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Lack of SC/pIgR-mediated epithelial transport of a human polymeric IgA devoid of J chain: *in vitro* and *in vivo* studies

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SUMMARY

Three human polymeric IgA (pIgA) myeloma proteins of tetrameric size were compared for their J-chain content, their *in vitro* secretory component (SC)-binding ability, and their capacity to be transcytosed by polymeric immunoglobulin receptor (pIgR)-expressing epithelial cells *in vitro* and rat hepatocytes *in vivo*. One of the three pIgA preparations, pIgA-L, was shown to lack J chain and was unable to combine with purified free human and rat SC, whereas pIgA-G and pIgA-C contained J chain and combined readily with SC. Furthermore, pIgA-L was not transferred into rat bile after intravenous injection, and was hardly transported apically by polarized Madin–Darbey canine kidney cell monolayers expressing the human pIgR, whereas pIgA-G and pIgA-C were efficiently transported in both test systems. Together with our recent demonstration that antibodies to human J chain block the SC/pIgR-mediated epithelial transport of pIgA, these data unanimously confirm the proposed key role of J chain in the epithelial transport of polymeric immunoglobulins into exocrine secretions.

INTRODUCTION

The selective enrichment of secretory IgA (sIgA) relative to other immunoglobulin classes in mammalian exocrine secretions depends on active transport of polymeric IgA (pIgA) through glandular epithelial cells expressing transmembrane secretory component (SC) or the polymeric immunoglobulin receptor (pIgR). Following basolateral interaction between this receptor and pIgA that is mostly locally produced, the epithelial cells internalize and transcytose the pIgR–ligand complexes in vesicles that subsequently fuse with the apical membrane. Proteolytic cleavage finally releases sIgA and free SC (FSC, the cleaved unoccupied receptor) into the secretions.[1–4]

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Abbreviations: AE, agarose gel electrophoresis; FITC, fluorescein isothiocyanate; FSC, free secretory component; IEP, immunoelectrophoresis; KO, knock-out; MDCK, Madin–Darbey canine kidney; mIgA, monomeric IgA; PAGE, polyacrylamide gel electrophoresis; pIgA, polymeric IgA; pIgR, polymeric immunoglobulin receptor; SC, secretory component; SDGU, sucrose density gradient ultracentrifugation; SDS, sodium dodecyl sulphate; sIgA, secretory IgA; TCA, trichloroacetic acid.

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The selectivity of epithelial pIgA transport resides in the high non-covalent affinity between SC/pIgR (mainly the first of its five extracellular immunoglobulin-like domains) and the Fc-portion of pIgA.[5,6] Monomeric IgA (mIgA) and IgG do not bind SC/pIgR and are only passively transferred to the secretion in smaller amounts.[7,8] For its binding to SC/pIgR, pIgA must contain J chain,[9] a 15 000 MW phylogenetically well-conserved[10] glycoprotein synthesized by plasma cells and incorporated intracellularly into pIgA. J-chain-rich, in contrast to J-chain-deficient, pIgA binds to FSC in solution[11] and to pIgR-expressing epithelial cells *in vitro*.[12] Moreover, antibodies to J chain inhibit this interaction.[9,13] Recently, we showed that antibodies to J chain, as well as their $F(ab')_2$ and Fab' fragments, do bind to J-chain-containing pIgA, thereby inhibiting its apical transcytosis in polarized pIgR-expressing epithelial cells *in vitro* and its biliary transport through rat hepatocytes.[14]

Here we report that a tetrameric pIgA1 myeloma protein totally lacking J chain, did not bind to human or rat FSC. In addition, it was neither transported efficiently by human pIgR-expressing epithelial cells *in vitro* nor into rat bile *in vivo* compared with two separate J-chain-containing tetrameric pIgA1 myeloma proteins. This result firmly supported the notion[9,12] that J chain is required for SC/pIgR-mediated transport, and agrees with the low IgA content found in bile and faeces of J-chain knock-out (KO) mice.[15]

