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Purification and Characterization of Chimeric Human IgA1 and IgA2 Expressed in COS and Chinese Hamster Ovary Cells[1]

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Abstract. Ag-specific chimeric human IgA molecules, of the two human subclasses, IgA1 and IgA2, have been expressed in two mammalian cell systems. Analysis of the secreted IgA molecules, purified in milligram quantities from stable Chinese hamster ovary transfectants by Ag affinity chromatography, has allowed a direct comparison of the biologic properties of the two subclasses. HPLC gel filtration analysis revealed that in both subclasses, the IgA molecules associate predominantly into dimers. The monomer units are presumed to interact noncovalently, inasmuch as no dimers are evident when the antibodies are subjected to SDS-PAGE. The recombinant antibodies are glycosylated, inasmuch as a lectin blotting procedure revealed that the H chains of both subclasses are recognized by Con A. When subjected to digestion by preparations of IgA1-specific proteases secreted by two pathogenic streptococcal strains, *Streptococcus sanguis* and *Streptococcus oralis*, the recombinant IgA molecules behave just as their natural equivalents. Thus, only the chimeric IgA1 molecule is cleaved, with the IgA2 remaining intact. In terms of interaction with natural effector molecules, both recombinant IgA isotypes were shown to interact with Fcα receptors on calcitriol-stimulated HL-60 cells with similar affinity, but neither antibody was found to interact with human C1q. The expression system described readily permits manipulation of the human IgA genes, which should lead to a fuller molecular understanding of how this important antibody mediates its function. *Journal of Immunology,* 1993, 151: 4743.

IgA is not only a major serum Ig in humans but is also the predominant class of Ig found in external secretions. These secretions include those of the respiratory, gastrointestinal, and genitourinary tracts as well as saliva, tears, milk, and colostrum. The mucosal surfaces, bathed by these secretions, represent a major site of exposure to the environment, having a surface area approximately 200 times greater than that of skin (1). As the chief antibody at this surface, IgA may well form the first line of defense against many invading pathogens.

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In humans, two subclasses of IgA occur, termed IgA1 and IgA2, with two allotypic variants of IgA2 (IgA2 m(1) and IgA2 m(2)) having been described. Monomeric IgA consists of two H (or α) chains of about 60 kDa and two L chains (25 kDa) arranged to form the Fab arms and Fc region common to antibody molecules. Each H chain folds into four homology regions: the N-terminal variable domain (V_H), followed by three constant domains Cα1, Cα2, and Cα3. A flexible hinge region, much longer in IgA1 than in IgA2, lies between the Cα1 and Cα2 domains. The L chain is composed of two domains, V_L and C_L, and is common to all Ig. The human α-chain constant region genes termed α1 and α2, for IgA1 and IgA2, respectively, have been cloned and sequenced (2). Each homology domain is encoded by a single exon, with the hinge being encoded within the Cα2 exon.

IgA in serum exists in predominantly monomeric form, with an IgA1:IgA2 ratio of about 9:1 (3). A small proportion of serum IgA occurs as dimers, or sometimes larger polymers. In contrast, the IgA found in secretions, termed

secretory IgA, is predominantly dimeric in form, with some higher molecular mass polymers also being present. Dimeric IgA in serum comprises two monomer IgA units linked by disulfide bonds to each other and to another small polypeptide termed J chain. This dimer may be transcytosed across epithelial cells lining mucosal sites into seromucous secretions, in a process mediated by the poly-Ig receptor. During this secretion process, a portion of the receptor, termed secretory component, is proteolytically cleaved and becomes complexed with the dimer, yielding the larger secretory IgA molecule. The ratio of IgA1:IgA2 in secretions varies but is normally approximately 6:4 (3).

In terms of function, secretory IgA may serve to aggregate and hence immobilize bacteria and inhibit their adherence to mucosal surfaces (4). Furthermore, aggregated serum and secretory IgA both appear capable of activating the alternative pathway of human C (5–7). Both species of IgA also interact with specific Fcα receptors present on human granulocytes, monocytes, and macrophages, which can mediate phagocytosis, superoxide generation, enzyme release, and clearance of immune complexes (3).

Despite the abundance of IgA in the body, relatively little is currently understood, in molecular terms, about how this antibody functions, how it polymerizes, or how it interacts with the poly-Ig receptor to allow transcytosis into seromucous secretions. This poor understanding stems, in part, from the previous lack of an abundant source of Ag-specific monoclonal IgA of both human subclasses. The present report describes the establishment of systems expressing recombinant forms of human IgA1 and IgA2, thus facilitating their isolation and allowing the use of genetic manipulation to probe their biologic functions.

The expression of chimeric mouse:human antibodies of defined specificity in myeloid cells has been described previously (8). Indeed, a matched set of chimeric antibodies, including chimeric human IgA2 but not IgA1, has been expressed in the mouse myeloid cell J558L (9). The constant domains of the human H chain were inserted into expression vectors downstream of a mouse variable domain, V_{NP}, and co-expression with a compatible mouse L chain generated chimeric molecules with specificity for the hapten NIP.[3] Here, we have employed a similar principle to express chimeric human IgA2 and, for the first time, human IgA1. However, in this case, the nonmyeloid COS and CHO cell expression systems used allowed higher antibody expression levels to be achieved. The isolation of purified IgA1 and IgA2 has facilitated a direct comparison of the biologic properties of the two subclasses.

