Identification of Residues in the CH2/CH3 Domain Interface of IgA Essential for Interaction with the Human Fcα Receptor (FcαR) CD89*

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Cellular receptors for IgA (FcαR) mediate important protective functions. An extensive panel of site-directed mutant IgAs was used to identify IgA residues critical for FcαR (CD89) binding and triggering. Although a tailpiece-deleted IgA1 was able to bind and trigger CD89, antibodies featuring CH3 domain exchanges between human IgA1 and IgG1 could not, indicating that both domains but not the tailpiece are required for FcαR recognition. To further investigate the role of the interdomain region, numerous IgAs, each with a point substitution in either of two interdomain loops (Leu-257—Gly-259 in Co2; Pro-440—Phe-443 in Co3), were generated. With only one exception (G259R), substitutions produced either ablation (L257R, P440A, A442R, F443R) or marked reduction (P440R) in CD89 binding and triggering. Further support for involvement of these interdomain loops was provided by interspecies comparisons of IgA. Thus a human IgA1 mutant, LA441–F443R (CD89R), was found, like mouse IgA, not to bind CD89. In contrast, bovine IgA1, identical to human IgA1 within these interdomain loops despite numerous differences elsewhere in the Fc region, did bind CD89. We have thus identified motifs in the interdomain region of IgA Fc critical for FcαR binding and triggering, significantly enhancing present understanding of the molecular basis of the IgA-FcαR interaction.

Human IgA is both a major serum immunoglobulin and the most abundant antibody class in seromucous secretions (1). The mucosal surfaces bathed by these secretions, such as those of the respiratory, gastrointestinal, and genitourinary tracts, are major potential sites of invasion due to their vast surface area. IgA therefore serves as a key first line of defense against many invading pathogens. Like all antibodies, IgA is capable of recognizing the foreign invader and triggering its elimination. The latter process is frequently mediated by the interaction of the Fc region of IgA with FcαR receptors (FcαR)

1 present on the surface of neutrophils, macrophages, monocytes, and eosinophils (2, 3). The human myeloid FcαR, CD89, possesses two extracellular Ig-like domains and displays homology to the three classes of human IgG Fc receptors (FcγRI, FcγRII and FcγRIII) and the high affinity IgE receptor FceRI, albeit at a lower level than these receptors do to each other (4). Interaction of CD89 with IgA, aggregated either by binding to antigen or artificially, acts as a potent trigger for an array of myeloid cell functions including phagocytosis, antibody-dependent cell-mediated cytotoxicity, superoxide generation, enzyme and inflammatory mediator release, and clearance of immune complexes (3). A detailed understanding of the molecular basis of the IgA-FcαR interaction is clearly important if the increasingly appreciated potential of recombinant IgA in numerous therapeutic applications (5–7) is to be fully realized.

Although others have described expression of human IgA in insect (8) and plant cells (5), we have expressed hapten-specific recombinant human IgA of both subclasses, IgA1 and IgA2, in mammalian cell hosts (9–11). Here, we have used an extensive panel of chimeric and site-directed mutant IgAs expressed in CHO K1 cells to identify residues critical for FcαR binding. We have constructed domain swap antibodies through exchange of the homologous C-terminal CH3 domains between human IgA1 and IgG1 in order to ascertain the contribution of each Fc domain to the interaction with FcαR. An IgA1 lacking the C-terminal 18 amino acid tailpiece has also been assayed for ability to bind the receptor. To allow more precise localization of the interaction site, a number of IgA1 mutants with single substitutions in loop regions lying at the interface of the CH2 and CH3 domains have been generated. The effects of such mutations on the ability to bind FcαR are consistent with the interdomain region of the Fc playing a critical role in binding to the receptor. Further support for this proposal is lent through correlation of the binding ability of IgAs derived from other species with sequence differences in these loops.

