A Human Immunoglobulin (Ig)A Cα3 Domain Motif Directs Polymeric Ig Receptor-mediated Secretion

By J. Mark Hexham, Kendra D. White, Leonidas N. Carayannopoulos, W. Iodeck M. andekci, Renee Brisette, Yih-Sheng Yang, and J. Donald Capra

From the Molecular Immunogenetics Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104

Summary

Polymeric immunoglobulins provide immunological protection at mucosal surfaces to which they are specifically transported by the polymeric immunoglobulin receptor (pIgR). Using a panel of human IgA1/IgG1 constant region “domain swap” mutants, the binding site for the pIgR on dimeric IgA (dIgA) was localized to the Cα3 domain. Selection of random peptides for pIgR binding and comparison with the IgA sequence suggested amino acids 402–410 (QEPSQGTTT), in a predicted exposed loop of the Cα3 domain, as a potential binding site. Alanine substitution of two groups of amino acids in this area abrogated the binding of dIgA to pIgR, whereas adjacent substitutions in a β-strand immediately NH2-terminal to this loop had no effect. All pIgR binding IgA sequences contain a conserved three amino acid insertion, not present in IgG, at this position. These data localize the pIgR binding site on dimeric human IgA to this loop structure in the Cα3 domain, which directs mucosal secretion of polymeric antibodies. We propose that it may be possible to use a pIgR binding motif to deliver antigen-specific dIgA and small-molecule drugs to mucosal epithelia for therapy.

Key words: immunoglobulin A • secretory immunoglobulin A • polymeric immunoglobulin receptor • J chain • mucosal immunity

Materials and Methods

Baculovirus Expression. Arsonate hapten-specific chimeric IgA1 and IgA1/IgG1 domain swap mutants were expressed as previously described (16, 17). Dimeric IgA was generated by coexpression of IgA with J chain. Affinity purification was carried out on arsonate-sepharose. Antibodies were eluted with 200 mM arsonillic acid (Sigma Chemical Co.) in 200 mM Tris-HCl, pH 8.0, which was removed by extensive dialysis against PBS. Mono- and dimeric IgA were detected by SDS-PAGE analysis. The hexahistidine-tagged human pIgR extracellular domain was expressed in a similar manner and purified on a Ni-NTA Agarose (Qiagen) column (18).

Construction of Mutant IgA Antibodies for Baculovirus Expression. The Cα3 loop mutants L1, L2, and L3 were constructed by PCR SOEing (splicing by overlap extension; reference 19) using the following complementary pairs of sense (S) and antisense (AS) primers: L1S 5′-GAGCCCAGCGCGGGCGCCGCCCTTC-GCTGTG-3′, L1AS 5′-CTCGGGTCGCGCCCGGCGGC-GGAAGCGACAC-3′; L2S 5′-TACCTGACTGCGGCAGCGCGCAGGAGCCC-3′, L2AS 5′-ATGGACTGACGCCGTCGGCGCGTCCTCGGG-3′; and L3S 5′-CGGCAGGGACCCGCGCCGCGCCGCGCCGGCCGCGCGCCGGGGC-3′, L3AS 5′-GCCGTCCTCGGGG-3′. The outer primers B1-2 (5′-CCTATAACCATGGAGCTACCC-3′), spe-
specific for the 5' leader of the VH region of this chimeric IgA heavy chain, and Cα3-3' (5'-CCCTCTAGATTTAGAACGAGTGC- CGTCCAC-3'), specific for the 3' tailpiece-encoding sequence of the IgA1 gene, were used with the above primer pairs L1-L3 to generate pairs of 5' and 3' fragments with complementary overlaps. These fragments were gel purified then spliced in a further PCR reaction using the outer primers B1-2 and Cα3-3'. Modified IgA1 genes were cloned into the baculovirus transfer vector using XbaI and NcoI digestion and the insert sequences were verified. Recombinant baculovirus was produced using the BacPAK system (Clontech).

FACS® Analysis. Min-ad-Derby canine kidney (MDCK) cells were plated in serum-free MEM plus Earle's salts (Mediatech, Inc.) 16 h before the experiment. Cells were harvested in 10 mM EDTA in PBS and washed in PBS 0.1% BSA. plgR binding of human IgA1 antibodies and mutants was assessed by incubation of 100 µl antibody in PBS/BSA with ~10^3 cells for 1 h. Cells were washed three times in PBS/BSA and bound antibody was detected with 100 µl of an anti-human κ FITC conjugate (Sigma Chemical Co.) diluted 1:100. Cells were washed as above and resuspended in 1 ml PBS/BSA. FACS® analysis was carried out on a Becton Dickinson FACScan® instrument. Data collection and analysis were performed with the LSYSII (Becton Dickinson) and WINMDI (http://facs.stanford.edu) or with the Cellquest programs (Becton Dickinson).