[3] Abbreviations used in this paper: NIP, 3-nitro-4-hydroxy-5-iodophenylacetate; NP, 3-nitro-4-hydroxyphenylacetate; CHO, Chinese hamster ovary; GS, glutamine synthetase; PBST, PBS/0.02% Tween 20; HCMV, human CMV.

Materials and Methods

Introduction of human IgA H chain genes into expression vectors

A 45-kb cosmid clone containing the human α1 gene (2), kindly provided by Dr. T. H. Rabbitts, was mapped using restriction enzymes. Southern blot analysis (10) using 20-mer Cα1 and Cα3 domain-specific probes revealed the presence of a *Hin*cII site and a *Bam*HI site flanking the α1 gene. These sites were used to subclone a 5.6-kb α1-containing fragment into the *Eco*RV and *Bam*HI sites of the cloning vector pSP73 (Promega, Madison, WI). From this construct, a smaller 2.7-kb α1-containing *Bgl*II fragment was subcloned into the unique *Bam*HI site of the mammalian cell expression vector pEE6.HCMV (11). This vector already contained the V_{NP} gene and the selectable marker gpt.

Restriction mapping of an α2-containing plasmid, pSV-VNPα2 (9), kindly provided by Dr. M. Neuberger, indicated a *Hin*dIII site on each side of the α2 gene. A 2.6-kb *Hin*dIII fragment was then subcloned into the multiple cloning site of the cloning vector pBluescript SK- (Stratagene, La Jolla, CA). The α2 gene was then transferred as a *Bam*HI-*Sal*I fragment into the V_{NP}- and gpt-containing pEE6.HCMV vector.

Sequencing of the double stranded expression vector templates using a Cα1 domain-specific primer was performed by the dideoxy chain termination method (12).

The cDNA coding for a mouse λL chain, cloned from the anti-NP hybridoma S43 (13), was similarly introduced into pEE6.HCMV along with the selectable marker GS.

Transfection of expression vectors into COS cells

COS cells were maintained in DMEM supplemented with 10% FCS, glutamine (2 mM), penicillin (100 IU/ml), and streptomycin (100 μg/ml). On the day before transfection, they were subcultured and seeded at 10^5 cells/ml in 24 × 1.5-cm multiwell culture plates. Transfection with expression vectors containing H and L chain genes were carried out according to the method of Lopata et al. (14). Cells in each well were transfected with 4 μg DNA by DEAE-dextran treatment for 6 h with subsequent shocking with 10% DMSO in HEPES-buffered saline. After washing, they were incubated for 5 days in DMEM with 10% FCS. Supernatants were then harvested, and cell debris were removed by centrifugation at 13,000 rpm for 5 min, and stored at 4°C before analysis.

Transfection of expression vectors into CHO-K1 cells

CHO-K1 cells were maintained in Glasgow minimum essential medium supplemented with 10% FCS, nonessential amino acids (GIBCO-BRL, Uxbridge, United Kingdom),

glutamate (60 μg/ml), asparagine (60 μg/ml), sodium pyruvate (1 mM), adenosine (7 μg/ml), guanosine (7 μg/ml), cytidine (7 μg/ml), uridine (7 μg/ml), thymidine (2.4 μg/ml), penicillin (100IU/ml), and streptomycin (100 μg/ml). Transfections were carried out by seeding 10^6 cells in a 9-cm petri dish. After 24-h incubation, each dish was transfected with 10 μg vector DNA by calcium phosphate coprecipitation followed by glycerol shock (15). After 24-h additional incubation in growth medium, appropriate selection was added. After 14 to 21 days, with weekly addition of fresh selective medium, resistant colonies were picked and expanded in mass culture. Supernatants were harvested, rid of cell debris as for COS cells, supplemented with protease inhibitors (0.02% sodium azide, 10 mM PMSF, and 50 mM iodoacetamide) and stored at 4°C before analysis.

Two rounds of transfection and selection were necessary. In the first round, the vector containing the L chain gene was introduced, and selection for the GS selectable marker on the vector was achieved by culture in growth medium supplemented with methionine sulfoximine (20 μM). A clone secreting high levels of L chain was then chosen for the second round in which the H chain gene was introduced. Selection for the gpt selectable marker on the H chain vector was achieved in growth medium supplemented with hypoxanthine and thymidine (HT supplement; GIBCO BRL), xanthine (0.25 mg/ml), and mycophenolic acid (10 μg/ml).

Affinity chromatography purification of anti-NIP antibodies

Anti-NIP IgA1 and IgA2 antibodies were purified from supernatants of transfected CHO-K1 cells on NIP-Sepharose affinity columns, prepared by coupling NIP-caproate to AH-Separose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions with *N*-ethyl-*N'*(3-dimethylaminopropyl)carbodiimide hydrochloride as the coupling agent. After passing samples through the column followed by extensive washing with PBS containing 0.02% sodium azide, antibody was eluted with 0.5 mM NIP-caproate in PBS and dialyzed exhaustively against PBS/0.02% azide. Antibody samples were then centrifuged to remove aggregates, concentrated in Centriprep concentrators (Amicon Ltd., Stonehouse, United Kingdom), and stored at −20°C.