To more readily assess receptor interaction, we have developed stable CHO cell transfectants expressing high levels of CD89, which have allowed, for the first time, comparison of the relative binding affinities of the different IgA molecules. As an additional, more physiologically relevant test for function, we have also assessed the ability of the antibodies to elicit a respiratory burst in neutrophils. The combined use of these receptor systems with targeted mutagenesis of IgA has facilitated identification of motifs on human IgA critical for interaction with FcαR and subsequent triggering via the receptor, further dissecting the molecular basis of this important antibody-receptor interaction.

EXPERIMENTAL PROCEDURES

Generation of Human IgG/IgA Domain Swap and Mutant IgA1 Expression Vectors—Chimeric IgG/IgA vectors were constructed by

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1 The abbreviations used are: FcαR, Fco receptor; CHO, Chinese hamster ovary; PCR, polymerase chain reaction; NIP, 3-nitro-4-hydroxy-5-iodophenylacetate; PBST, phosphate-buffered saline containing 0.1% Tween 20; BSA, bovine serum albumin.
POS PCR overlap extension mutagenesis (12) using Pfu polymerase (Stratagene) and plasmid vectors containing wild-type human IgG1 γ chain and IgA1 α chain genes as templates, as described previously (10, 11). In each case, the mutated PCR products were ligated into unique restriction sites in the expression vectors, replacing the wild-type sequence in the appropriate region. To facilitate this approach, appropriate base pairs 3′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking primer (5′-AGGACTCTTACTCCCTCGAGCAG-3′) annealed 5′ of a unique BstEI site in the Cγ1 exon, whereas the 3′-flanking primer (5′-ATAACCGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~100 base pairs 3′ of the stop codon and incorporated a SacI site. For the domain swap experiments, a 5′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking primer (5′-AGGACTCTTACTCCCTCGAGCAG-3′) annealed 5′ of a unique BstEI site in the Cγ1 exon, whereas the 3′-flanking primer (5′-ATAACCGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~100 base pairs 3′ of the stop codon and incorporated a SacI site. For the domain swap experiment, a 5′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking primer (5′-AGGACTCTTACTCCCTCGAGCAG-3′) annealed 5′ of a unique BstEI site in the Cγ1 exon, whereas the 3′-flanking primer (5′-ATAACCGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~100 base pairs 3′ of the stop codon and incorporated a SacI site. For the domain swap experiment, a 5′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking primer (5′-AGGACTCTTACTCCCTCGAGCAG-3′) annealed 5′ of a unique BstEI site in the Cγ1 exon, whereas the 3′-flanking primer (5′-ATAACCGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~100 base pairs 3′ of the stop codon and incorporated a SacI site. For the domain swap experiment, a 5′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking primer (5′-AGGACTCTTACTCCCTCGAGCAG-3′) annealed 5′ of a unique BstEI site in the Cγ1 exon, whereas the 3′-flanking primer (5′-ATAACCGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~100 base pairs 3′ of the stop codon and incorporated a SacI site. For the domain swap experiment, a 5′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking primer (5′-AGGACTCTTACTCCCTCGAGCAG-3′) annealed 5′ of a unique BstEI site in the Cγ1 exon, whereas the 3′-flanking primer (5′-ATAACCGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~100 base pairs 3′ of the stop codon and incorporated a SacI site. For the domain swap experiment, a 5′-flanking primer, which anneals 5′ of a unique XhoI site in the α chain vector, has been described previously (10), whereas the 3′ flank of an appropriate vector as the template and the 5′-flanking primer (5′-ATACGGTGTCGACGCTTTATTTCCATGCTG-3′) annealed at ~240 base pairs downstream of the stop codon and incorporated an XhoI site. For the 5′ flank of the chain, the 5′-flanking prime }
BBA) to each well, the plate was transferred to a Microlumat LB96P luminometer, and the chemiluminescence was measured at regular intervals for 1 h.

