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Immunology

Recombinant human IgA expressed in insect cells

(baculovirus/glycosylation/immunoglobulin A receptor/J chain)

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ABSTRACT **IgA serves as the first line of humoral defense at all mucosal surfaces and is present in large quantities in serum. To map the sites of interaction of immune effector molecules with the IgA constant region (C_α), we have expressed soluble, chimeric human IgA in insect cells using recombinant baculoviruses. This antibody is correctly assembled into heavy chain/light chain heterodimers, N-glycosylated, and secreted by the insect cells; further, when coexpressed with a human J chain, the antibodies can assemble into dimers. The recombinant protein is authentic by a number of criteria, including antigen-binding, recognition by monoclonal antibodies, complement fixation via the alternative pathway, and specific binding to the monocyte IgA Fc receptor. We have also constructed viruses which encode structurally altered IgA heavy chains. Using one of these variant viruses, we have shown that glycosylation of the second domain of C_α is required for interaction with the monocyte IgA Fc receptor. This system should prove useful in further characterization of the structure–function relationships in human C_α.**

Immunoglobulins mediate humoral immunity by attaching to foreign antigens and then recruiting effectors—complement, granulocytes, cytotoxic T cells, etc.—to destroy and clear the offending entities. Due to the obvious immunologic and clinical relevance of immunoglobulin–effector interactions, antibody structure–function is the subject of intense and fruitful research (reviewed in ref. 1). IgA has several unique properties. (*i*) IgA can intracellularly associate with J chain via a cysteine in the C-terminal "tail" to form secreted dimers (2) which are then specifically recognized by the polymeric-Ig receptor (pIgR) pathway and transported across mucosal epithelia (3, 4). The IgA thus transported serves as the first line of humoral defense at mucosal surfaces (reviewed in ref. 5). Consequently, it is also the target of specific receptors and proteases produced by a number of pathogenic bacteria (6). Blood also contains a large quantity [average, 2 mg/ml (7)] of predominantly monomeric IgA; this circulating pool is largely independent of the mucosal pool in humans (8). (*ii*) IgA binds to an Fc_α receptor [monocyte/macrophage ($mFc_\alpha R$) (9–11)] on the surface of eosinophils (12), neutrophils (13), and monocytes/macrophages (9), triggering effector responses in so doing. (*iii*) B lymphocytes and T lymphocytes possess surface receptors for Fc_α through which immunoregulatory signals are thought to be transmitted (14). (*iv*) IgA is thought to activate complement through the alternative pathway (15). Thus, IgA not only plays a role in host defense against extracellular viruses and bacteria but is also potentially critical for neutralization of intracellular viruses in tissues expressing pIgR (16) and for destruction of helminths, protozoans, and other eukaryotic parasites (17). Several immunologic disease processes are mediated by IgA, including IgA glomerulonephritis (18) and possibly the exacerbation of allergic asthma (19, 20). Each of these properties of IgA depends on the ability of effector molecules such as complement component C3 and $mFc_\alpha R$ to recognize specific sites on the surface of the IgA heavy-chain constant region (C_α); however, none of these sites has been well defined.

To investigate the above-mentioned functions of IgA, we have established its production in insect cells utilizing recombinant baculoviruses (21). The baculovirus system allows production of mutant antibodies as well as combinatorial expression of immunoglobulin with other polypeptides (J chain, chaperonins, etc.) much more rapidly than with stably transfected mammalian lines. Here we describe production of immunologically and functionally authentic human IgA with hapten specificity and explore the utility of this expression system in addressing structure–function correlations in C_α.

MATERIALS AND METHODS

Synthesis of Ig Coding Regions. DNA fragments corresponding to the heavy- and light-chain variable (V) regions of monoclonal antibody (mAb) 93G7 were amplified by PCR from plasmids pHγ1-360E and pHκ-360E (22), respectively. The oligonucleotide primers for these amplifications included a 5′ *Nco* I site at the initiation codon and a 12-nt antisense overlap with C_α at the 3′ end. The coding regions of human $C_\alpha 1$ and C_κ were obtained from human peripheral-blood leukocyte RNA by reverse transcription–PCR (23); in this case, the primers included a 12-nt sense overlap with the appropriate V region at the 5′ end and an *Xba* I site at the 3′ end. The appropriate V and C regions were joined by PCR overlap extension (24).

