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Secretory Antibody Formation: Conserved Binding Interactions between J Chain and Polymeric Ig Receptor from Humans and Amphibians

Ranveig Braathen,* Valerie S. Hohman,† Per Brandtzaeg,* and Finn-Eirik Johansen2*

Abs of the secretory Ig (SIg) system reinforce numerous innate defense mechanisms to protect the mucosal surfaces against microbial penetration. SIgAs are generated by a unique cooperation between two distinct cell types: plasma cells that produce polymers of IgA or IgM (collectively called plgs) and polymeric Ig receptor (pIgR)-expressing secretory epithelial cells that mediate export of the plgs to the lumen. Apical delivery of SIgAs occurs by cleavage of the pIgR to release its extracellular part as a pIg-bound secretory component, whereas free secretory components are derived from an unoccupied receptor. The joining chain (J chain) is crucial in pIgSIgA formation because it serves to polymerize Igs and endows them with a binding site for the pIgR. In this study, we show that the J chain from divergent tetrapods including mammals, birds, and amphibians efficiently induced polymerization of human IgA, whereas the J chain from nurse shark (a lower vertebrate) did not. Correctly assembled polymers showed high affinity to human pIgR. Sequence analysis of the J chain identified two regions, conserved only in tetrapods, which by mutational analysis were found essential for pIgA-pIgR complexing. Furthermore, we isolated and characterized pIgR from the amphibian Xenopus laevis and demonstrated that its pIg binding domain showed high affinity to human pIgA. These results showed that the functional site of interaction between pIgR, J chain and Ig H chains is conserved in these species and suggests that SIgAs originated in an ancestor common to tetrapods.

Secretory IgA (SIgA)3 has been recognized as the major Ab class in mammals for >40 years. The conventional explanation for this huge SIgA production has been that it must protect the large mucosal surfaces against a many different bacterial and viral pathogens. Recently, however, the role of SIgA in host interactions with the commensal bacteria in the intestine has been revealed (1). Thus SIgA is important both for defense against mucosal pathogens but also to restrict the indigenous gut flora to the lumen.

Animals are adapted to live with vast number of bacteria colonizing their mucosal surfaces. Such mutualistic microorganisms provide benefit to the host, not only as competitors of pathogenic bacteria but also through direct positive tissue interactions (2, 3). However, if the mucosal barrier is breached the indigenous micro-biota may cause life-threatening infections or a severe tissue-damaging response from the systemic immune system. In mammals, luminal bacteria induce production of IgA Abs (4) that cooperate with innate defense mechanisms on the mucosal surfaces to provide protection against pathogens and to restrict commensals to the lumen (5–7).

Plasma cells located in the mucosal lamina propria of the gastrointestinal and respiratory tracts are preferentially switched to IgA and coexpress the joining chain (J chain). Therefore, mucosal plasma cells secrete mostly polymeric IgA (pIgA; predominantly dimers) with the capacity to bind to the polymeric Ig receptor (pIgR) (8, 9). SIgA production is initiated when pIgA binds pIgR expressed on the basolateral side of epithelial cells lining the mucosal surfaces. Endocytosis and intracellular trafficking of the receptor-ligand complex to the apical plasma membrane is mediated by sorting signals in the cytoplasmic tail of the pIgR, and luminal delivery of SIgA occurs by cleavage of the receptor near the plasma membrane (10). Covalently bound secretory component (SC; the extracellular cleaved piece of the pIgR) confers mucophilic properties and proteolytic resistance to the SIgA molecule (11). Similarly generated SIgM Abs are mainly important in the neonate and in individuals with selective IgA deficiency (12), whereas constitutive transcytosis of unoccupied pIgR leads to the secretion of free SC (12).