Chimeric mouse-human IgG1 anti-NIP antibody was isolated in a similar manner from the supernatant of a J558L myeloma transfectant (9) obtained from Public Health Laboratory Service, Porton Down, United Kingdom, and maintained in RPMI 1640 with 10% FCS.

Pooled human IgG, used as a control on some SDS gels, was prepared from serum by standard methods of ammonium sulfate precipitation, and ion-exchange and gel filtration chromatography (16).

Analysis of Ig by Western blotting

Affinity-purified human IgA antibodies were analyzed on 10% SDS-PAGE gels under reducing and nonreducing conditions (17). For Western blotting, proteins were transferred to nitrocellulose membranes that were then blocked by incubating overnight with shaking in 5% nonfat milk powder in PBS. After washing three times with PBS containing 2% Nonidet P-40 and 0.2% SDS (PBS/detergent), the filters were further incubated for 2 h with either sheep anti-human IgA Fc-peroxidase conjugate (Serotec, Oxford, United Kingdom) or an anti-mouse λL chain-peroxidase conjugate (Nordic Immunological Laboratories, Maidenhead, United Kingdom) diluted 1:1000 and 1:500, respectively, in PBS/detergent according to manufacturer's instructions. The filters were then washed as before, substrate (a mixture of 12 mg diaminobenzidine in 18 ml of 50 mM Tris HCl buffer (pH 7.6), 2 ml of 0.3% nickel chloride, and 20 μl 30% hydrogen peroxide) added and the blot allowed to develop for 5 to 10 min.

Alternatively, blotted proteins were assessed for their ability to bind to Con A. In this case, the nitrocellulose membranes were blocked for 1 h in PBST containing 1% BSA and then incubated for 1 h in a 1-μg/ml solution of biotinylated Con A (Sigma Chemical Co., Ltd., Poole, United Kingdom) in PBS/1% BSA. After washing three times in PBST/1% BSA, the filters were incubated for 30 min with streptavidin-peroxidase conjugate (Sigma) at a concentration of 1 μg/ml in PBS/1% BSA. After washing as above, the blot was developed by addition of substrate as before.

HPLC analysis

Samples of affinity-purified IgA1 and IgA2 at 1 mg/ml were run on a DuPont GF250 column in either 200 mM sodium phosphate buffer, pH 7.0, or 500 mM arginine chloride, pH 6.0.

ELISA analysis of Ag-binding by COS cell transfectant supernatants

Microtiter plates (Nunc Immunoplate Maxisorp; GIBCO BRL) were coated overnight with approximately 0.25 μg/well NIP-BSA in 0.05 M sodium carbonate buffer, pH 9.6. After washing once with PBST and four times with water, 100 μl of blocking buffer (0.05 M sodium carbonate buffer, pH 7.2, containing 0.5% casein or PBST containing 5% nonfat milk powder) were added to each well and incubated for an additional hour at room temperature. After washing as above, 100 μl of transfectant supernatant were added to each well and incubated for an additional hour at room temperature. After washing as above, 100 μl of a 1:1000 dilution of an anti-human IgA Fc-peroxidase conjugate (Serotec) in 0.1 M Tris HCl buffer, pH 7.0, with 0.02% Tween 20 and 0.2% casein, were added to each well. After

incubation for 1 h at room temperature and washing as above, 100 μl of fresh substrate (0.1 mg/ml tetramethylbenzidine and 0.005% H_2O_2 in 0.1 M sodium citrate buffer, pH 6.0) were added to each well. After 20-min incubation at room temperature, A_{630} was measured on a Dynatech Laboratories (Billinghurst, United Kingdom) MR600 plate reader.

ELISA to detect binding to C1q

Microtiter plates were coated with NIP-BSA as described above and blocked for 1 h with 200 μl/well PBST/1% BSA. Plates were then washed once with PBST and 10 times with water before addition of 100 μl antibody, diluted in PBST, to each well. Affinity-purified IgA1, IgA2, and IgG1 were used at concentrations of 1 μg/ml. For IgM, neat culture supernatant from an anti-NIP IgM J558L transfectant (9) was used. After incubation for 1 h at room temperature, the plates were washed as above, and 100 μl neat human serum were added per well as a source of C1q. After additional incubation for 1 h at room temperature, 100 μl/well of anti-human C1q-peroxidase conjugate (Serotec), diluted 1:400 in PBST according to the manufacturer's instructions, were added. The plates were incubated for an additional hour and washed as above, and 200 μl of substrate (0.1 M citrate buffer, pH 4.0, containing 0.6 mg/ml 2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid) and 0.02% H_2O_2) were added to each well. After 5 to 10 min, A_{414} was measured on an Anthos HTII plate reader (Denley, Billinghurst, United Kingdom).

Cleavage by IgA1 protease preparations

One-liter 18-h cultures of *Streptococcus sanguis* (NCTC 7863) and *Streptococcus oralis* (NCTC 11427) were kindly provided by Dr. I. Douglas. Cells were removed by centrifugation at 10,000 rpm for 20 min. Supernatants were precipitated with ammonium sulfate (60% saturation), and the pellets were redissolved in a small volume of PBS. After extensive dialysis against PBS, the protease preparations were aliquoted and stored at −70°C before use.

Affinity-purified IgA1 and IgA2 at 1 mg/ml in PBS were incubated at 37°C with equal volumes of either crude protease preparation or PBS. After 72 h, the antibodies were analyzed on 10% SDS-PAGE gels under nonreducing conditions.