Synthesis of J-Chain Coding Region. To construct an expression plasmid encoding human J chain, a cDNA library from a human B-cell line (25) was screened with a 1.6-kb *Xba* I fragment including exons 3 and 4 from the human genomic J-chain gene (26). A clone encoding the 137-aa mature protein predicted from the previously characterized genomic clone (26) as well as a 22-aa N-terminal signal peptide was obtained.

Construction of Baculovirus Transfer Vectors. The inserts were digested, purified, and ligated into the unique *Nco* I and *Xba* I sites in pH-360EX by standard techniques (27). Sequencing, oligonucleotide-directed mutagenesis, and plasmid isolation were as described (27).

Production of Recombinant Viruses. Transfer plasmid (4 μg) was cotransfected with linear wild-type baculoviral DNA into *Spodoptera frugiperda* cells (Sf9; ref. 28) by using cationic liposomes (Invitrogen) as instructed by the manufacturer, except that 0.25 μg of linear DNA was used. Growth, plaque purification, and titration of viruses were

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Abbreviations: Ars, *p*-azophenylarsonate; BSA, bovine serum albumin; mAb, monoclonal antibody; $mFc_\alpha R$, monocyte/macrophage Fc_α receptor; pIgR, polymeric-Ig receptor.

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standard (21). For protein production, infected cells were maintained in spinner flasks at a density of 2×10^6 per ml.

Isolation of Recombinant Proteins. Cell supernatants were adjusted to 20 mM Tris Cl, pH 7.5/10 mM EGTA/10 mM EDTA/1 mM phenylmethanesulfonyl fluoride and centrifuged at 90,000 × *g* for 40 min. Antibodies were isolated from the resulting supernatant by precipitation with ammonium sulfate at 40% saturation. This fraction was either used directly or further purified on *p*-azophenylarsonate (Ars)-agarose (22).

Protein Analysis. Pellets and supernatants were analyzed by SDS/PAGE (29). For Western immunoblotting, proteins were transferred onto 0.2-μm Immobilon membranes (Millipore) in 10% methanol/10 mM Caps·NaOH, pH 11. Membranes were blocked with 2% nonfat dry milk in TBST [20 mM Tris Cl pH 7.5/0.05% bovine serum albumin (BSA)/150 mM NaCl/0.05% Tween 20]. Proteins were detected by appropriate antisera conjugated to alkaline phosphatase, followed by nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (BRL).

Glycosylation Analysis. Purified proteins were blotted onto Immobilon as described above. Lectin reactivity was assessed with the glycan differentiation kit (Boehringer Mannheim). Alternatively, cells were treated with tunicamycin (5 μg/ml) following infection; resultant proteins were analyzed by Western blotting.

ELISA. IgA concentrations were measured by sandwich ELISA. Anti-IgA1 mAb (courtesy of Roy Jefferis, University of Birmingham Medical School) was coated onto wells (200 ng per well) of Maxisorp (Nunc) 96-well plates which were then blocked by incubation with 2% nonfat dry milk in TBST (200 μl per well) at 25°C for 2 hr. Recombinant protein sample or purified serum IgA1 standard was then diluted into 100 μl of TBST, applied to the coated wells, and detected with goat anti-human IgA1 coupled to alkaline phosphatase. Plates were developed with *p*-nitrophenyl phosphate (1 mg/ml in 50 mM Tris Cl, pH 9.5/100 mM NaCl/50 mM $MgCl_2$). Ars-specific antibody was detected by sandwich ELISA as above except that Ars-derivatized BSA was used as the capture reagent. mAb reactivity was determined by coating wells with goat anti-mouse IgG (300 ng), which was then used to capture the mAb, which in turn captured IgA from the sample; final detection was accomplished as above.

IgA1 Protease Treatment. Supernatants from *Haemophilus influenzae* strains (Rd^- and isogenic negative control courtesy of Andrew Plaut, Tufts New England Medical Center; DL42 courtesy of Leslie Cope and Eric Hansen, University of Texas Southwestern Medical Center) were used as a source of IgA1 protease (30). Cleaved Fc regions were analyzed by Western blotting.

Complement Fixation. The ability of antibodies to fix complement component C3 was assessed essentially as described (31) with the following alterations. Ars-BSA or Ars-gelatin was used to capture hapten-specific antibody and normal human serum was used as the source of complement. Deposited C3 was detected with sheep anti-human C3 (The Binding Site, San Diego) followed by goat anti-sheep IgG conjugated to alkaline phosphatase (Sigma).