RESULTS

Expression of IgA in CHO K1 Cells—DNA sequence analysis confirmed that the desired domain swaps/mutations had been correctly incorporated into each expression vector and that no misincorporations had occurred. After expression in CHO K1 cells and hapten affinity purification, the integrity and purity of each antibody was confirmed by SDS-polyacrylamide gel electrophoresis. For each antibody produced, reactivity with specific antibodies both in solution (in hapten binding enzyme-linked immunosorbent assays) and on nitrocellulose filters (on Western blots) confirmed that introduction of mutations had affected neither antigen binding ability nor epitope recognition, suggesting that no gross conformational changes had been introduced. Fig. 1 shows Western blotting results with wild-type IgA1 (lanes 1, 4, and 7), domain swap γ1γ2α3 (lanes 2, 5, and 8), and domain swap α1α2γ3 (lanes 3, 6, and 9) run under reducing conditions and probed with anti-IgA Fc (lanes 1–3), anti-IgG γ chain (lanes 4–6), and anti-Cγ3 domain (lanes 7–9).

Functional Analysis of Domain Swap and Tailpiece-deleted Antibodies—The ability of the domain swap antibodies, γ1γ2α3 and α1α2γ3, and the tailpiece-deleted mutant PTerm455 to interact with CD89 was assessed by rosetting both with neutrophils and with CD89 transfectants (Fig. 2). Wild-type IgA1 and wild-type IgG1 mediated rosette formation with neutrophils presumably via the constitutively expressed FcαR and FcγRII/FcγRIII, respectively, since no rosettes formed in the absence of coating antibody. However, the two domain swap antibodies, each coated on erythrocytes at comparable levels with wild-type antibody, were unable to mediate rosette formation. The tailpiece-deleted mutant, in contrast, appeared similar to or slightly more effective than wild-type IgA1 at rosette formation when coated at comparable levels. Similar results were obtained using the CD89 transfectants, with the exception that wild-type IgG1 did not bind these cells, since they express only FcγR and not FcαR.

To investigate the relationship between FcαR recognition and triggering of FcαR-mediated cellular responses, we studied the capacity of the antibodies to trigger a neutrophil respiratory burst. The oxidative burst is a biologic response of major importance in both host defense and in inflammatory diseases. The ability of the antibodies to trigger neutrophil respiratory burst by FcαR was found to correlate very well with their capacity to trigger a respiratory burst in neutrophils when presented in an aggregated form on a hapten-coated surface (Fig. 3). Hence, although wild-type IgA1 and PTerm455 both elicited strong respiratory bursts, as measured by luminol chemiluminescence, the two domain swap antibodies did not produce significant bursts. Furthermore, we have shown earlier that the mutant N263A, which lacks the N-linked sugar moieties normally attached to the Cα2 domain, binds CD89 with affinity comparable with that of wild-type IgA1 (16). Here, our results show that N263A triggers a burst comparable with that of wild-type IgA1 (Fig. 3), reaffirming the correlation between binding to CD89 and ability to trigger the neutrophil respiratory burst.

Our results suggest that the presence of regions in both the Cα2 and Cα3 domains of IgA is required for efficient interaction with CD89 and for triggering via this receptor. Our earlier results showed that both N263A and IgA1 mutant C311S, in which Cys-311 lying on the surface of Cα2 is changed to Ser, bound to CD89 with affinity comparable with wild-type IgA1 (11, 16). Thus, it appears that neither residue 263 and the carbohydrate attached to it nor residue 311 are directly involved in interaction with CD89. It is likely, therefore, that regions of the Cα2 domain other than those proximal to these residues form the receptor interaction site. Furthermore, the presence of the tailpiece is not essential for FcαR recognition or triggering, indicating that part of the Cα3 globular domain itself must be required for efficient FcαR binding.