Rosette assay

SRBC were derivatized by incubation for 1 h at room temperature with 10 μg/ml NIP-caproate-*O*-succinimide in isotonic borate buffer, pH 8.5, washed three times with PBS, and sensitized with chimeric IgA anti-NIP antibody (50 to 200 μg/ml) as described previously (18). HL-60 cells were maintained in RPMI 1640 containing 10% FCS and stimulated with 1,25-dihydroxyvitamin D3 (10^{-9} M) for 6 days before use. Rosetting of sensitized RBC to HL-60 cells was performed in V-bottomed microtiter plates as described previously (18), scoring an effector cell with three or more RBC attached as a rosette.

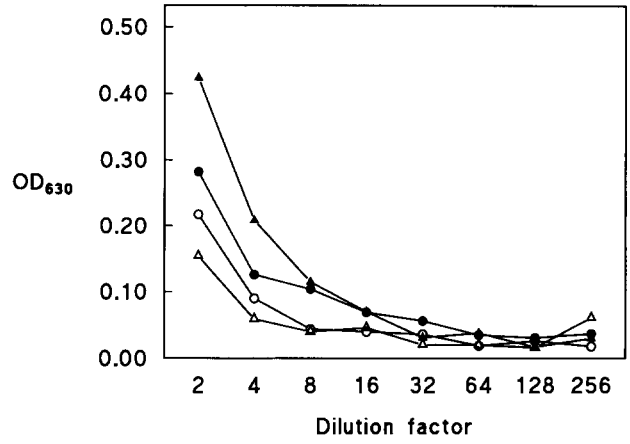

FIGURE 1. Ag-binding ELISA of IgA antibodies present in COS cell supernatants 5 days after transfection with L and H (α) chain vectors. ●, IgA1 H chain transfectant; ○, ▲, △, IgA2 H chain transfectants.

Results

Construction of vectors for expression of chimeric human IgA1 and IgA2 and characterization by transient expression

Convenient restriction enzyme sites were located on each side of the human α1 gene in a previously isolated cosmid clone (2) by Southern blotting. These sites were then used to introduce the α1 gene into the mammalian cell expression vector pEE6.HCMV downstream of a mouse V_H domain, V_{NP}. This plasmid contains the strong promoter/enhancer transcriptional control element from the HCMV (19) upstream of the mouse V_{NP} gene. An SV40 origin of replication is provided by the SV40 early promoter fragment, which drives a selectable marker, either gpt for the H chain genes or GS for the L chain gene. The vector also contains an ampicillin-resistance gene allowing selection and propagation in bacterial hosts. Sequencing of the construct, using a Cα1 domain-specific primer, confirmed the presence of the α1 gene.

The human α2 gene (coding for the IgA2 m(1) allotype) was similarly subcloned from a previously described plasmid (9) into the V_{NP}-containing pEE6.HCMV expression vector. Sequencing, as described above, confirmed that the vector contained the α2 gene.

The two H chain expression constructs were each separately co-transfected with a L chain expression vector into COS cells. After incubation for 5 days, the cell supernatants were analysed by ELISA for binding to Ag. The results are shown in Figure 1. They reveal that for both the IgA1 and IgA2 H chain constructs, co-transfection with the mouse L

chain vector resulted in expression of antibodies that were recognized by the anti-IgA Fc second antibody and were specific for the hapten NIP. Having confirmed that transient expression could be achieved from the vectors, we went on to develop stable CHO transfectants.

Analysis of chimeric IgA antibodies purified from the supernatants of stable CHO transfectants

The IgA1 and IgA2 H chain expression vectors were each separately transfected into a clone of CHO-K1 cells, previously transfected with the L chain vector and submitted to GS selection, which stably expressed the compatible λL chain. After selection for the gpt selectable marker, resistant colonies were picked and expanded. ELISA analysis of Ag binding, as for COS cell transfectants, allowed selection of clones secreting high levels of antibody. Supernatants were harvested and antibodies purified by affinity chromatography on NIP-Sepharose columns. After elution, extensive dialysis to remove NIP-caproate and centrifugation to remove aggregates, samples were analyzed by SDS-PAGE and Western blotting as shown in Figure 2.

Under nonreducing conditions, the purified IgA1 antibody displays a major band of about 170 to 180 kDa molecular mass, reactive with both anti-IgA H chain and anti-L chain antibodies. A small proportion of higher molecular mass aggregates is also present. A faint band of about 120-kDa molecular mass is reactive with both anti-H chain and anti-L chain antibodies. This band may possibly represent a complex of one H chain and one L chain. After reduction of the interchain disulfide bonds, the molecule dissociates into the H and L chains, with molecular masses of about 60 and 25 kDa, respectively.

The purified IgA2 shows a different pattern on SDS gels. Under nonreducing conditions, two major bands of about 120 and 50 kDa molecular mass are evident, with some higher molecular mass aggregates being again present. Western blotting shows that these major bands correspond to disulfide-bonded H chain dimers and L chain dimers, respectively. In the IgA2 m(1) allotype expressed here, in contrast to most antibody molecules, disulfide bonds do not form between H and L chains, rather, the L chains bond to each other (20). Upon reduction, the IgA2 m(1) molecule dissociates into its constituent 60-kDa H chain and 25-kDa L chain.