The J chain has been characterized in a range of species including mammals (human (13, 14), mouse (15), bovine (16), rabbit (17), and brushtail possum (18)), birds (chicken (19)), reptiles (turtle (20)), amphibians (Xenopus laevis (21) and Rana catesbeiana (22)), and cartilaginous fish (nurse shark (23) and clearnose skate (24)). In humans, this unique 15-kD polypeptide contains eight Cys residues that form three intrachain bridges and two bridges to the tailpieces of IgA or IgM in human plgs (25). No crystal structure is available for SIgA or SIgM, but several lines of evidence suggest that J chain and pIgR interact directly, although this remains controversial (8, 9, 25, 26).

Monomeric IgA (composed of two H chains and two L chains) readily form polymers when coexpressed with a J chain (13). Earlier investigations into the function of the J chain showed that...
cloning into a by QuikChange PCR (Stratagene) or by splice overlap extension and subcloned into pCEP4. Mutagenesis of the human J chain was performed expression. The J chain open reading frame from mouse (15), chicken (28), cation, HygR for antibiotic selection, and CMV promoter for cDNA tained ori P and encoded EBV nuclear Ag (EBNA) for episomal repli-
The plasmid for episomal expression of wild-type human J chain (pCEP4- Furthermore, we identify two conserved regions in the J chain that different tetrapod species is capable of inducing polymerization of human IgA and that the resulting pIgA binds human pIgR/SC. Furthermore, we identify two conserved regions in the J chain that are necessary for the latter function only. Finally we characterize the first amphibian pIgR and show that its pIg binding domain is capable of binding human pIgA. These findings reveal a remarkable conservation of protein-protein interaction sites in secretory Abs from different species, suggesting an evolutionary significance of this first-line defense system.

Materials and Methods

Plasmid construction

The plasmid for episomal expression of wild-type human J chain (pCEP4-wt chain) has been described previously (27); this expression vector contained ori P and encoded EBV nuclear Ag (EBNA) for episomal replication, HygR for antibiotic selection, and CMV promoter for cDNA expression. The J chain open reading frame from mouse (15), chicken (28), X. laevis (21), bullfrog (22), and nurse shark (23) was isolated by PCR and subcloned into pCEP4. Mutagenesis of the human J chain was performed by QuikChange PCR (Stratagene) or by splice overlap extension and subcloning into a HindIII- and BamHI-digested pCEP4. The entire open reading frame of all plasmids was verified by DNA sequencing (Medigenomics).

A plasmid for recombinant expression of human SC was constructed by PCR with a forward primer producing a Kozac sequence at the ATG initiation codon and a reverse primer inserting a stop codon at the cleavage site for SC. The resulting PCR product was ligated into pCDNA3.1 directional TOPO (Invitrogen Life Technologies). A TBLASTN search with the amino acid sequence of the extracellular domain 1 (D1) of human or chicken pIgR as the query both identified an expressed sequence tag putatively encoding X. laevis pIgR. The clone (IMAGE 4031968) was obtained from Geneservice and sequenced in full (GenBank accession no. EF079676). An expression plasmid for xD1-hD2D5-SC was constructed by fusing the D1 of X. laevis pIgR with the domain 2 (D2) from human pIgR by splice overlap extension and subcloning as described above (domain boundaries are indicated in Fig. 3). An expression plasmid for hD2D5-SC was constructed by deleting the D1 from the human SC expression plasmid by QuikChange PCR.

Production and purification of recombinant IgA and SC

The 5-iodo-4-hydroxy-2-nitrophenylacetyl (NIP)-specific IgA1-producing Chinese hamster ovary (CHO) cell line D2 (27) was cultured in Ham’s-F12 medium supplemented with 10% FCS, 2 mM glutamine, and 50 μg/ml gentamicin. Semistable transfections (episomal expression) were performed as previously described (27). Briefly, cells were seeded at 10–15% confluence in 6-well trays on day 1 and transfected on day 2 with 1.4 μg DNA and 3 μl of FuGENE (Roche Diagnostics) according to the manufacturer’s protocol. On day 3, the cells received fresh medium and on day 4 they were trypsinized and seeded into 10-cm plates in medium supplemented with 300 μg/ml hygromycin B (Roche Diagnostics). Cells received fresh medium with hygromycin B every 3–4 days; after 10–12 days each 10-cm plate contained at least 500 hygromycin B-resistant colonies that were pooled and seeded into 10-cm plates or large flasks for IgA production. The vector pCEP4 without insert was used as negative control (mock).