Rosetting. HL-60 promyelocytic leukemia cells express $mFc_\alpha R$ following treatment with 0.5 μM calcitriol for 5–7 days (32, 33) and thus were used in these experiments. Sheep erythrocytes (Colorado Serum, Denver) were derivatized with Ars exactly as described (34) and then were washed five times in 100 volumes of Hanks' balanced salts solution (HBSS). The haptenated erythrocytes were then coated with sensitizing antibody or random serum Ig (in HBSS) for 12 hr at 4°C and washed further. Rosetting was assessed as in ref. 33. Induction of $mFc_\alpha R$ on HL-60 cells was checked by standard flow cytometry using the My43 (IgM) anti-$mFc_\alpha R$ mAb (33) and by immunoblotting using a distinct, IgG-class mAb (35).

RESULTS

Insect Cells Can Synthesize, Assemble, and Secrete Monomeric IgA Antibodies. Transfer vectors, one encoding a mouse/human chimeric IgA1 heavy chain and the other a mouse/human chimeric κ light chain, both with leader peptides, were used to create recombinant viruses which direct synthesis of the respective immunoglobulin chain in lieu of the baculoviral polyhedrin gene (21). Each chain consists of the V region belonging to the murine, anti-Ars mAb 93G7 (22) linked to the appropriate human C region; the C_α gene possesses 3′ sequences encoding a secreted immunoglobulin. Thus, an insect cell infected with both the $V_{Ars}C_\alpha 1$ and $V_{Ars}C_\kappa$ viruses would presumably export a human IgA1 antibody possessing the Ars specificity of the mouse mAb. Immunoblot analysis (Fig. 1) showed that Ig heavy (*Upper*) and light (*Lower*) chains were produced by Sf9 cells infected with the appropriate viruses; coinfected cells (lanes 5 and 6) expressed both chains. Sf9 cells coinfected with heavy- and light-chain-encoding viruses produced IgA of a size consistent with monomeric IgA (lanes 10 and 13); no multimeric IgA was observed. Sandwich ELISA using Ars-derivatized BSA as the capture agent was employed to determine the time course of antigen-specific IgA secretion (data not shown). By 72 hr, the concentration of Ars-specific human IgA in the medium reached a plateau at ≈0.75 μg per 10^6 cells; therefore, all subsequent cultures were harvested 3 days after infection. Structural authenticity of the recombinant protein was assessed by the criteria described below.

A human IgA1 heavy chain has seven carbohydrate moieties (36, 37). Five are O-linked disaccharides coupled to serine residues in the hinge region between C_H1 and C_H2, and two are complex oligosaccharides N-glycosidically linked to asparagine residues, 271 (in C_H2) and 483 (in the C-terminal

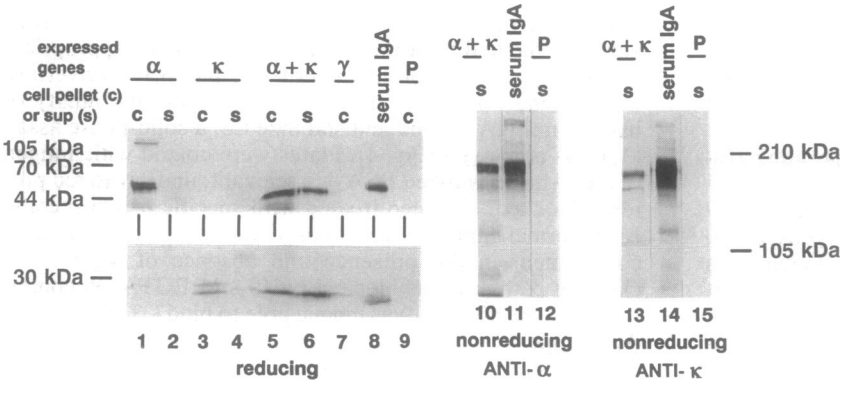

FIG. 1. IgA heavy and light chains encoded by cells infected with various recombinant viruses were assayed by SDS/PAGE followed by immunoblotting. Lysates (lanes 1, 3, 5, 7, and 9) or culture supernatants (sup) (lanes 2, 4, and 6) of cells infected with IgA1 heavy-chain virus alone (lanes 1 and 2), κ light-chain virus alone (lanes 3 and 4), heavy- *and* light-chain viruses (lanes 5 and 6), IgG heavy-chain virus alone (lane 7), or polyhedrin (P)-expressing virus alone (lane 9) were immunoblotted with anti-α (*Left*, upper rank) and anti-κ (*Right*, lower rank) antisera. Lane 8, human serum IgA. Samples were reduced and electrophoresed in SDS/10% polyacrylamide gels. Lanes 10, 11, and 12 and lanes 13, 14, and 15 show the same samples as lanes 6, 8, and 9, respectively, but unreduced and run in 6% polyacrylamide gels.