Comparison of purified IgA1 and IgA2

HPLC analysis of affinity-purified IgA1 and IgA2, in both cases, revealed that in aqueous solution the majority of antibody exists as a 250- to 300-kDa molecular mass species with some larger aggregates also being present (Fig. 3). Comparable HPLC traces were found when either sodium phosphate or arginine chloride buffers were used. This finding implies that the recombinant IgA antibodies predominantly associate as dimers. The monomer units presumably interact in a noncovalent fashion, since no dimers are evident on SDS gels.

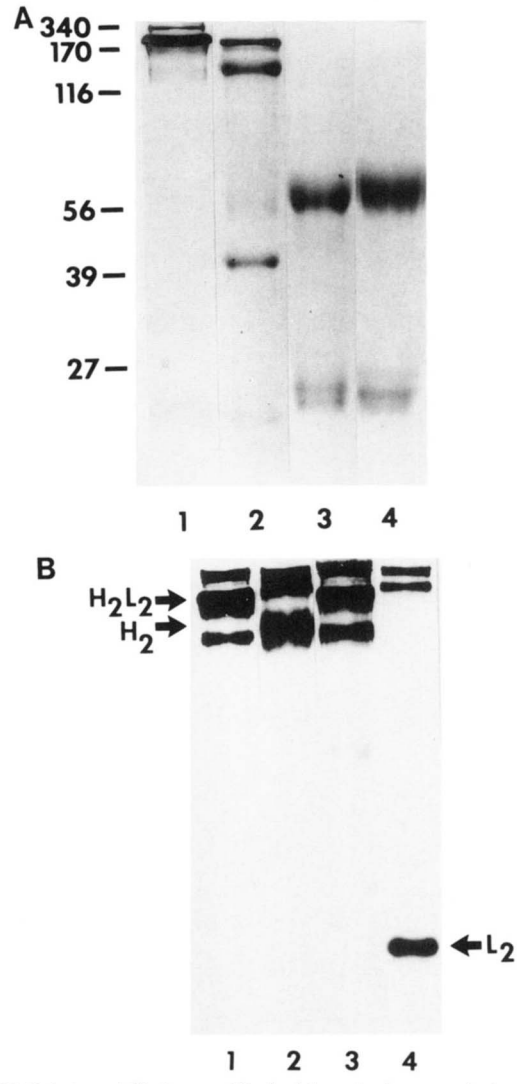

FIGURE 2. Affinity-purified chimeric human IgA antibodies expressed by CHO-K1 transfectants. *A*, SDS-PAGE analysis. Under nonreducing conditions IgA1 (*Lane 1*), IgA2 (*Lane 2*); under reducing conditions IgA1 (*Lane 3*), IgA2 (*Lane 4*). *B*, Western blot analysis under nonreducing conditions. Probing with an anti-human IgA Fc antibody IgA1 (*Lane 1*), IgA2 (*Lane 2*); probing with an anti-λ chain antibody IgA1 (*Lane 3*), IgA2 (*Lane 4*).

Purified IgA1 and IgA2, run on SDS gels and blotted onto nitrocellulose membranes, both showed reactivity with the lectin Con A, which binds to the N-linked carbohydrate moieties of Ig (21). Figure 4 demonstrates that both IgA1 and IgA2 possess on their H chains carbohydrate moieties recognized by Con A. In this highly sensitive assay, the Con A appears also to show reactivity with the mouse L chain and with other trace contaminants.

Crude protease preparations were isolated from two

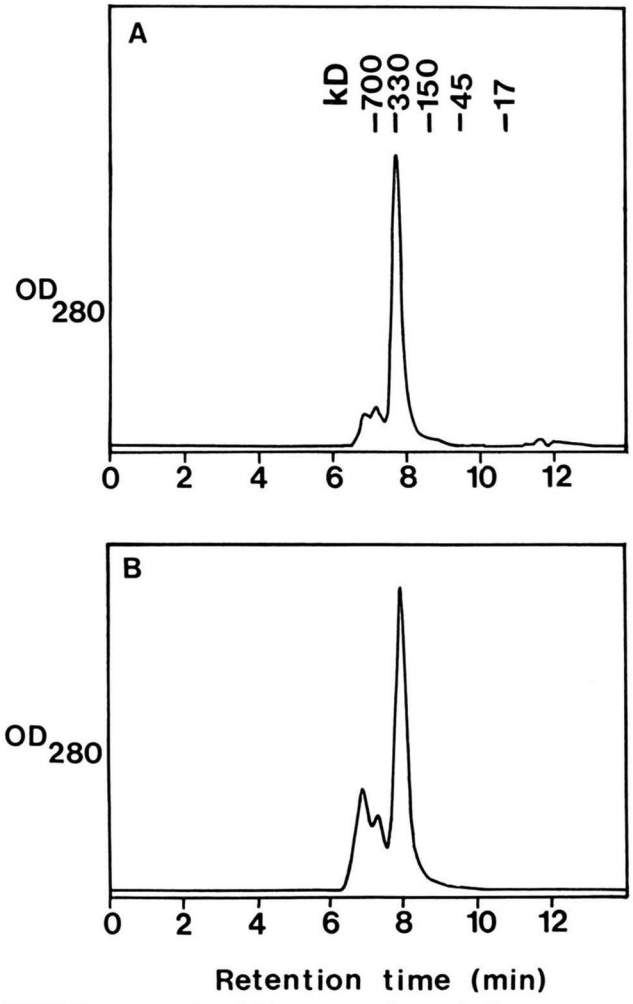

FIGURE 3. HPLC gel filtration analysis of affinity-purified chimeric human IgA antibodies run in 200 mM sodium phosphate buffer, pH 7.0. *A*, IgA1. The retention times of molecular mass markers are indicated above the profile. *B*, IgA2.