Functional Analysis of Panel of IgA1 Mutants—Since a clear requirement for both domains of IgA Fe in FcαR binding had been established, we chose to determine if the interdomain region might be directly involved in interaction with the receptor. We generated a panel of IgA1 antibodies, each with a single amino acid substitution in either of two interdomain loops,
transfectants, with half-maximal rosette formation with CD89 P440R, however, had a reduced ability to form rosettes with the P440A unable to form rosettes. Hence, residue 440 also appears approximately 5-fold. Interestingly, conversion of the same residues in the two interdomain loops of interest, was found not to mediate rosetting. Indeed, it served as a mimic of the Arg seen in human IgG3 m15 and m21 allotypes at position 435 in the protein A site (Fig. 4). These allotypes do not bind protein A, whereas the IgG3 m15,16 allotype with His at this position does bind protein A. Model building (21) has indicated that the lengthy side chain of arginine prevents favorable IgG-protein A contact formation, thereby resulting in the inability of such IgG3 molecules to bind protein A.

We also made amino acid alignments of IgAs from different species and discovered that mouse IgA, previously found not to bind human FcR (2), differed from human IgA1 at only two residues in the two loops of interest (Fig. 4). Therefore we generated a human IgA1 mutant, termed LA441–442MN, which through replacement of Leu-Ala with Met-Asn at positions 441–442, mimicked mouse IgA in these two loops. In addition, we expressed bovine IgA1, since this antibody is identical to human IgA1 within these two interdomain loops (Fig. 4), despite numerous differences elsewhere in the Fc region.

When this panel of antibodies was assessed for ability to form rosettes via CD89 on either neutrophils or CD89 transfectants, we found that the majority of point substitutions in the two loops resulted in loss of ability to bind the receptor on either neutrophils or the CD89 transfectants (Table I), suggesting that these two loops do contribute to FcR binding. Only two of the IgA1 point mutants remained capable of mediating rosette formation, namely G259R and P440R. G259R exhibited an ability to form rosettes similar to that of wild-type IgA1, forming comparable numbers of rosettes with either neutrophils or CD89 transfectants. Hence, conversion of the sequence within these two interdomain loops was found not to mediate rosette formation with either neutrophils or the CD89 transfectants. Hence, conversion of the sequence within these regions to those of mouse IgA conferred binding characteristics similar to those of the murine antibody, again supporting the notion that the conformation of these two interdomain loops is critical for FcR binding.

Erythrocytes coated with bovine IgA were able to form rosettes with both neutrophils and the CD89 transfectants (Table I). Half maximal rosette formation was achieved at coating concentrations of around 250 μg/ml (Fig. 5), consistent with an affinity approximately 2.5-fold less than that of wild-type human IgA1. Despite numerous differences elsewhere in the Fc region, bovine IgA resembles human IgA1 in the two loops of interest, and its ability to bind FcR with relatively high affinity is consistent with a role for these interdomain loops either directly or indirectly in FcR binding.

In keeping with results for the domain swap antibodies above, we observed a strong correlation between the ability of the IgA1 mutant antibodies to mediate rosette formation via FcR and their capacity to trigger a respiratory burst in neutrophils. Hence, only P440R and G259R elicited respiratory bursts, with L257R, P440A, A442R, F443R, and LA441–442MN failing to trigger the neutrophils (Fig. 6). The burst generated by G259R mirrored that of wild-type IgA1, consistent with a role for these interdomain loops either directly or indirectly in FcR binding.

*(2) M. A. Kerr and W. W. Stewart, personal communication.*
ent with broadly comparable affinities for the receptor. Bovine IgA elicited a strong burst, whereas P440R triggered a smaller burst, consistent with the respective intermediate and lower affinities for receptor suggested by their rosetting profiles.