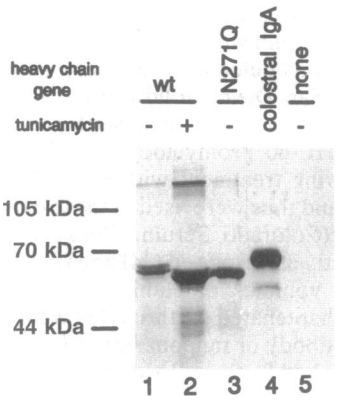

FIG. 2. Immunoblot analysis of IgA heavy chains in various states of glycosylation. Cell lysates from infected cells were harvested 3 days after infection with virus encoding wild-type IgA1 heavy chain and after incubation in the absence (lane 1) or presence (lane 2) of tunicamycin (5 μg/ml). Lysate N271Q (lane 3) is from cells grown without tunicamycin and infected with a virus encoding mutant IgA1 lacking one of two N-linked oligosaccharides. Purified colostral IgA1 was run as a positive control (lane 4), while lysate from polyhedrin-expressing, nonrecombinant virus was run as a negative control (lane 5). Samples were reduced before SDS/7.5% PAGE.

tail) [numbering according to Kabat *et al.* (38)]. To establish the glycosylation state of recombinant IgA1, immunoblot analysis was used to compare various lysates. IgA heavy chain expressed from $V_{Ars}C_{\alpha}1$ in cells not exposed to tunicamycin (Fig. 2, lane 1) migrated more slowly than that from the lysate of tunicamycin-treated (5 μg/ml) cells (lane 2), indicating that the heavy chain is indeed N-glycosylated. A heavy chain with glutamine at position 271 (termed N271Q) was created by site-directed mutagenesis, eliminating an N-glycosylation site while minimally altering the amino acid side-chain chemistry. The mutant heavy chain (Fig. 2, lane 3) displayed an apparent molecular mass lower than that of the wild-type molecule but greater than that of the completely unglycosylated heavy chain. Cumulatively these results are consistent with N-glycosylation of wild-type $V_{Ars}C_{\alpha}$ heavy chain at both sites, of the mutant at position 483 alone, and of tunicamycin-treated wild type at neither site. Cell lysates were used for this comparison because the tunicamycin-treated cells secreted IgA very poorly, consistent with previous reports (39, 40). To examine the type of glycosylation present on the recombinant antibodies, the reactivity of baculoviral IgA1 with various lectins was compared with that of serum IgA1 (data not shown). These results were consistent with the presence on the recombinant molecule of both O-linked Galβ(1-3)GalNAc disaccharides and N-linked high-mannose oligosaccharides. As expected (36), serum IgA possessed the O-linked disaccharides and complex, sialylated N-linked oligosaccharides.

The authenticity of the baculoviral IgA monomer was investigated further by examining its reactivity with several IgA1-specific mAbs (M4C11, M4D8, 2D7, N1F2; all courtesy of Roy Jefferis) as well as its susceptibility to cleavage by *H. influenzae* IgA1 protease. By these criteria, the recombinant molecule was indistinguishable from human serum IgA1 (data not shown).

Insect Cells Can Assemble IgA Dimers Incorporating Human J Chain. A baculoviral vector which encodes human J chain was assembled and used to express the J chain in Sf9 cells (Fig. 3*A*); presumably, this protein was N-glycosylated, since treatment of infected cells with tunicamycin altered its migration in an SDS/polyacrylamide gel (lane 5). Cells not expressing Ig heavy and light chains failed to secrete J chain (lane 6) except when treated with millimolar amounts of 2-mercaptoethanol (ref. 41 and data not shown); however, J