IgA Abs secreted from pools of >500 transfectants were affinity-puri-
fied on a NIP-Sepharose column (13). Fractions containing monomeric or pIgA were separated by ion-exchange (MonoQ) chromatography (27). Briefly, IgA was eluted by a linear salt gradient from 2.5–500 mM NaCl in 20 mM Tris-HCl (pH 7.1) and collected in 300-μl fractions. Peak fractions containing pIgA were pooled and analyzed for molecular size by native gel
electrophoresis and by ELISA for binding to free SC and for J chain content (see below).

The J558L transfectoma cell lines expressing human Fc-containing IgA1, IgM, and IgG1 have been described previously (29, 30). Crude cell supernatants were used in ELISA for SC binding analyses.

Production of recombinant human free SC and derivatives were performed essentially as described (31). Briefly, one 10-cm plate with 293E cells was transiently transfected with 6 μg of each expression vectors using FuGENE. Supernatants were harvested 3–7 days, and the SC was precipitated by ammonium acetate, resuspended, and dialyzed against PBS.

Immunoprecipitations, gel electrophoresis, and Western blotting

Approximately 500 ng of IgA from CHO cell supernatants were immunoprecipitated with rabbit anti-human IgA (diluted 1/100; DakoCytomation) and sheep anti-rabbit Ig-coated magnetic beads (7 × 10^7 beads in 1 ml; Dynal). Samples were resolved by nonreducing SDS-PAGE on a 5% (w/v) acrylamide gel and transferred onto a polyvinylidene fluoride membrane (Millipore) that, after air drying, was blocked with PBST (10% skimmed milk in PBS plus 0.05% Tween 20). Subsequent incubations were all performed in PBST. The membrane was incubated with a mouse antiserum to human IgA (diluted 1/3000; gift from T. Lea, Institute of Immunology, Rikshospitalet-Radiumhospitalet Medical Center, University of Oslo, Norway) for 90 min, washed three times for 10 min, and then incubated with HRP-conjugated sheep anti-mouse IgG (diluted 1/3000; Amersham Biosciences) for 90 min. After three washes, the membrane was incubated for 10 min with SuperSignal (Pierce) and the substrate was detected with a light-sensitive camera (Chemidoc; Bio-Rad).

For analysis of NIP-purified IgA, 30 ng were resolved by nonreducing SDS-PAGE on a 5% (w/v) acrylamide gel with the Criterion system (Bio-Rad) and transferred and probed as described above except that the membrane was first incubated with a rabbit antiserum to human IgA (diluted 1/3000; DakoCytomation) and next incubated with HRP-conjugated donkey anti-rabbit IgG (diluted 1/3000; Amersham Biosciences). For J chain immunoblotting, the membrane was stripped in buffer (53 mM Tris (pH 6.8), 1.6% SDS, and 14.3 mM 2-ME) at 60°C (15 min) and then blocked again with 10% (w/v) skimmed milk in PBST and incubated with our rabbit antiserum to human J chain (previously absorbed with monomeric IgA) diluted 1/800 (32). Secondary Ab and the revealing reaction were as described above.

For native immunoblots, 60 ng of IgA was resolved on a 5% (w/v) acrylamide gel as described above. SDS was omitted from all buffers and the pH of the loading buffer was 8.5. The gel was transferred to a polyvinylidene difluoride membrane and the membrane was probed as for analysis of NIP-purified IgA as described above.

ELISAs

For all Ag-specific ELISAs, microtiter plates were coated with 3 μg/ml NIP-BSA and blocked with 1% (w/v) BSA in PBS. For total IgA quantification, the primary Ab was mAb against human IgA (diluted 1/30 000; a gift from T. Lea, Institute of Immunology, Rikshospitalet University Hospital, Oslo, Norway) and the secondary Ab was alkaline phosphatase-conjugated rabbit anti-mouse Ig Ab (diluted 1/1000; DakoCytomation).