MATERIALS AND METHODS

Human monoclonal pIgA

Three pIgA1 myeloma proteins, pIgAκ-G, pIgAλ-C and pIgAκ-L, were purified from the respective patient sera by gel filtration on Ultrogel AcA22 and Jacalin–Sepharose affinity chromatography, as described earlier.[16] The purity was verified by agarose gel electrophoresis (AE), immunoelectrophoresis (IEP), and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) with and without reduction. Gradient PAGE without SDS[16] was used to assess the size of pIgA. Because pIgA-L was mostly tetrameric (Fig. 1a), tetramers of pIgA-G and pIgA-C were also purified,[16] allowing for better comparison of the three protein fractions. This precaution was taken mainly to avoid dissimilarities in diffusion properties because transcytosis of dimeric and tetrameric IgA[17]

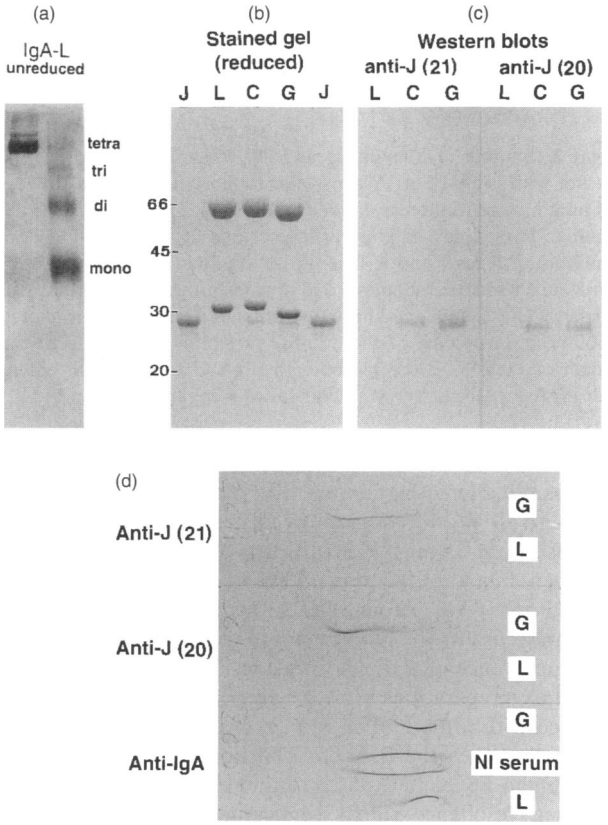

Figure 1. Lack of J chain in tetrameric pIgA-L. (a) Tetrameric size of pIgA-L (left lane) shown by gradient PAGE (4–20%) without SDS, as compared to a fraction of the same serum containing IgA mono- (160 000 MW), di- (335 000 MW), tri- (495 000 MW) and tetramers (755 000 MW) in decreasing concentration (right lane); anode at the bottom. (b) Stained SDS–PAGE of reduced pIgA-C (C), pIgA-G (G) and pIgA-L (L) (50 μg each), with lack of J-chain band in (L). Note identical mobility of the reference J chain (J) (20 μg) and J chain released from (C) and (G). MW markers: 66 000, 45 000, 30 000 and 20 000, for bovine serum albumin, ovalbumin, carbonic anhydrase and trypsin inhibitor, respectively. (c) Western blots with two different anti-J-chain antibodies (20, 21) of reduced pIgA run on SDS–PAGE as in (b), with same markers. Reduced pIgA-L did not react with either anti-J-chain antibody. (d) Immunoelectrophoresis (anode at the left) of mildly reduced-alkylated pIgA-G (G) and pIgA-L (L) revealed with antisera to human J chain, and of native (G) and (L) as well as normal serum (Nl serum) with antiserum to human α-chains.

as well as J-chain-containing pentameric IgM[18] through Madin–Darbey canine kidney (MDCK) epithelial cells expressing human pIgR was shown to be quite comparable. IEP, AE, PAGE and SDS–PAGE were stained with Coomassie Brilliant blue R-250.

Human and rat FSC

FSC was purified from human milk and rat bile by a combination of gel filtration, affinity-purification on human pIgA–Sepharose, protein A– or G–Sepharose, and various antibody immunoadsorbents to remove contaminants.[19] Purity was checked by SDS–PAGE; only one band (80 000 MW) was found with a load of up to 15 μg protein. In addition, rat FSC only gave a single precipitin arc upon IEP with antiserum to whole rat bile, and no arc with antiserum to whole rat serum. Also, human FSC did not react with antiserum to whole human serum.