**Insertion of Potential Binding Motifs into Domain Swap Antibody a1o2γ3—**Our results with IgAs having point mutations within the two interdomain loops comprising Leu-257—Gly-259 and Pro-440—Phe-443 strongly implied a role for these regions in FcαR binding. We therefore sought to determine whether it was possible through introduction of whole loop elements to convert a nonbinding molecule into an antibody capable of binding the receptor. Hence, we made use of our domain swap antibody a1o2γ3 as a template, replacing the existing His-433—Asn-434—His-435—Tyr-436 loop in the γ3 domain with the analogous Pro-Leu-Ala-Phe loop from IgA1 to create mutant a1o2γ3PLAF. Thus we generated an antibody possessing the two IgA1 motifs of interest, with one of the loops contributed by the appropriate IgA domain, and the other introduced into the analogous position in an IgG domain.

When assayed for ability to bind FcαR, a1o2γ3PLAF mutant was found not to mediate rosette formation with either neutrophils or CD89 transfectants (Table I), nor could it elicit a neutrophil respiratory burst (Fig. 6). Attempts to similarly introduce the Leu-257—Gly-259 loop into the γ1γ2o3 domain swap antibody also yielded an antibody (γ1γ2o3LLLG) unable to specifically bind to FcαR (data not shown). These results indicate that it is not feasible to readily convert a previously nonbinding antibody to one displaying appreciable affinity for FcαR through introduction of key residues into these interdomain loops. However, the earlier ablation of binding in point mutants lends strong support for the notion that loops Leu-257—Gly-259 and Pro-440—Phe-443 play a critical role in the binding of IgA1 to CD89.

**Fig. 6. Stimulation of neutrophil respiratory bursts by IgA antibodies attached to NIP-BSA-coated microtiter plates.** Chemiluminescence (CL, arbitrary units) was induced in A by wild-type IgA1 (●), bovine IgA (●), P440R (■), LA441—442MN (○), P440A (▲), no antibody (negative control) (X); in B by wild-type IgA1 (●), G259R (○), A442R (▲), L257R (■), F443R (▲), a1o2γ3PLAF (△), no antibody (negative control) (●). Each point shown is the mean of triplicate determinations. Each experiment was performed three times with neutrophils from different donors. The results shown are those from a typical experiment.

**DISCUSSION**

There is now a growing appreciation of the importance of IgA in immune protection, and attention has turned to the effector functions mediated by this abundant antibody. As with IgG and IgE, there is much interest in the relationship between the structure of this immunoglobulin and its function, particularly in terms of FcαR interaction.

We have shown earlier, using a CHO K1 system, that loss of N-linked sugar moieties from the CH2 domain of IgA1 (through mutagenesis of the Asn-263 attachment site to Ala) did not perturb binding to CD89 (16). This finding was in contrast to an earlier report where the mutation of Asn-263 to Glu in IgA expressed in insect cells produced an antibody incapable of binding FcαR (8). The different expression systems may have contributed to these functional differences, perhaps partly because insect cells tend to attach larger and differently processed oligosaccharides than those of mammalian cells, possibly affecting the suitability of the insect cell-derived wild-type antibody for comparative purposes. The different natures of the substituting residues ( Ala and Glu) may also have contributed in part to the contrasting results. As an example of such an effect, substitution with either Arg or Ala at residue 440 in this study was found to produce differing levels of reduction in FcαR binding activity. Regardless of the reason behind them, the conflicting results underline the importance of using a range of different approaches in order to fully elucidate the molecular basis of an interaction such as that between IgA and FcαR.