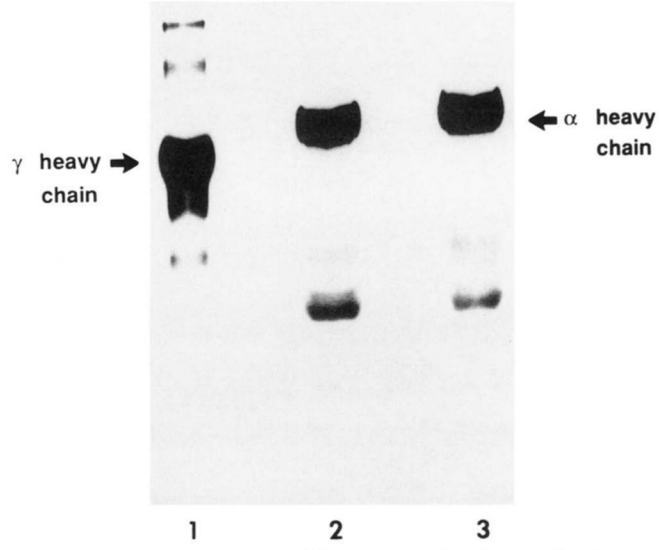

FIGURE 4. Reactivity of human antibodies with Con A. After SDS-PAGE under reducing conditions and transfer to nitrocellulose, the blot was probed with Con A-peroxidase conjugate. *Lane 1*, pooled human IgG; *Lane 2*, chimeric human IgA1; *Lane 3*, chimeric human IgA2.

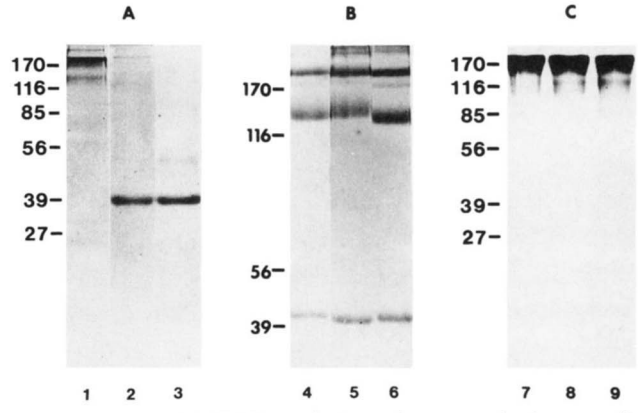

FIGURE 5. SDS-PAGE analysis under nonreducing conditions of digestion products of antibodies treated with streptococcal protease preparations. *A*, Chimeric human IgA1. *B*, Chimeric human IgA2. *C*, Pooled human IgG. *Lanes 1, 4,* and *7*, untreated; *Lanes 2, 5,* and *8*, treated with *S. sangius* protease; *Lanes 3, 6,* and *9*, treated with *S. oralis* protease.

streptococcal strains, *S. sanguis* and *S. oralis*, known to secrete human IgA1-specific proteases (22). When affinity-purified IgA1 was incubated with either of the protease preparations, the antibody was cleaved to yield a major band at about 40-kDa molecular mass (Fig. 5). In contrast, purified human IgA2 was resistant to attack from either of the proteases (Fig. 5). Pooled human IgG was similarly resistant.

Fcα receptors are expressed on human neutrophils, monocytes, and macrophages (3). Their expression can also be induced on a number of neutrophil- and monocyte-like cell lines after stimulation with certain agents (23). Stimulation of the human promyelocytic cell line HL-60 with 1,25-dihydroxyvitamin D3, for example, is reported to induce expression of Fcα receptors (24). We found that after 5 to 6 days of such stimulation, about 35% of a population of HL-60 cells were capable of forming rosettes with both purified IgA1- and IgA2-sensitised RBC (Fig. 6), presumably through interaction with Fcα receptors, inasmuch as no rosettes were seen in the absence of IgA. The two IgA subclasses appear to interact with the receptor with broadly similar affinity, since around 50% of total rosette formation corresponded to 75-μg/ml concentrations of either IgA1 or IgA2 used to sensitize the E.

The purified IgA1 and IgA2 were assayed by an ELISA technique for binding to human C1q, the first component of the classical complement pathway. Chimeric human anti-NIP IgG1 and IgM molecules were used as positive controls in the assay. In each case, the anti-NIP antibodies were