Antibodies

Two different rabbit antibodies to human J chain were described earlier,[20,21] as were the antisera used against human α-chains, human SC[8] and rat SC.[19]

Identification of J chain in pIgA

Four methods were used to demonstrate J chain in pIgA:

(1) SDS–PAGE with reduction[16] showing stained J-chain band, identical to J chain purified from monoclonal IgM[22] used as reference;

(2) Positive Western blot after reduced SDS–PAGE with rabbit antibodies to J chain, and peroxidase-labelled anti-rabbit immunoglobulin;[16]

(3) J-chain precipitin arc with rabbit antibodies to J chain upon IEP of mildly reduced-alkylated pIgA;[20] and

(4) Size increase of the ^{125}I-labelled[23] pIgA preparations after exposure to a large molar excess of rabbit anti-J chain antibody, as shown by sucrose density gradient ultracentrifugation (SDGU).[14]

Binding of FSC to pIgA

Four methods were used to study the binding of human or rat FSC to pIgA:

(1) Human pIgA was subjected to AE in the presence or absence of rat or human FSC; disappearance of the FSC band indicated SC binding to pIgA;

(2) Similarly, pIgA, rat or human FSC, and their mixtures, were submitted to IEP developed with antibodies to SC and α-chains; a change in mobility and/or shape of the SC precipitin arc indicated binding;[24]

(3) A two- to sixfold molar excess of pIgA was subjected to SDGU in the presence or absence of ^{125}I-labelled[23] or fluorescein isothiocyanate (FITC)-labelled rat or human FSC; increased sedimentation rate of most of the ^{125}I or FITC label indicated binding;[14] and

(4) Surface plasmon resonance analysis was used as described by the manufacturer (BIAcore™ 2000 system, Pharmacia Biosensor, Uppsala, Sweden) with a carboxymethylated dextran sensor chip coupled[25] to human FSC in order to monitor its binding to the different pIgA preparations [0·5 mg/ml at 20 μl/min in 0·01 M phosphate-buffered saline (PBS) with 3 mM ethylenediaminetetraacetic acid (EDTA) and 0·005% surfactant P20] in real time for 5 min; after 2 min of

dissociation with the same buffer, the chip was regenerated by running 0·1 M citrate buffer, pH 2·5, for 1·5 min, and a control sensorgram (chip subjected to coupling procedure without FSC) was subtracted from each experimental sensorgram.

Secretion into rat bile

Purified pIgA was ^{125}I-labelled and intravenously (i.v.) injected [260 000–280 000 counts per minue (c.p.m.)] into bile duct-cannulated rats.[24] Recovery of trichloroacetic acid (TCA) (10% w/v final) -insoluble ^{125}I-labelled pIgA in bile over 3 hr was measured with a gamma-counter.

Apical transcytosis of cold and ^{125}I-labelled pIgA by human pIgR-expressing polarized MDCK cells

Confluence and polarization of MDCK cells on filter inserts (0·4-μm pore size) were assessed by microscopy and transepithelial electrical resistance.[14] Cold pIgA (100 μg/ml) was added to the basolateral medium (0·5 ml), and its transport into the apical medium (0·5 ml) after 24 hr was measured (in duplicate on three inserts for each pIgA) by a sandwich enzyme-linked immunosorbent assay (ELISA) with affinity-purified antibodies to human α-chain as coat in the microwells, and the same biotinylated antibodies and Extravidin-coupled alkaline phosphatase (Sigma, St. Louis, MO) as developing reagent. Each pIgA preparation provided its own standard curve. Transport of the ^{125}I-labelled pIgA (3 μg/ml) by triplicate filters was measured by apically TCA-precipitable c.p.m. after 24 hr,[14] and expressed as ng/filter, based on specific radioactivity.

RESULTS

Identification of J chain in the pIgA preparations

Reduced pIgA-G and pIgA-C released a faint fast J-chain band visible on stained SDS–PAGE and with the same mobility as *bona fide* purified J chain;[22] it was different from the κ- or λ-chain bands and did not appear in reduced pIgA-L (Fig. 1b). After blotting and probing with antibodies to J chain, a fast band in reduced pIgA-G and pIgA-C, but not in pIgA-L, reacted with both antisera to J chain (Fig. 1c). When mildly reduced, alkylated pIgA-G, pIgA-C and pIgA-L were subjected to IEP developed with antibodies to J chain, only pIgA-G and pIgA-C displayed a J-chain arc (Fig. 1d). Finally, when ^{125}I-labelled pIgA-G, pIgA-C and pIgA-L were incubated with anti-J-chain IgG, only ^{125}I-labelled pIgA-G[14] and pIgA-C displayed a change in sedimentation behaviour (not shown). These data collectively established firmly the presence of J chain in pIgA-G and pIgA-C, and its absence in pIgA-L.