For J chain-specific ELISA (Table I) and the SC-affinity ELISA (Figs. 1C and 2B), 800 ng/ml, 400 ng/ml, 200 ng/ml, and 100 ng/ml purified recombinant pIgA was incubated in triplicate wells overnight.

For J chain-specific ELISA, the microtiter plates were washed and fixed with 2% glutaraldehyde for 30 min then incubated with 6 M urea (pH 3.0) at 37°C for an additional 30 min to reveal bound J chain by denaturation (27). After washing, the plates were incubated with our rabbit antiserum to human J chain (diluted 1/300) and alkaline phosphatase-conjugated goat anti-rabbit IgG (diluted 1/2000; DakoCytomation). The J chain content of each pIgA sample was normalized to pIgA with a wild-type human J chain. For SC affinity measurement, the microtiter plates were washed and incubated with 8 μg/ml free SC purified from human colostrum, then rabbit antiserum to human SC (DakoCytomation; diluted 1/1000), and finally goat anti-rabbit IgG as above. The values were expressed as the slope of the linear regression of OD 405 vs IgA concentration and normalized to the corresponding slope obtained for NIP-specific myeloma pIgA (27).

ELISA for recombinant human and chimeric SC was performed by coating microtiter plates with 2 μg/ml SpA surface protein from S. pneumoniae, which requires intact human D3D4 for SC/pIgR binding (31). Detection was with a mAb against human pIgR D2 (present in all SC variants) (1/5000; Sigma-Aldrich) followed by HRP-conjugated goat anti-mouse IgG (1/2000; Sigma-Aldrich) and color reaction with tetramethylbenzidine peroxidase substrate (Kirkegaard & Perry Laboratories).

FIGURE 1. J chains from different vertebrates facilitate polymerization of human IgA and binding to human SC. A, NIP-specific recombinant IgA without a J chain (mock) or containing a J chain from different species was immunoprecipitated with polyclonal anti-human IgA serum, subjected to nonreducing SDS-PAGE, and immunoblotted with a mAb against human IgA. Migration of monomers and dimers are indicated. The percentage of polymers for each sample is indicated above each lane. B, Binding of recombinant human SC to CHO cell-produced IgA with different J chains was analyzed by ELISA as described in Materials and Methods. Mean OD values + SD from quadruplicate wells in one of three similar experiments is shown. C, The polymeric fractions shown in A were purified by chromatography and tested for binding to native human secretory component in an ELISA as described in Materials and Methods. Mean ± SEM of three independent experiments. Binding values that differ from wild-type J chain or from mock control (p < 0.05; two-tailed Student’s t test) are indicated by a star (*) or a closed circle (○), respectively.
**FIGURE 2.** Two conserved regions in the J chain are critical for binding of polymeric IgA to SC. A, The amino acid sequences of the J chains from human, mouse, chicken, bullfrog, *X. laevis*, and nurse shark were aligned with ClustalW. Dashes denote identity to residues in the human sequence, and dots denote gaps. Amino acid numbering, predicted β-strands, and surface accessibility (bold font) of human J chain are indicated. SS marks the three intrachain disulfide bridges and “TP” marks the Cys paired with the secretory tail piece of IgA or IgM. The conserved N-linked glycosylation site is indicated by a star (*). Secondary structure prediction and surface were performed with Jpred (http://www.compbio.dundee.ac.uk/). B, NIP-specific pIgA containing mutants of human J chain was purified and analyzed as described in the legend to Fig. 1. Names of mutants include original amino acid (left), the position as indicated in A, and the new amino acid (right). Where several amino acids were altered, the first amino acid in the range is indicated. Mean ± SEM of four independent experiments. Binding values that differ from wild-type J chain or from mock control (p < 0.05; two-tailed Student’s t test) are indicated by a star (*) or a closed circle (○), respectively.
The binding of recombinant human and chimeric SC to pIgA or pentameric IgM or IgG1 was performed by coating microtiter plates with NIP-BSA as described above and saturating with NIP-specific J558L transfectoma-produced Ig (1.5 μg/ml). Dilution series of SC were added and detected as in ELISA for recombinant SC. For the binding of recombinant human and chimeric SC to CHO-produced IgA containing human, mouse, chicken, Xenopus, bullfrog, or nurse shark J chain, CHO cell supernatants were precipitated with 40% (w/v) ammonium acetate, resuspended in PBS, and the concentration was adjusted to 1.5 μg/ml. ELISA for SC binding analysis was performed as described above.