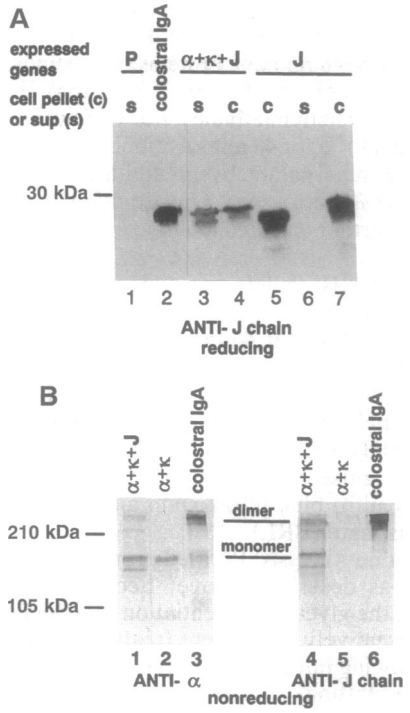

FIG. 3. Expression of human J chain in Sf9 cells. (*A*) Reducing SDS/10% PAGE and anti-J chain immunoblot analysis. Lane 1, negative control supernatant from nonrecombinant virus; lane 2, J chain from secretory IgA from human colostrum; lanes 3 and 4, culture supernatant and cell lysate, respectively, from cells triply infected with heavy-, light-, and J-chain viruses; lanes 6 and 7, supernatant and cell lysate, respectively, from cells infected with J-chain virus alone; lane 5 is a lysate from tunicamycin-treated cells expressing J-chain alone. (*B*) Nonreducing SDS/6% PAGE and anti-J chain immunoblot analysis. Lanes 1 and 4, supernatant from cells triply infected with heavy-, light-, and J-chain viruses; lanes 2 and 5, supernatant from cells infected with heavy- and light-chain viruses alone; lanes 3 and 6, secretory IgA from human colostrum. Lanes 1–3 were stained with anti-IgA antiserum; lanes 4–6 were stained with anti-J chain antiserum.

chain did appear in the medium when expressed with IgA (lane 3). Insect cells triply infected with viruses expressing Ig light chain, heavy chain, and J chain were able to assemble dimeric IgA (Fig. 3*B*, lanes 1 and 4); curiously, the majority of extracellular IgA appeared to be monomeric in association with J chain (lanes 1 and 4). No multimeric IgA appeared in the absence of J chain.

Functional Characterization of Baculoviral IgA1 Monomers. The utility of the recombinant antibody in future functional studies hinges on the integrity of the surfaces, as yet undefined, on which effector proteins interact with C_{α}. To address this issue, the ability of baculoviral IgA to interact with complement factor C3 and with $mFc_{\alpha}R$ was assessed.

The C3b(Bb) convertase is pivotal in the activation of the alternative pathway of complement (42). Strong evidence supports the ability of bound IgA to serve as an appropriate stabilizing surface for the alternative-pathway convertase (ref. 15 and references therein). To examine the ability of baculoviral IgA to bind and stabilize C3, a solid-phase assay (31) was employed (Fig. 4). Plates were coated with antigen (either Ars-derivatized BSA or irrelevant, underivatized protein), blocked, and then treated with specific or nonspecific IgA; normal human serum was then used as a source of complement in the presence and absence of Mg^{2+}. The classical pathway was blocked by 10 mM EGTA. Recombinant Ars-specific IgA was indeed able to bind C3 (Fig. 4, trial A) in a manner dependent on the amount of antibody. This binding depended on antigen specificity, since plates lacking

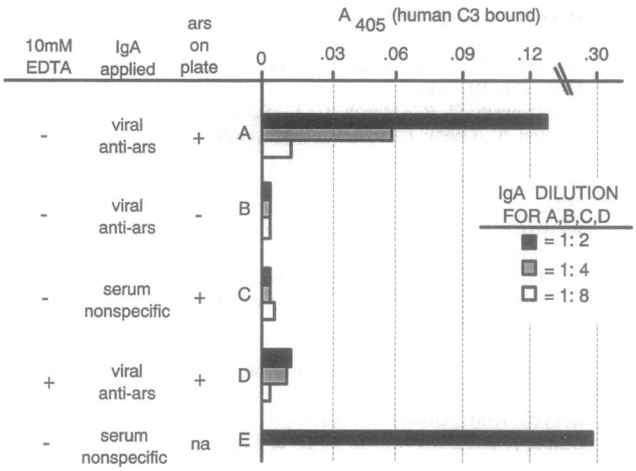

FIG. 4. C3 deposition on surfaces coated with baculovirally expressed IgA1. Normal human serum was used as the source of complement; sheep anti-human C3 was used as the detecting reagent. Trial A, Ars-coated plates incubated with supernatant from insect cells expressing IgA1 with Ars specificity; trial B, non-Ars-coated plates incubated with the same supernatant; trial C, Ars-coated plates incubated with supernatant from cells expressing polyhedrin supplemented with 500 ng of irrelevant human serum IgA1; trial D, same experiment as trial A, but following addition of 10 mM EDTA; trial E, positive control using 500 ng of serum IgA1 directly coated onto plastic before blocking.