Phage Display Peptide Library Selection. The random 40-mer peptide library was constructed in the pCANTAB5e vector and has an insert total diversity of 1.55 × 10^10 (20). The random 40-mer is flanked by two peptide tag sequences, preceded by a leader peptide and fused to the membrane-proximal domain of the M13 phage coat protein III. 1–2 × 10^6 MDCCK cells were harvested in 5 ml PBS plus 10 mM EDTA at 37°C, washed twice in 15 ml PBS, and resuspended in 1.8 ml PBS at 4°C. 100 µl phagemid library stock (4.5 × 10^12 CFU) was added and incubated for 1 h at 37 or 4°C. The cells were then washed five times with 15 ml PBS at 4°C. Bound phage were eluted with 2 ml of 0.1 M glycine/HCl, pH 2.2, containing 0.1% BSA for 10 min and neutralized immediately with 400 µl of 2 M Tris base. Phage rescue and amplification were carried out in Esherichia coli strain TG1 (Pharmacia) according to standard procedures (21).

DNA Sequencing and Analysis. DNA sequencing was carried out on double-stranded plasmid or phagemid DNA using an ABI 377 Prism (Applied Biosystems, Inc.) automated sequencer. Alignments of deduced peptide sequences and Ig-constant regions were carried out using the MAP (22) and PIMA (23) software.

Results and Discussion

Chimeric human IgA1 (16) and a panel of IgA1/IgG1 constant region domain swap mutants (24) with murine-encoded arsotne specificiy were expressed in baculovirus as both monomer and dimer, affinity purified, and used to define the plgR binding site. dIgA was operationally defined as an IgA preparation generated by coexpression of IgA with J chain. MDCCK cells, transfected with rabbit plgR (25), were used to measure binding of recombinant IgA1 mutants to the receptor by FACS® analysis (Fig. 1a). Specific binding was observed with dIgA and not with monomeric IgA (Fig. 1b), a medium control (Fig. 1b) or IgG (data not shown). Mutant VGAA, in which the Cα1 domain was substituted with the Cγ1 domain, bound to the plgR in a manner similar to wild-type IgA1 (Fig. 1c). The dimeric molecule (Fig. 1c, heavy line) bound to the receptor, whereas the monomer (light line) did not. Similarly, the VGGA mutant, in which both Cα1 and Cα2 including the hinge of IgA were replaced with the analogous domains from IgG, bound as a dimer but not as a monomer (Fig. 1d). Thus, the Cα1 and Cα2 domains of dIgA are not necessary for plgR binding, suggesting that the presence of the Cγ3 domain is required.

dIgA contains four Cα3 domains and the covalently bound J chain which, together with the IgA tailpiece, are responsible for IgA polymerization. To reduce the complexity of this problem, a library of random 40-mer peptides, expressed as a phage display library (20), was selected against plgR-expressing MDCCK cells. The goal was to identify putative plgR binding sites within IgA by reducing them to a minimum peptide binding unit, a proven approach for several receptor–ligand interactions (26–28). Selection was carried out on live plgR-expressing MDCCK cells in suspension with negative selection on nonreceptor-expressing cells. Bound phage were eluted with acid or by cell lysis. Recovery of both acid-eluted and cell-associated phage increased gradually from ~6 × 10^4 to 5 × 10^5 CFU over 4–6 successive rounds, indicating enrichment for specific binding clones. Individual clones were randomly selected from the final panning from the acid-eluted and cell-associated phage and further tested for their ability to bind to IgA1 of the IgA1/IgG1 domain swap panel. Twenty clones were selected for further sequencing. Sequencing of the phage increased gradually from ~6 × 10^4 to 5 × 10^5 CFU over 4–6 successive rounds, indicating enrichment for specific binding clones. Individual clones were randomly selected from the final panning from the acid-eluted and cell-associated phage.
membrane-associated fractions and sequenced. Binding of
the enriched phage populations to recombinant human
pIgR, as measured by ELISA, increased with successive
rounds of panning and was inhibited by polymeric IgM
(data not shown). Sequencing of phagemid DNA showed
that 20 out of 32 acid-eluted clones and 12 out of 32 cell-
associated clones had open reading frames (Fig. 2). There
is little clonality among these two groups of sequences,
although the A22 peptide was recovered three times. These
peptides were aligned for maximum homology with the
human IgA1 Cα3 region amino acid sequence (Fig. 2) us-
ing the PIMA program (23). Many of the peptides, particu-
larly A12 (9 out of 30 identical amino acids) (Fig. 3 a),
show homology with human IgA1 Cα3 domain, prompt-
ing a further examination of the amino acid sequence and
structure in this area.