Binding of FSC to the pIgA preparations

By AE both rat and human FSC bound to pIgA-G and pIgA-C, but not to pIgA-L; thus, the FSC bands persisted unchanged when mixed with the latter, but disappeared when mixed with the two former pIgA preparations (Fig. 2a). Similarly, by IEP rat and human FSC bound clearly to pIgA-C and pIgA-G, but not to pIgA-L (Fig. 2b), as shown by a visible change in electrophoretic mobility and/or shape of the SC precipitin arc.

When human FSC was ^{125}I-labelled and mixed with the three pIgA preparations before SDGU, the ^{125}I-labelled FSC peak, which alone sedimented slower than pIgA, was not significantly altered by mixture with pIgA-L, whereas pIgA-C and pIgA-G produced a much faster sedimentation peak, demonstrating binding of SC to pIgA[14] (not shown).

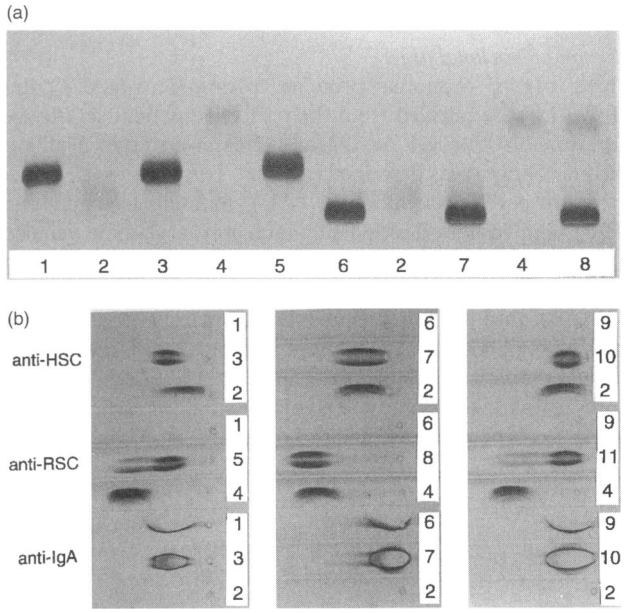

Figure 2. Binding of human or rat FSC with pIgA-G and pIgA-C, but not with pIgA-L. (a) Agarose electrophoresis (anode at the top); (b) immunoelectrophoresis (anode at the left). Lane 1, pIgA-C; lane 2 human FSC; lane 3 mixture of lanes 1 and 2; lane 4 rat FSC; lane 5 mixture of lanes 1 and 4; lane 6 pIgA-L; lane 7 mixture of lanes 6 and 2; lane 8 mixture of lanes 6 and 4; lane 9 pIgA-G; lane 10 mixture of lanes 9 and 2; and lane 11 mixture of lanes 9 and 4. Electrophoretic mobility and/or shape of the anti-SC precipitin arcs changed when human or rat FSC is complexed with pIgA-C and pIgA-G, but not with pIgA-L. All pIgA : FSC molar ratios were 2 : 1.

When human FSC was FITC-labelled and mixed with pIgA-G or pIgA-C before SDGU, there was a strong decrease of fluorescence at the level of fluorescent FSC (upper band), with appearance of a heavier peak of FITC–SC bound to the two pIgA preparations (lower band), contrasting the result obtained with pIgA-L (Fig. 3).

Finally, when the BIAcore 2000 sensor chip was coupled with human FSC and exposed to the three pIgA preparations, a strong resonance signal (indicating good binding) was observed only with pIgA-G and pIgA-C (Fig. 4).