Here we sought, through a program of site-directed mutagenesis and sequence comparisons, to investigate the site on IgA responsible for binding to FcαR. Earlier work using insect cell-derived IgA had identified two residues at the CH2/CH3 domain interface critical for FcαR binding (22). For the reasons outlined above, our aim was to conduct an investigation using CHO cell-derived IgA to provide a detailed evaluation of the structural requirements for efficient FcαR binding so that a more definitive picture of the recognition process could emerge. Our experiments involved both IgA domain swap and point mutations. Since immunoglobulin molecules are composed of globular domains joined by relatively flexible linker regions, each domain in a domain swap antibody is likely to adopt its native conformation, allowing its contribution to function to be determined. Regarding the introduction of single or adjacent double amino acid substitutions, although these are generally well tolerated in terms of overall structure (23), the possibility of some degree of structural perturbation of the protein cannot be ruled out. However, the point mutations introduced in this study involved residues predicted to lie on loop regions of the immunoglobulin, and sequence comparison between members of the Ig superfamily indicates that the loops of family members are quite variable without giving rise to changes in the overall protein fold. Thus mutagenesis of these loop residues in IgA most probably does not disrupt native protein structure. Furthermore, all the antibody mutants generated still retained reactivity with antigen and were recognized by specific antibody reagents. The mutations are therefore unlikely to have produced any gross structural aberrations, allowing us to draw conclusions on the relative contributions of the mutated residues to FcαR binding.

The CD89 transfectant cell line, with its high levels of receptor expression, enabled us to more accurately determine the relative affinity of the various antibodies for the receptor. This approach had the advantage that we were able to probe the relative contributions of the residues in the two loops to the interaction. We found single point mutation of residues Leu-257, Pro-440, Ala-442, and Phe-443 to arginine and joint mutation of Leu-441 and Ala-442 to Met and Asn, respectively,
resulted in loss or reduction of FcαR binding activity. These findings are consistent with an earlier report in which point mutations of Leu-258 and Leu-441 to Arg obviated receptor binding, although no indication as to the scale of change in affinity was given (22). Our results therefore confirm and extend this earlier study (22) in showing that residues in these two regions clearly play important roles in the binding of FcαR to IgA. Since a change of Gly to Arg was tolerated at position 259 without apparent reduction in receptor affinity, it seems that this residue is less critical for interaction with the receptor than others in the loops. Residue Pro-440 may also play a more minor role in the interaction since substitution to Arg only reduced affinity partially, although conversion to Ala resulted in loss of receptor binding activity. Since Ala and Pro have relatively similar sizes and hydrophobicities, Ala might be expected to be a better substitute than Arg if Pro-440 was directly involved in contact with the receptor. The finding that conversion to Ala had a more marked effect than that to Arg may therefore argue against a direct role for this residue in receptor interaction.

A molecular model for human IgA1 based on small angle x-ray and neutron scattering data has recently been generated (20). Fig. 7 shows a view of the Fc region of the model in which the loop residues in the proposed FcαR binding site are highlighted. The model confirms that the two loops implicated in binding, although on different domains, lie close in three-dimensional space, and it is feasible that an FcαR domain (or domains) could readily interact with both loops (or close-lying residues) simultaneously. Interestingly, residue Gly-259 is seen to lie on the outer edge of the interdomain “pocket,” perhaps explaining why substitution with the bulky arginine side chain at this residue does not affect FcαR docking.

There are essentially two possible explanations for the observed effects of the mutations to loops 257–259 and 440–443. The mutations may either elicit their effects on binding affinity through removal of direct binding interactions, or they may trigger alterations in the conformation of close-lying residues, which are providing the binding contacts. The lack of effect of mutation of Gly-259, which lies immediately adjacent to other residues tested, may argue to some extent against the latter possibility, but in either case, the loops serve as important markers of the FcαR binding site.

A recent report of direct relevance to this question identifies residues in the first domain of human FcαR essential for interaction with IgA (24). Residues His-85 and Arg-82, which lie in a loop predicted to occupy the apical tip of the domain, were found to play a critical role in binding to IgA, whereas Arg-87 makes a minor contribution to the interaction. The basic side chains of these receptor residues might be predicted to interact with acidic residues on the ligand. A search for such negatively charged amino acids in the vicinity of the two loops studied here reveals Glu-254, Asp-255, and Glu-261 flanking the Leu-257—Gly-259 loop and Glu-437 lying close to the Pro-440—Phe-443 loop (Fig. 4). On the molecular model (Fig. 7), all these residues appear to lie in quite close proximity immediately adjacent to loops 257–259 and 440–443, possibly forming a negatively charged surface to which the receptor might dock. One explanation for the effects on FcαR binding of mutation to loops 257–259 and 440–443 could thus be that indirect conformational effects on close-lying acidic residues perturbs charge-matching interactions with basic residues on FcαR. However, even if the aforementioned loops do not participate directly in contacts with the receptor, they appear to play a critical role in conformational maintenance of the receptor binding site.