The human Cα3 domain is 40% identical and 62% ho-
mologous to the corresponding region of human IgG1 at
the amino acid level. In addition, all the sequence hall-
marks of the immunoglobulin superfamily fold are con-
served. Accordingly, the human IgG1 crystal structure (29)
was used to predict the likely positions of the major struc-
tural motifs (β-strands and loops) within the IgA1 se-
quence, an approach used previously to map the FcεR1
(CD89) binding site on IgA1 (24). Fig. 3 a shows the align-
ment of the peptide A12 with the IgA1 sequence and the
corresponding IgG1 sequence with its secondary structural
features. The A12 peptide is homologous to a region that
in the IgG structure forms an exposed 6-amino acid loop
between two β-strands. However, in IgA1, this area contains
a 3-amino acid insertion to expand the loop to 9-amino
acids. The flanking β-strand sequences and part of the loop
are conserved between IgA and IgG, which suggests that
gross structural features are also conserved. Fig. 3 b shows
alignment of this region in the CH3 domain of five mam-
malian IgA molecules aligned with the four human IgG
subclasses. Despite sequence differences in the loop, all IgA
sequences have the three additional amino acids, whereas
the IgG sequences do not. Similar to IgA, the sequence of
IgM contains a 2-amino acid insertion at this site (data not
shown). On the basis of these observations, three mutant
IgA1 molecules were constructed and expressed in bacu-
lovirus to examine the effect of amino acid changes in this
area on pIgR binding (Fig. 3 c). Mutations were made in
the loop itself (L1 and L3) and in the β-strand NH2-termi-
nal to the loop (L2) as a negative control. Binding was then
measured to the physiologically relevant human receptor
by ELISA using the purified recombinant extracellular do-
main of human pIgR expressed in baculovirus as previously

![Figure 2. Alignment of deduced peptide sequences from selection of phage display pep-
tide library against pIgR receptor-expressing cells with the human Cα3 domain amino
acid sequence. Peptides designated A or M
are from the acid-eluted and cell-associated
fractions, respectively. Numbering of IgA1 is
corresponding to reference 5.](image)

![Figure 3. Comparison of IgG1 and IgA1 CH3 sequences and IgG1 structure in the area homologous to several phage-derived peptides. (a) The A12 peptide alignment with human IgG1 and IgG3. IgG1 indicates structural features of IgGs where < denotes a β-strand running in a descending orientation (i.e., hinge to CH3 direction), > denotes a β-strand running in an ascending direction (i.e., CH3 to hinge direction), and – denotes a loop or open structure (29). (b) Comparison of several mammalian IgA sequences with the four human IgG subclasses showing the additional IgA-specific amino acids present in the loop at positions 402-410 in the IgA sequence. hu, human; gr, gorilla; mur, murine; rab, rabbit. (c) IgA1 Cα3 mutants L1, L2, and L3 aligned with the Cα3 and Cε3 wild-type sequences and Cε3 structure (IgGε3). – denotes sequence identity in the mutants — denotes a space introduced in the IgG sequence to maximize homology, and IgGε3 is labeled according to panel a. Numbering of IgA1 and IgG1 is according to references 5 and 29, respectively.](image)
described (18). Fig. 4 shows the binding of IgA1 monomer, IgA1 dimer, and IgG compared with the monomeric and dimeric forms of the L1, L2, and L3 mutants to purified human plgR. Only dimeric wild-type IgA1 and dimeric L2 mutant, in which the mutations are in the β-strand NH₂-terminal to the loop, show binding. Mutations within the loop itself, namely L1 and L3, abrogate the binding of the dimeric IgA1 mutant molecules to the plgR. Similar binding patterns were obtained with the loop mutants and rabbit plgR-expressing cells as measured by FACS® (data not shown). These results indicate that this Cα3 loop is the major binding motif for the plgR on dlgA.

IgA is, in functional terms, closely related to IgM, sharing its ability to polymerize and be secreted. However, the overall IgA domain organization resembles that of IgG. The presence of amino acid sequence insertions in all the polymeric Igs that are ligands for this receptor and the absence of insertions from non-plgR-binding Igs (Fig. 3 b) supports its role in Ig secretion. The variation in the insertion size and the actual IgA and IgM sequences may reflect differences in fine structure of these polymeric antibodies or in their affinity for plgR binding.

The fact that monomeric IgA is not secreted suggests that either a conformational change induced by polymerization is required for dlgA binding to the receptor or that the binding requires a polyvalent interaction of these Cα3 sites with the receptor. The presence of J chain is required for optimal IgA (or IgM) polymerization but its precise role in Ig secretion remains to be elucidated. The increase in binding observed with dimeric L3 when compared with monomeric L3 (and to a lesser extent with the L1 mutants) suggests that J chain and/or polymerization may play a role in binding (Fig. 4). Although amino acids 402–410 in the Cα3 domain of dlgA define a major plgR binding site, other dlgA structures may be involved. J chain-deficient mice express lower levels of polymeric IgA and have impaired hepatic transport of IgA (which humans lack) but normal levels of IgA at mucosal epithelial sites, compared with wild-type mice (30, 31). J chain thus may not be necessary for secretion of IgA but still required for stable binding to the secretory component in the mucosal environment; however, alternative secretory mechanisms may also be involved. Further studies are underway with peptides and additional mutations to examine the nature of the interaction between IgA and the plgR as well as the role of J chain. The ability of a peptide sequence to confer mucosal secretion upon a molecule may prove a powerful means of delivery of therapeutic molecules to mucosal areas where they may prevent the entry of pathogens.


