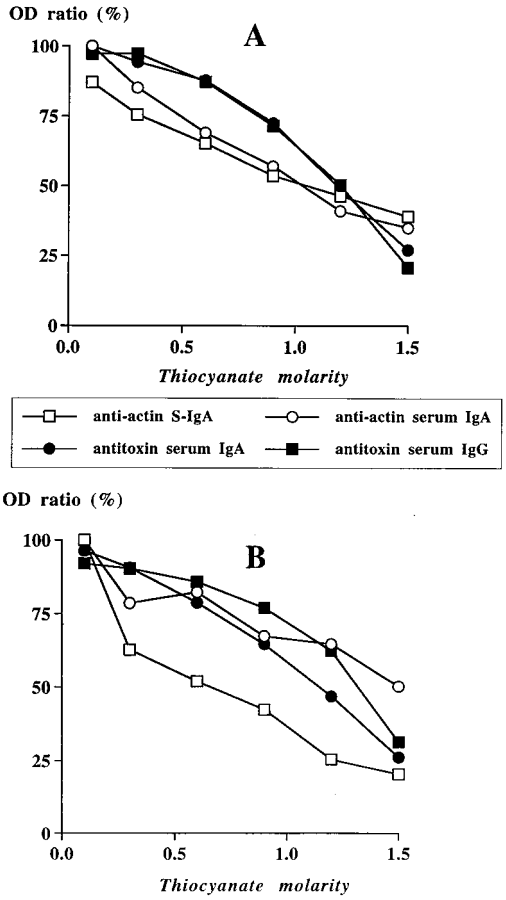

FIG. 6. Comparison of the functional affinities of natural and vaccine Abs, using the Ag-Ab dissociation method with increasing molarities of sodium thiocyanate. In this method, the slope of the curve is inversely proportional to the affinity of the Ab; i.e., horizontal lines correspond to the highest affinities. Autologous salivary S-IgA, serum IgA, and serum IgG were isolated from subjects 1 (A) and 2 (B). The dissociation curves show that autoimmune complexes have a higher sensitivity to low molarities of thiocyanate than those of autologous tetanus antitoxins of high intrinsic affinity (K_a of serum antitoxins from subject $1 = 5.6 \times 10^{10}\ M^{-1}$). In contrast, the resistance of the other immune complexes to the high molarities was similar. The symbols represent median values of triplicate measurements, with the range being smaller than 5%. Reproducibility was excellent in two additional experiments with the same specimens.

only 2 to 3 days. The lumen can also contain cross-reactive xeno-Ags from food. It is likely that secretory S-IgA auto-Abs are involved in the clearance of these Ags and that they can prevent the occurrence of pathogenic autoimmunity. It has been shown that injections of natural Abs have a beneficial effect on spontaneous lupus in mice (25). It is thus possible that S-IgA natural Abs display a similar regulatory function, as suggested by the high frequency of autoimmune diseases in IgA-deficient subjects (32).

Since the major classical role assigned to S-IgA is protection against microorganisms, we investigated natural Abs for their specificity to *S. pyogenes*, a common pathogen of the oropharynx. Surface (Fig. 4) and secreted (Fig. 5) components were selected because secretory Abs are useful mainly by reacting with many accessible Ags, irrespective of their pathogenic roles. Abs of natural specificities were affinity purified either with DNP, an artificial Ag not encountered under normal conditions, or with human actin, an auto-Ag having no cross-reactivity with *S. pyogenes*. The polyreactive S-IgA gave Ab spectra which differed slightly according to the Ag used for immunoabsorption. These variations were due to unbalanced purifications of the natural Abs, in agreement with the nonrandom association of the reactivities of polyreactive serum IgG with different self Ags. For instance, polyreactive serum Abs to actin contain 12.5% of the total reactivity to tubulin but as much as 49.6% of that to fetuin (23). DNP- and actin-purified Abs thus correspond to different populations with overlapping specificities. An alternative explanation for IgA binding to auto-Ags was a nonimmune lectin-like interaction between the CHO of the IgA and molecules of the self. Both *N*- and *O*-linked CHO are known to be attached to the α chain and to display a high diversity (45). It has been shown that this prosthetic moiety can be reactive with bacterial fimbriae (57). Here, the large number of bands was not in favor of a nonimmune interaction, which was ruled out by selective denaturation of the protein-folding structure and thus of the Ab function. Similarly, a possible interference of the 42-kDa streptococcal IgA-binding receptor, protein *Arp*, was also ruled out by the failure to solubilize this molecule from group A streptococcal cells and by the need for a gene transfer into an *Escherichia coli* recipient for its purification (33).