Transport of pIgA by pIgR-expressing epithelial cells *in vivo* and *in vitro*

Because the above data were obtained only with soluble proteins *in vitro*, the need for J chain in epithelial SC/pIgR-mediated pIgA transport was further studied in living epithelial cells by comparing first, biliary transport of the three i.v. injected, ^{125}I-labelled pIgA preparations in bile duct-cannulated rats; and second, apical transcytosis of the three cold and ^{125}I-labelled pIgA preparations by human pIgR-expressing MDCK cell monolayers cultured on filter inserts.

The hepatic transport kinetics of i.v. injected ^{125}I-labelled pIgA in rats is shown in Fig. 5. The recovery of the three preparations in 3 hr bile (Table 1) was, as expected, about

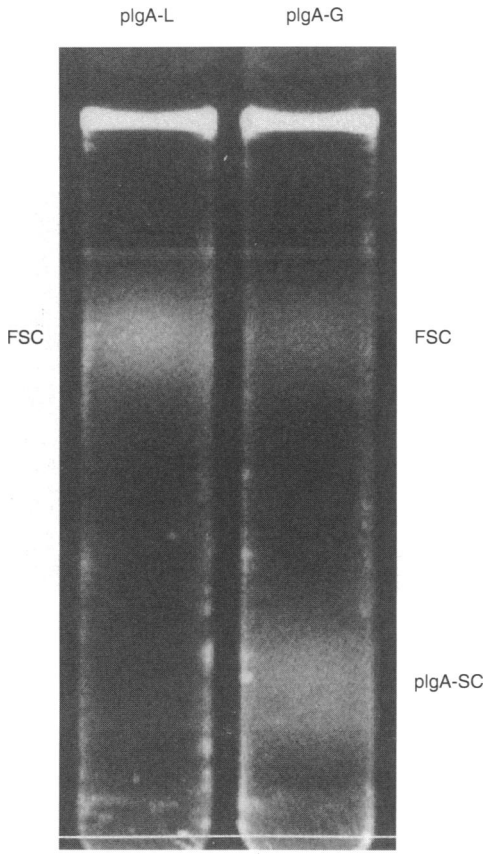

Figure 3. Sucrose density gradient ultracentrifugation of FITC-labelled human FSC mixed with 3·8 molar excess of pIgA-G (right tube) or pIgA-L (left tube). FITC–FSC mixed with pIgA-L sedimented at the same level (FSC) as FITC–FSC alone (not shown), whereas the majority sedimented much faster when mixed with pIgA-G (pIgA-SC), indicating complex formation. Tubes were scanned under ultraviolet light.

Table 1. Recoveries of i.v. injected ^{125}I-labelled pIgA in 3 hr rat bile

Tetramer preparation	Mean	*n*	SD	Range
pIgA-G	41·64*	4	2·83	(38·0–44·1)
pIgA-C	38·50	5	6·38	(31·6–47·3)
pIgA-L	1·84†	5	0·26	(1·68–2·31)

*Recoveries expressed as percentage of the i.v. injected c.p.m. amount.

†Overestimated because TCA-precipitability of ^{125}I-labelled labelled pIgA-L in bile was $\leqslant$78.3% versus $\geqslant$97.9% for ^{125}I-labelled pIgA-C and pIgA-G.

40% for pIgA-G and pIgA-C, but only about 2% for pIgA-L that lacked J chain. Furthermore, Fig. 6 illustrates that MDCK cells expressing the human pIgR transported pIgA-G and pIgA-C much better than pIgA-L; the apical gain of ^{125}I-labelled pIgA-L in 24 hr was only $0{\cdot}47 \pm 0{\cdot}08$ ng/insert (mean $\pm$ SD), whereas it was $2{\cdot}21 \pm 0{\cdot}24$ ng/insert, or roughly five times more, for pIgA-C. Similar apical transport of ^{125}I-labelled pIgA-G was reported earlier.[17]

DISCUSSION

Based on four methods, our results clearly established the presence of J chain in two tetrameric monoclonal human pIgA molecules, pIgA-G and pIgA-C, and its absence in tetrameric pIgA-L. In agreement with the notion that J chain is required in pIg for its binding to SC/pIgR,[9,12] we further demonstrated with different methods that this was also the case for our three pIgA preparations because pIgA-L was totally unable to bind human or rat FSC *in vitro*. All comparisons between the three monoclonal pIgA preparations were performed under virtually identical conditions, including selection of tetrameric test fractions. Our data were supported by studies with pIgR-expressing living cells, employing: polarized MDCK mono-