Our results, in agreement with an earlier study (22), indicate that the presence of the IgA1 tailpiece is not important for binding CD89. The molecular model above (20) is consistent with the tailpiece adopting a rather open conformation lying over the surface of the Fc, such that the penultimate Cys of the tailpiece could reach the exposed Cys-311 lying on the surface of the CH2 domain. If this arrangement is the case, it does not appear to influence the conformation of the region binding CD89 to any significant degree. Interestingly, mutation of Cys-311 to Ser does not obviate CD89 binding (11), suggesting that if tailpiece bridging to Cys-311 does occur, it is not important for CD89 binding.

The inability of the two mutant domain swap antibodies α1α2γ3PLAF and γ1γ2α3LLG, each with an engrafted interdomain loop, to interact with the receptor gives a clear indication that the relative orientation in three-dimensional space of the two binding site loops (or regions affected by their conformations), one to the other, is absolutely critical for efficient receptor binding. Thus the engrafted loops held in the context of a IgG domain apparently did not adopt identical conformations to those they assume in the context of the homologous IgA1 domain, despite in the case of α1α2γ3PLAF, identical flanking residues in Cα3 and Cγ3 either side of the loop (Fig. 4). This inability to successfully transfer binding motifs between IgA1 and IgG domains probably reflects the differing arrangements of the CH2 domains relative to each other in the two antibody classes, evident from the molecular model of IgA1 (20), which could well translate to give differences at the Fc interdomain surface. Alternatively, or in addition, these results may suggest that there are important FcαR contacts besides those provided by these two engrafted loops.

The FcαR binding site at the Fc interdomain region is strikingly dissimilar to the site localization for the homologous FcγR receptors on IgG. Human FcγRI and FcγRII interact with distinct but overlapping sites on human IgG lying in the lower hinge region (25–27). The molecular model of human IgA1 suggests that the average conformation of the antibody is probably T-shaped, unlike the average Y-shape of IgG (20). The extended reach of IgA1 afforded by this arrangement may facilitate simultaneous binding to distally placed repetitive antigen molecules and may have evolved for improved recognition of mucosal pathogens. However, the T-shaped arrangement may reduce the accessibility of the N terminus of the Fc,
and thus, evolution of a FceRI recognition site at the interdomain region of the Fc may have been favored. An additional or alternative evolutionary pressure arising from the susceptibility of the IgA1 hinge region to cleavage by specific proteases secreted by a number of important pathogenic bacteria has also been suggested as a selective force favoring unique IgA-FceRI interaction sites (24).

Although FceRI and FcyRI may not recognize homologous sites on their ligands, there may be some degree of similarity between the docking of IgA to FceRI and IgE to the high affinity FcRI. The binding site for FceRI is known to lie in the Cε3 domain of human IgE (28, 29). A loop region at the Cε3/Cε4 domain interface, encompassing the region analogous to the Leu-257—Gly-259 loop on IgA, appears important for binding to FcRII (30, 31), whereas an N-terminal Cε3 region analogous to the FcyRII site on IgG is also essential for maintenance of the IgE-Fc conformation necessary for efficient FcRII ligation and subsequent signaling (31, 32).

There is increasing interest in the potential of recombinant IgA for numerous clinical applications. For the first time, we have demonstrated that a major physiological effector function of IgA, namely FcA, have demonstrated that a major physiological effector function of the IgA1 hinge region to cleavage by specific proteases secreted by a number of important pathogenic bacteria has also been suggested as a selective force favoring unique IgA-FcA interaction sites (24).

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