Their polyreactivity allows the whole population of S-IgA natural Abs to react with a large variety of Ags at the surface of bacteria; this is in agreement with the seminal study of Brandtzaeg et al., showing that oral bacteria become coated with IgA in human saliva in vivo (13), and with a recent study reporting that at least 24 to 74% of bacteria are coated with IgA in the human feces (55). However, these Abs must display sufficient efficiency to form a permanent barrier against commensal microbes and to protect against low levels of infective agents. Despite the current opinion that the polyreactive monoclonal Abs of the IgM isotype have low affinity, the present method showed variable degrees of functional affinity which can reach those of vaccine antitoxins (Fig. 6). This is in agreement with previous data showing significant affinities of polyreactive mouse monoclonal Abs (52). The very high functional affinities revealed here by the thiocyanate dissociation method could be related to a competition between natural Abs. Under these experimental conditions, the molecules of highest affinity are favored within a random Ab population lacking Ag-driven selection. Similarly, the nonimmune binding with pFv (Fig. 7A) is of major interest because it shows that secretory polyreactive Abs are involved in the macromolecular immune fortresses of the gut lumen (11). The binding of pFv to the V_H domain can both maintain secretory Abs under a polymeric state and allow other S-IgA molecules to amplify the antimicrobial activity of polyreactive Abs by their simultaneous presence in the nonimmune complexes (Fig. 7B).

The results presented here are in agreement with a hypothesis based on observations of murine intestinal tissues (43), showing that there is a high percentage (more than 40%) of B-1 ($CD5^+$) lymphocytes in the intestinal effector sites but that these cells are virtually absent from the Peyer's patches (30). The B-1 lymphocytes synthesize polyreactive and antibacterial Abs (6, 14, 15), and it has recently been shown that the percentage of multireactive cells is significantly higher (85% versus 50%) in this subpopulation than in B-2 cells (17). Although there is no evidence for a similar contribution of B-1 cells in the human gut, a fascinating hypothesis would be that two different immune systems can coexist in the gut mucosa. The "primordial" system would consist of T-independent self-replenished B cells, which produce polyreactive Abs to auto-Ags, foods, and bacteria. In contrast, the "conventional" B-cell system would be T dependent, renewed in the bone marrow and

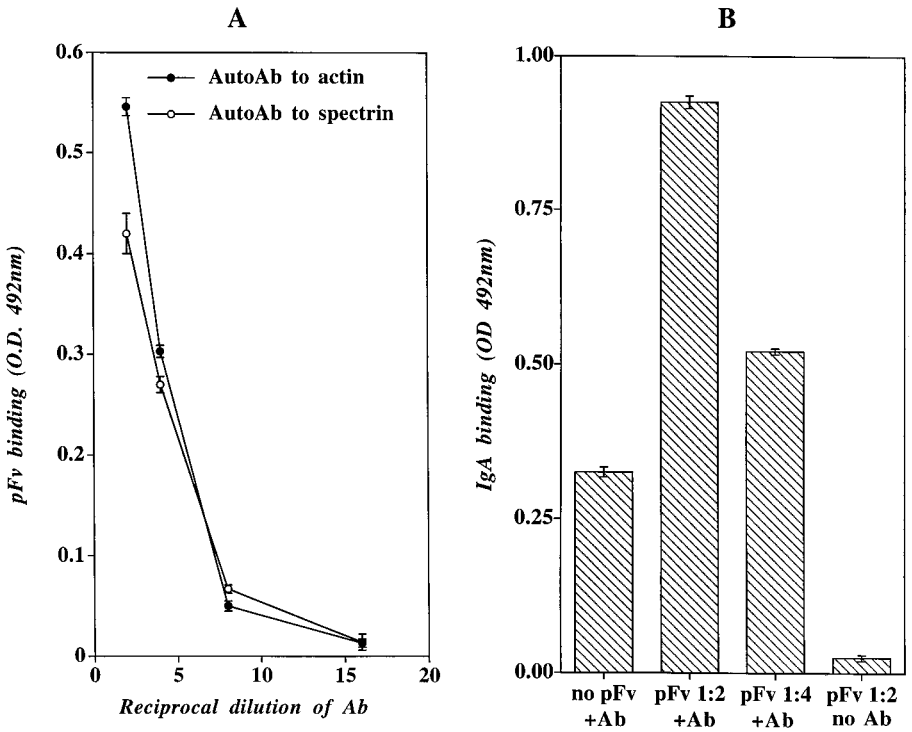

FIG. 7. Complexing of intestinal protein Fv (pFv) with S-IgA auto-Abs increases S-IgA binding to auto-Ags. (A) Dilutions of colostral S-IgA were incubated with human actin- and human spectrin-coated plates. The captured Abs reacted with further added human pFv. (B) A constant dilution of S-IgA was preincubated with two dilutions of pFv. The mixture showed an apparently increased auto-Ab activity to human actin, due to the simultaneous presence of irrelevant S-IgA molecules in the Ab-pFv nonimmune complexes. Symbols and columns represent mean values in duplicate, with ranges indicated.

in the Peyer's patches, and produce mainly Ag-induced monoreactive Abs.

By analogy with the classical "key-and-lock" model of the Ag-Ab reaction, the natural Abs in human secretions might represent a "passe-partout" system (set of skeleton keys) of germ line-encoded Abs, leading to a background level of basic protection against most food Ags, pathogens, and commensal microbes. This primordial secretory system, acting mainly in the intestinal lumen (preventive role), can reinforce the conventional "private-key" Ab system specifically induced by pathogens and vaccines.

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