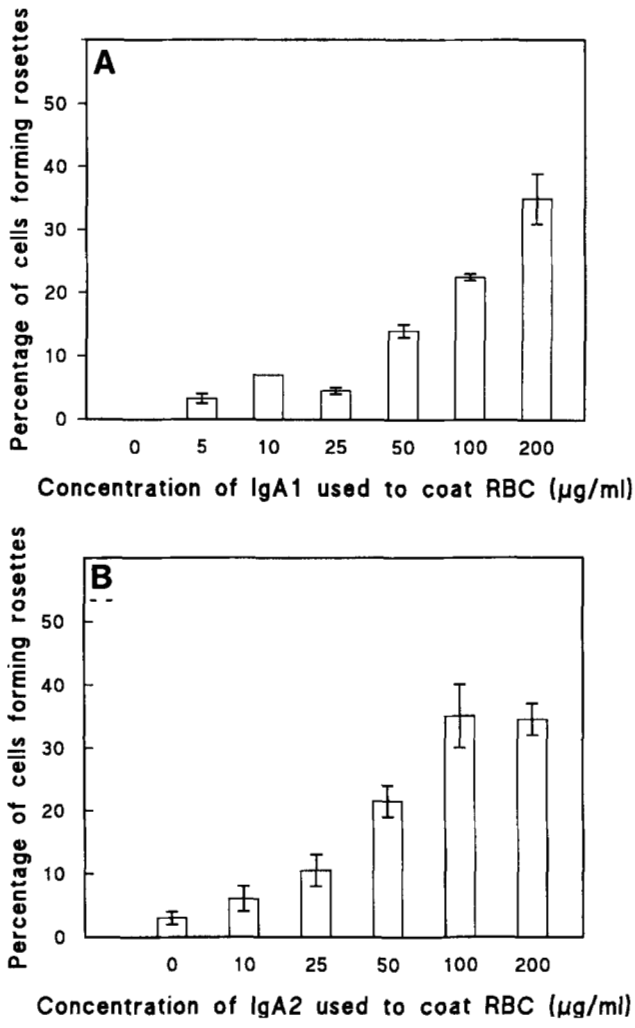

FIGURE 6. Binding of chimeric human IgA to Fcα receptors on calcitriol-stimulated HL-60 cells, assessed by rosette formation. *A*, IgA1; *B*, IgA2.

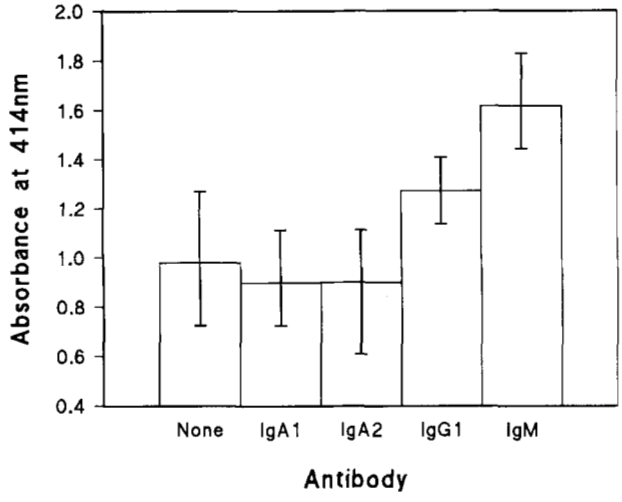

FIGURE 7. Binding of anti-NIP chimeric human antibodies to human C1q assessed by ELISA.

captured by NIP-BSA coated on to microtiter plates. Human serum was then added as a source of C1q and incubated for 1 h. After extensive washing, an anti-human C1q-peroxidase conjugate was added, which, on addition of substrate, served to reveal which of the antibodies bound human C1q. Figure 7 shows that human IgM bound C1q well. Under the conditions of the assay, human IgG1 showed, at best, only low levels of C1q binding, much less than those seen with IgM. Neither subclass of human IgA was found to show any interaction with human C1q.

Discussion

Many chimeric human IgG molecules have now been expressed and used to investigate the relative efficacy of the four subclasses in numerous biologic functions. Similarly, chimeric human IgE has also been expressed. However, to date, only one of the two human IgA subclasses had been expressed in this way (9). Hence, it has not been possible to make quantitative functional comparisons between monoclonal forms of the two human IgA subclasses. Here we describe, for the first time, the establishment of systems to overexpress chimeric human IgA1 and IgA2. We have confirmed that the antibodies made in CHO cells exhibit the mobilities in SDS-PAGE and reactivity with human IgA-specific antisera expected of antibodies possessing human Cα regions.

After affinity purification of the chimeric molecules, we were able to make direct comparisons between the properties of human IgA1 and IgA2. Although both subclasses appear to be secreted from the CHO cell transfectants as noncovalently associated dimers, this association does not appear to affect the function of the antibodies in any of the assays used in this study. By comparison, the majority of serum IgA exists in monomeric form with some J chain-containing IgA dimers also being present. In contrast, certain myeloma IgA proteins have been reported that associate as dimers lacking J chain (25).

It is noteworthy that mouse IgM, which also possesses a tailpiece, when secreted by nonmyeloid glioma cell or CHO-K1 transfectants, is also capable of assembling as polymers in the absence of J chain (26, 27). Similarly, in human macroglobulinemia, covalently associated hexameric IgM may be produced in the absence of J chain (28). Thus, in both myeloid and nonmyeloid expression systems that lack J chain, Ig molecules possessing tailpieces appear to associate into polymers. This polymerization may be somewhat uncontrolled, inasmuch as unusual polymer forms, e.g., hexameric IgM, tend to predominate. In the case of IgM, these polymers are covalently linked, whereas IgA molecules appear able to associate through either covalent or noncovalent interactions.