Ars (trial B) failed to display C3 deposition, as did Ars-coated plates exposed to serum IgA1 of irrelevant specificity (trial C). C3 deposition was also dependent on Mg^{2+}, as shown in trial D which represents experiment A done in the presence of 10 mM EDTA. Trial E is a positive control: serum IgA1 was directly coated onto the plates, followed by blocking solution and then serum.

The second major effector pathway utilized by IgA involves recruitment of macrophages and granulocytes to antigens coated with antibody; this depends on the interaction of bound immunoglobulin with $Fc_\alpha R$ on the surface of the effector cells. To assess the ability of recombinant IgA1 to recognize the $mFc_\alpha R$, rosetting of Ars-derivatized IgA-coated erythrocytes by $mFc_\alpha R^+$ cells was examined. Calcitriol-induced HL-60 cells were used as a model for human monocytes. HL-60 is a promyelocytic cell line which upregulates $mFc_\alpha R$ and assumes a monocytoid morphology upon treatment with 0.1 μM calcitriol for 5–7 days (32, 33). Baculoviral anti-Ars IgA1 mediated rosetting between HL-60 cells and Ars-coated erythrocytes. The binding was specific since underivatized erythrocytes failed to rosette as did derivatized erythrocytes exposed to irrelevant IgA. Furthermore, the rosetting was inhibited by excess IgA (of either subclass) and anti-$mFc_\alpha R$ mAb My43 (33) but not by excess IgG. To probe the role of the $C_\alpha 2$ N-linked oligosaccharide in the interaction between Fc_α and $Fc_\alpha R$, we employed the N271Q mutant described previously. An amount (as assessed by ELISA) of Ars-specific mutant equal to that of the wild-type antibody used above was employed to coat Ars-derivatized erythrocytes; strikingly, IgA1 lacking the $C_\alpha 2$ sugar was completely unable to mediate rosetting. This result has interesting implications for the location of the receptor-binding site on C_α, as discussed below.

DISCUSSION

We have shown that insect cells can assemble and secrete high levels of immunologically authentic IgA1 monomer which binds both complement factor C3 and $mFc_\alpha R$. As with natural IgA1, these molecules possess O- and N-linked carbohydrate, although the insect cells do not process the N-linked sugars beyond the high-mannose stage. When the heavy and light chains are coexpressed with human J chain, a dimeric, J chain-containing species is produced, albeit inefficiently; no dimeric IgA appears in the absence of J chain. Furthermore, a mutant IgA1 which lacks the $C_\alpha 2$ oligosaccharide was used to begin a structure–function analysis of C_α; this partially unglycosylated molecule failed to mediate rosetting between coated erythrocytes and $mFc_\alpha R^+$ cells. This suggests that, as in IgG (43, 44) and IgM (45), N-linked glycosylation is important to maintaining proper conformation of effector binding sites. It also suggests that $mFc_\alpha R$ recognizes a region on Fc_α involving the "lower" part of $C_H 2$ and the "upper" part of $C_H 3$—analogous to the region which protein A recognizes on Fc_γ (ref. 46; Fig. 5). This contrasts with the paradigm established with IgG and IgE, whose respective FcRs recognize sites encompassing the lower hinge (or $C_\varepsilon 2$ domain in IgE) and the "upper" part of the penultimate Fc domain (Fig. 5). In the interaction between IgG and $Fc_\gamma RI$ and $Fc_\gamma RII$, the N-linked sugar of IgG is attached to the top of $C_H 2$, where it influences the conformation of the binding site on Fc_γ; unglycosylated IgG displays a much lower affinity for both receptors and fails to mediate rosetting between $Fc_\gamma R^+$ cells and coated erythrocytes (43, 44). However, in IgA1 the $C_H 2$ carbohydrate attaches to position 271, three residues "below" the classical intradomain cystine, and thus may influence a large loop at the "bottom" of $C_H 2$ which forms part of the region between $C_H 2$ and $C_H 3$ in three-dimensional space (L.C. and A. Edmundson, unpublished modeling data). The proposed site is attractive on evolutionary grounds as well. Because it is far from the C_α hinge, IgA is free to mutate in the hinge and upper $C_\alpha 2$ regions while maintaining its ability to bind receptor. This freedom is evidenced by the selection and maintenance of the hinge-deleted IgA2 isotype by virtue of its resistance to IgA proteases. In IgA2, not only is most of the hinge lost, but the remaining stump of the lower hinge differs in sequence and glycosylation from IgA1; furthermore, the hinge deletion certainly brings the Fab arms much closer to Fc [similarly to the Mcg paraprotein (47)] potentially restricting access to much of the upper $C_H 2$. Despite these differences, both IgA1 and IgA2 effectively compete for receptor binding