Results

J chains from divergent tetrapods support pIgA and SIgA formation with human IgA and human secretory component (SC)

We coexpressed the J chain from human, mouse, chicken, bullfrog, X. laevis or nurse shark with human IgA and analyzed the secreted IgA for molecular size and pIgR affinity (Fig. 1). Although the human J chain induced the greatest level of polymerization, the J chain from mouse, chicken, or Xenopus also induced significant pIgA formation (Fig. 1A; Table I). We then tested the binding of secreted IgA to recombinant human free SC (Fig. 1B). We found that the J chains from different species that induced polymerization of human IgA efficiently also were part of a polymer with high affinity to human SC. Surprisingly, the small amounts of pIgA formed in the presence of the bullfrog J chain showed significant affinity to human SC. Conversely, the small amounts of pIgA formed in the absence of J chain (or when IgA was coexpressed with nurse shark J chain) showed virtually no affinity to SC. To eliminate possible bias in the SC binding experiments due to different levels of polymerization, we purified each pIgA fragment and analyzed its ability to bind native human free SC purified from the colostrum (Fig. 1C). These results confirmed the binding pattern described above.

Two motifs in human J chain are important for pIgA affinity to pIgR/SC

We identified two regions of high amino acid conservation in the J chain from tetrapods (regions 2 and 3), but only one of them (region 2) was conserved in the shark (Fig. 2A; Table II). We tested a series of point mutations of these two regions in the human J chain as well as a third amino-terminal region predicted to be in a surface-exposed loop (region 1) for the effect on pIgA formation and SC binding (Fig. 2B).

Several mutations in region 1 showed similar ability to support pIgA formation and SC binding as the wild-type J chain, suggesting no direct involvement of this region in polymerization or pIgR interaction. For region 2, we found that substitution of amino acids 50–52 (ISD) with Ala significantly reduced the affinity of pIgA to SC. Because glycosylation of the phylogenetically conserved Asn49 is directed by Ser51 the ISD mutant lacked the glycosylation of Asn49. However, the mutation of Asn49 to Ala (N49A) did not reduce pIgA affinity to SC, although a slight decrease in pIgA formation was seen (Table I) similar to that previously observed with mutagenesis of mouse J chain (34). Furthermore, the ISD52A mutant showed reduced SC affinity, although glycosylation would be maintained. Thus, glycosylation per se was apparently unimportant for the pIgR interaction, although an important motif for pIgR interaction was located in the highly conserved region 2 of the human J chain.

A region between the sixth and seventh Cys of the J chain was found to be highly conserved in all tetrapods examined, but not in the nurse shark (Table II; Fig. 2A). Again, we made several J chain mutations in this region and coexpressed the resulting J chain vari-

ants with human IgA. All mutants supported pIgA formation (Table I). When analyzing pIgA binding to human SC, we found that mutation of two Tyr residues (Tyr102 and Tyr104) to Ala was not tolerated, suggesting a critical role in pIgR interaction. Nevertheless, the residues were able to substitute in these positions without loss of SC binding. Therefore, we concluded that these Tyr residues are most likely required for a hydrophobic “core” rather than for direct pIgR interaction. Several different mutations that affected Arg106 significantly reduced SC binding to the pIgA produced. Substitution of this Arg for Ala or for a negatively charged Glu (R106E) had a similar effect (Fig. 2B; compare R106A with R106E). Altering the negatively charged Asp105 to Arg did not compensate for the R106E mutation (DR105RE). Therefore, it appeared that Arg-106 was essential for pIgR interaction. Although the triple mutation (RNK106AAA) tended to produce a slightly less functionally active pIgA than R106A, there was no statistical difference between these mutants. Furthermore, no other individual amino acid in this region resulted in a J chain with significant loss of function. Altogether, Arg106 was the only amino acid in region 3 found to be essential for pIgR interaction.