The nonmyeloid CHO-K1 host cell used in this study

appears to possess the enzymes necessary to mediate the disulfide bridge formation required in IgM polymer formation (27). In the case of IgA, however, we have found that the dimers formed are stabilized only by noncovalent interactions. This finding may suggest that disulfide bridge formation between monomers in IgA and IgM proceeds via somewhat different mechanisms, which may also serve to explain in part the preferred polymerization of IgA into dimers and IgM into pentamers or hexamers.

Since chimeric IgG molecules expressed in CHO-K1 cells are secreted solely as monomers (data not shown), it is tempting to speculate that the tendency of chimeric IgA molecules to associate noncovalently is due to their α-chain C-terminal tailpiece. Our preliminary studies with an IgA1 tailpiece-deletion mutant suggest that this may be a reasonable explanation. Furthermore, mutant mouse IgM molecules lacking the tailpiece are secreted as monomers from myeloid hybridomas or transfectants (29).

The loss of carbohydrate moieties from antibody molecules can result in important functional changes. For example, IgG molecules lacking N-linked oligosaccharides display a marked reduction in their ability to interact with Fcγ receptors (30, 31). Hence, before studying the interaction of the recombinant IgA antibodies with numerous effector and other molecules, we sought to verify that they were expressed in a glycosylated form from the CHO transfectants. Reactivity of both recombinant IgA subclasses with Con A revealed that their H chains possess N-linked oligosaccharides. More detailed analysis, of which preliminary data are reported elsewhere (32), has revealed as expected the presence of both N- and O-linked carbohydrate moieties in recombinant IgA1, and only N-linked sugars in IgA2. The expression system described here readily allows for the generation of antibodies with modified glycosylation profiles, permitting assessment of the effects of these changes on effector function and other biologic properties.

Antibodies of the human IgA1 subclass are susceptible to cleavage by a family of specific proteases secreted by a number of pathogenic bacteria of the genera *Neisseria*, *Haemophilus*, and *Streptococcus*. These bacteria are responsible for some of the major infectious diseases that gain entry to the body via the mucosal surfaces. For example, all three microorganisms associated with the cause of bacterial meningitis (*Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*) secrete IgA1 proteases. Certain important causes of genitourinary tract infection, e.g., *Neisseria gonorrhoeae*, and oral cavity infection, e.g., *S. sanguis*, also produce IgA1 proteases (33). The correlation between pathogenicity and IgA1-protease positivity is striking, particularly as other closely related species within these three genera neither cause disease nor secrete IgA1 proteases (34).

The protease enzymes display remarkable specificity, cleaving only IgA1 from humans, gorillas, and some apes. The sites of cleavage are the prolyl-seryl or prolyl-threonyl peptide bonds within the duplicated Pro-Ser-Thr-Pro-Pro-Thr-Pro-Ser sequence (residues 223 to 238) of the IgA1 hinge. Human IgA2 lacks this region and is thus not susceptible to attack.

Cleavage of IgA1 molecules within the hinge region generates Fab portions still capable of recognizing Ag molecules but devoid of Fc-dependent effector functions. The invading bacterium, on secretion of IgA1-protease, then becomes coated in specific Fab molecules, thus masking its antigenic surfaces from recognition by intact antibody molecules. As recruitment of effector molecules requires recognition of sites on the Fc region of the antibody, the Fab regions themselves are unable to mediate destruction of the bacterium by these means. Hence, the protease-secreting microoganism subverts the normal IgA-mediated defense mechanisms, and gains a foothold in the body (33, 35).

The protease secreted by *S. sanguis*, in common with other streptococcal IgA1 proteases, is reported to cleave IgA1 between residues Pro227 and Thr228 (36). The *S. oralis* enzyme is less well documented, but it is likely that it also attacks the same site. As expected, both enzyme preparations were able to cleave recombinant human IgA1 but not IgA2. In the future, genetic manipulation of the IgA1 hinge should allow a description of the broader regions around cleavage sites necessary for recognition by numerous proteases, and may generate protease-resistant IgA1 molecules with therapeutic possibilities.

Both subclasses of chimeric IgA antibodies produced here interact with Fcα receptors with similar apparent affinity. This is consistent with the findings of others studying the interaction of serum IgA with either cell surface or solubilized forms of the Fcα receptor (reviewed in Ref. 3). The affinity of the receptor for IgA has recently been estimated as about 5×10^7 M^{-1} (37). The antibody concentrations required to give half maximal rosette formation in our assays (around 75 μg/ml) are consistent with such a value.

The lack of reactivity of both chimeric human IgA1 and IgA2 with human C1q is in keeping with other reports in the literature. A recent study utilizing a panel of chimeric anti-NIP antibodies possessing different human constant regions found chimeric IgA2 not to bind C1q; chimeric IgA1, not available at that time, was not included in the study (7). In molecular terms, this inability to bind C1q may not be too surprising, inasmuch as neither human IgA1 nor IgA2 possesses the homologue of the three-residue motif (Glu218-Lys320-Lys322) critical for C1q binding to human IgG1 (38).

The expression system described here has numerous potential applications in the study of human IgA. Work underway aims to elucidate the structural requirements for the interaction of IgA with J chain, in the assembly of dimeric IgA. Ultimately, reconstitution of secretory IgA should also be possible, providing Ag-specific forms of this complex

IgA species. As the system permits manipulation of the IgA genes, studies using site-directed mutagenesis should aid localization of interaction sites for effector and other molecules, contributing to a molecular understanding of how this important Ig functions.

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