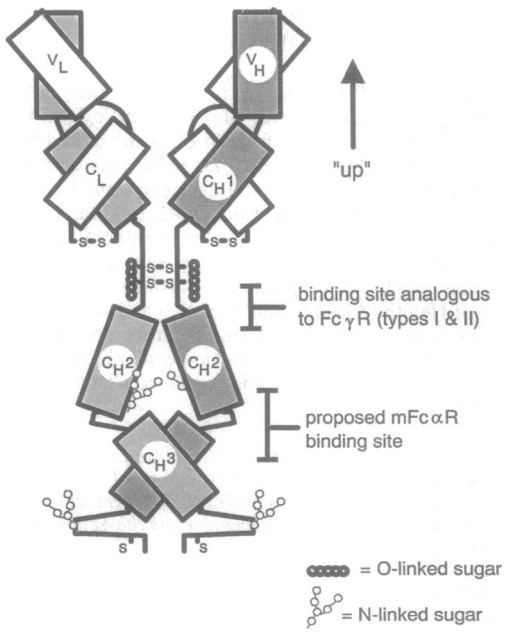

FIG. 5. Schematic of an IgA1 monomer including glycosylation. Each domain is represented by a rectangle. Some cysteines are shown (S). Arrow labeled "up" (as used in *Discussion*) is for orientation.

in the assay described above, demonstrating that the receptor site is insensitive to the intermolecular differences in the region around the lower hinge.

Recombinant expression systems play a vital role in our understanding of antibody structure–function relationships. Elegant studies using chimeric and/or mutated antibodies have helped define determinants of complement interaction, FcR recognition, and polymer assembly in the IgG (1), IgE (1), and IgM (45, 48) systems. Previous workers have expressed human IgA chimeric antibodies (15, 49) in mammalian transfectoma lines; however, we wished to establish an expression system for IgA and subject the product to more detailed characterization. The baculoviral system here described produces glycosylated human IgA1 in high yield. This system possesses the great advantage of rapid production of new recombinant proteins in amounts sufficient for the types of assays described in this paper. The time from initial transfection of the transfer vector to plaque-pure viral stock is ≈3 weeks, and many mutants can be processed in parallel. Additionally, once viral stocks are made, combinations of proteins (e.g., heavy chain, light chain, J chain, disulfide isomerase, and mutants thereof) can be coexpressed easily by coinfection with the various viruses. The last point is of particular importance to the study of pIgs such as dimeric IgA and pentameric IgM, whose assembly involves polypeptides other than immunoglobulin. Our results demonstrate the usefulness of the baculovirus–Sf9 expression system for Ig C-region functional experimentation. This system may also help provide insights into the roles of glycosylation and J chain in the folding and assembly of IgA. Our singly unglycosylated mutant IgA was secreted by the insect cells as efficiently as the wild-type molecule. The doubly unglycosylated IgA resulting from tunicamycin treatment, however, was secreted at low levels. Perhaps the second N-linked sugar at position 483 serves to solubilize or correctly position the C-terminal tail so that retention in the endoplasmic reticulum does not occur. Lastly, the cloning of human J chain cDNA and its assembly into dimeric IgA in this system should allow facile screening of mutants to determine which elements of J chain allow dimer assembly and which allow recognition by the pIgR.

Note. Morton *et al.* (50) described the production of human IgA1 and IgA2 in stably transfected CHO cells.

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