X. laevis pIgR binds human pIgA but not pentameric IgM

Having established that the J chains from birds and amphibians efficiently substituted for human J chain in the formation of pIgA with a pIgR docking site, we made the assumption that amphibians encode a pIgR. By BLAST search, we identified an expressed sequence tag encoding X. laevis pIgR and obtained the clone from Geneservice. The sequencing of cDNA from Xenopus revealed that its pIgR contained four extracellular Ig-like domains similar to the pIgR from chicken (Fig. 3) but in contrast to mammalian pIgR, which contains five Ig-like domains (18, 35, 36). The alignment of Xenopus pIgR with the pIgR from human, possum, and chicken and a sequence comparison of each Ig-like domain indicated that X. laevis pIgR lacks an Ig domain equivalent to mammalian D2 (Fig. 3; Table III). Interestingly, a Pro residue within the transmembrane helix present in mammalian and chicken pIgR was absent in Xenopus pIgR, suggesting that signaling through this receptor may be different in amphibians. However, the strong basolateral targetting signal present in all pIgRs was also conserved in Xenopus pIgR.

The initial noncovalent interaction of pIgA and pentameric IgM with pIgR is mediated by its extracellular D1 and the respective Ig Fc portions, including the J chain (37, 38). We wanted to test whether mammalian pIgA could bind amphibian pIgR and therefore constructed a chimeric SC molecule that contained D1 from Xenopus and D2 through D5 from human pIgR (xD1-hD2D5). The binding of this chimera to human pIgA or pentameric IgM or IgG was compared with that of human SC. The xD1-hD2D5 chimeric SC showed distinct binding to pIgA but no detectable affinity to pentameric IgM (Fig. 4A). Conversely, human SC bound both pIgA and pentameric IgM. A human SC lacking D1 showed no

<table>
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<tr>
<th>Region</th>
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<th>Xenopus</th>
<th>Bullfrog</th>
<th>Nurse shark</th>
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*Percentage of the amino acid identity of the J chain from the indicated species to the human J chain in region 1, region 2, region 3, and overall. See Fig. 2A for alignment and location of each region.
affinity to either ligand, demonstrating that human pIgA bound the Xenopus-human chimera because of Xenopus D1. No SC variant bound IgG (data not shown).

Binding of human pIgA to Xenopus pIgR is improved by substituting human J chain with that of Xenopus. We hypothesized that the J chain contributes directly to the pIgR interaction site of pIgA. It was therefore reasonable to assume that pIgA composed of human α H chains and the Xenopus J chain would show better binding to Xenopus pIgR than pIgA containing the human or mouse J chain. Therefore, we tested the binding of xD1-hD2D5 SC to pIgA containing the human, mouse, or Xenopus J chain and did indeed find that pIgA with Xenopus J chain was the better ligand for Xenopus pIgR D1 (Fig. 4B). Furthermore, IgA without J chain showed no binding to Xenopus pIgR D1, demonstrating J chain dependence for pIgR-pIg interaction also in this species.

**FIGURE 3.** Alignment of pIgR from *X. laevis* with human, possum, and chicken pIgR. Residues identical in all sequences, highly conserved sequences, or conserved sequences are indicated by a star (*), a colon (:), and a period (.), respectively. Gaps in the alignment are indicated by hyphens. The start of each extracellular domain, the cytoplasmic tail, and the cleavage site for human pIgR is indicated. The CDR1, CDR2, and CDR3 in D1 are written in bold font. The predicted signal peptide and transmembrane region are underlined. GenBank accession numbers: *Homo sapiens*, NP_002635; *possum (Trichostrongylus vulpecula)*, AAD41688; and *chicken (Gallus gallus)*, AAW71994.

**TABLE 3.** Conservation of secretory antibody formation in tetrapods by guest on April 13, 2017 http://www.jimmunol.org/ Downloaded from

**CONSERVATION OF SECRETORY ANTIBODY FORMATION IN TETRAPODS**
Discussion

Knockout mice for the J chain and pIgR have shown that both genes are critical for SIg generation (9, 39). In this study, we present a mutational analysis of human J chain supporting a direct interaction between these two polypeptides. Furthermore, we identify the first amphibian pIgR and demonstrate that protein-protein interaction between pIgR and the J chain is remarkably conserved between tetrapods.

The J chain probably originated in the first jawed vertebrate because it is present in rays and sharks. Intriguingly, it appears to be deleted in bony fish; despite near completion of the genome project for several fish species, no gene resembling this unique gene has been identified. Interestingly, we found that a region critical for pIgA-pIgR interaction was highly divergent between the J chains from tetrapods and the nurse shark. Furthermore, this C-terminal region is absent from the J-chain sequence of clearnose skate, suggesting that it is not required for normal J-chain function in Chondrichthyes (24). We therefore speculate that the highly conserved J chain region in tetrapods is required for pIgR interaction, thereby allowing active export of plgs to mucosal surfaces. Because the pIgR interaction site is not conserved in J chain from cartilaginous fish, its function here might solely be to facilitate Ig polymerization. Thus, Abs with higher avidity and better agglutinating capacity would be produced. Dooley and Flajnik (40) have proposed that absence of J chain expression in B cells that have undergone affinity maturation would provide monomers with enhanced tissue penetration in a memory response.

Because the Xenopus J chain showed high homology to the human J chain in two regions that were required for pIgR binding, we predicted that a pIgR homologue would be found in this species. Database searches identified such a gene and we deduced the primary sequence of the open reading frame by DNA sequencing of

Table III. Sequence comparison of the extracellular Ig-like domains from pIgR

<table>
<thead>
<tr>
<th>Domain 1</th>
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<td>6 27</td>
<td>11 20 28</td>
<td>21 21 22</td>
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</table>

*Percentage of amino acid sequence identity between the four extracellular Ig domains (Xd1, xD2, xD3, and xD4) of Xenopus laevis pIgR (indicated vertically on the left) compared with each of the five (human and possum) or four (chicken) Ig-like domains of pIgR from human (H), possum (P), and chicken (C). The Ig domains of the human, possum, and chicken most similar to each of the Xenopus Ig domains are indicated in bold font. Chicken and Xenopus have no equivalent of mammalian domain 2.

FIGURE 4. D1 of X. laevis pIgR/SC mediates pIgR binding to J chain-containing IgA. A, Recombinant human SC, a chimeric SC with X. laevis D1 fused to human D2 (xD1-hD2D5-SC) and human SC lacking D1 (hD2D5-SC), were tested for binding to J558L transfectedoma-produced plgA or pentameric IgM (mouse J chain and human Fc region). B, Human SC or xD1-hD2D5 SC were tested for binding to CHO cell-produced IgA without J chain (mock) or with J chain from different species in an ELISA. Mean ± SD of quadruplicate (A) or triplicate (B) wells are shown. Each experiment shown is representative of three or more similar experiments.
full-length cDNA. Although the Xenopus plgR clearly has an overall structure similar to that of plgR from other species, including four Ig domains (like chicken plgR, whereas mammalian plgR contain five Ig domains), a transmembrane region, and a cytoplasmic tail with a basolateral targeting motif, it was surprisingly divergent in the critical ligand-binding CDR1-like region in D1 (37, 41); the sequence VNRH, conserved in all plgRs published to date (resequencing of rabbit plgR has revealed this sequence in that species, although it was initially reported as VTRH) is substituted with ANKY in Xenopus plgR (Fig. 3). Additionally, CDR1 is one amino acid longer in *X. laevis* than in other plgRs published to date. However, structural modeling (Swiss Model; http://swissmodel.expasy.org/) predicted the CDR1 of Xenopus plgR would form an α helix characteristic of the plgR D1 (Ref. 42 and data not shown). Due to the relative divergence of CDR1, it was surprising that the *Xenopus* plgR D1 mediates fairly strong binding to mammalian plgA. A possible explanation for its failure to bind mammalian pentameric IgM is that this polymer adopts a different conformation than *Xenopus* J chain-containing IgM, which reportedly is hexameric (43). We have previously shown that differences in the CDR2 region between human and rabbit plgR D1 are primarily responsible for the disparity of IgM binding between these two species; only human plgR binds pentameric IgM with high affinity (38). It is therefore possible that differences between the CDR2 regions in *Xenopus* and human plgR contribute to the failure of *Xenopus* plgR to bind mammalian pentameric IgM.

An interesting difference between the mammalian plgR on the one hand and the plgR from chicken and *Xenopus* on the other is that the former contain five extracellular Ig-like domains while the latter contain four, lacking an equivalent to mammalian D2. In mammalian plgR, D2 and D3 are encoded by a single exon, thereby diverging from the rule “one domain, one exon.” Sequence comparisons of each individual Ig-like domain within and across mammalian and nonmammalian plgRs showed that mammalian D2 is most similar to mammalian D3, suggesting that it arose by a duplication of the exon encoding D3 (Table III and data not shown). There is no remnant of such a duplication event in the chicken plgR gene (36) or in the plgR gene of *Xenopus tropicalis* (data not shown), suggesting that his duplication event occurred after mammals diverged from birds and amphibians.

Amphibians express five Ig classes: IgM, IgY, and IgX (5) and the recently identified IgF and IgD (44). IgY exists as a monomer after mammals diverged from birds and amphibians. IgM, IgG, and IgX constitute the polymeric IgGs in *Xenopus*, but only IgM polymers incorporate a J chain (43, 45, 46). IgX has been suggested to be an analog to IgA because of its ability to form polymers and its expression chiefly in the gut (46). It is an intriguing possibility that IgX could be a J chain-independent ligand for this receptor. We found that *Xenopus* plgR only bind J chain-containing plgA and that the affinity improved when this plgR contained the *Xenopus* J chain compared with the human or mouse J chain, suggesting a J chain dependence for plg-plgR interaction also in this species. Importantly, our results demonstrated that the interaction site between plgR and J chain-containing plgA is conserved between mammals and amphibians.

In humans, SigA is the dominant Ab on a biosynthetic basis and reportedly protects against *Vibrio cholerae* and enterotoxicogenic *Escherichia coli*-induced diarrhea (47), in agreement with the notion that such Abs mediate mucosal immune exclusion (12). We have previously generated plgR knockout mice and have recently shown that SigA protect against horizontal spread of the mucosal pathogen *Salmonella typhimurium* even in naive animals, thereby providing herd immunity (39, 48). In addition, recent data have suggested that the large amounts of SigA generated in the mouse gut may serve to curtail the commensal microbiota within the mucosal compartment (7, 49). Thus, mucosal immune exclusion protects against pathogens and promotes luminal habitation of the beneficial indigenous microbiota, thereby preventing adverse systemic immune reactions.

In conclusion, our structural data imply that the J chain has served two roles in the evolution of a secretory Ab system. Its Ig-joining capacity allowed regulation of polymerization to form Abs with better agglutinating capabilities, while its interaction with the plgR allowed the export of such Abs to the mucosal surfaces where the bacterial load is high. The functional conservation between each polypeptide chain specifically involved in the generation of secretory Abs from amphibians, birds, and mammals suggests that this important first-line defense mechanism has been maintained in tetrapod evolution.

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Disclosures

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