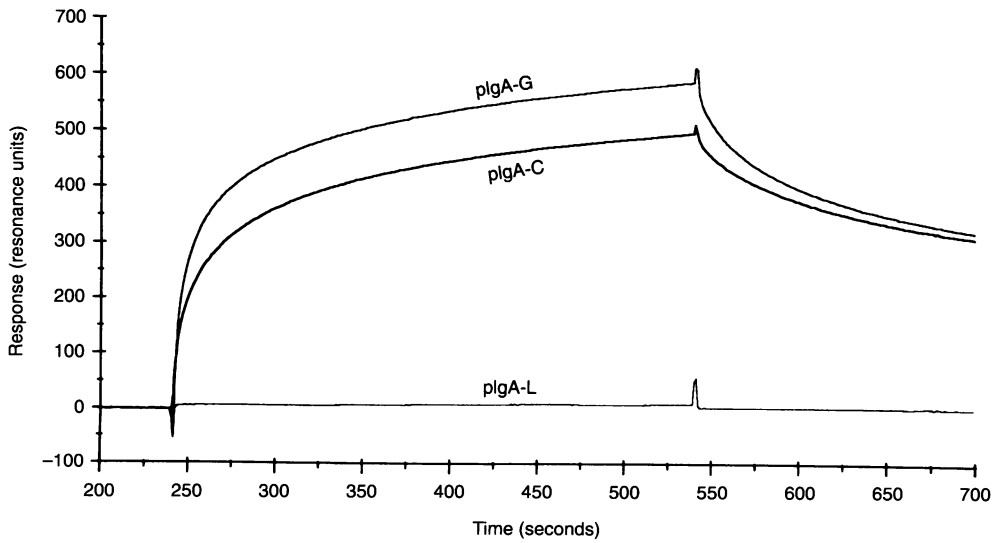

Figure 4. Surface plasmon resonance demonstration of binding of pIgA-G and pIgA-C, but not pIgA-L, to a human FSC-coupled sensor chip. Three representative sensorgrams are shown, as indicated. Control sensorgrams for each pIgA were subtracted (see Materials and Methods). Note total absence of SC binding to pIgA-L.

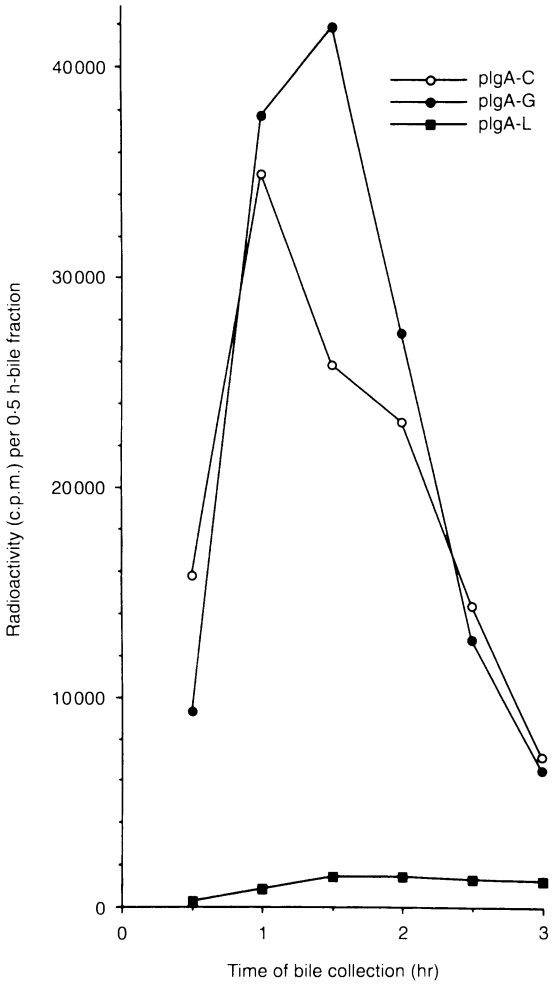

Figure 5. Kinetics of transport of ^{125}I-labelled pIgA-C, pIgA-G and pIgA-L into successive (30 min) rat bile samples. Each pIgA (260 000 280 000 c.p.m.) was i.v. injected into four or five rats before bile duct cannulation. A representative curve is shown for each labelled pIgA preparation.

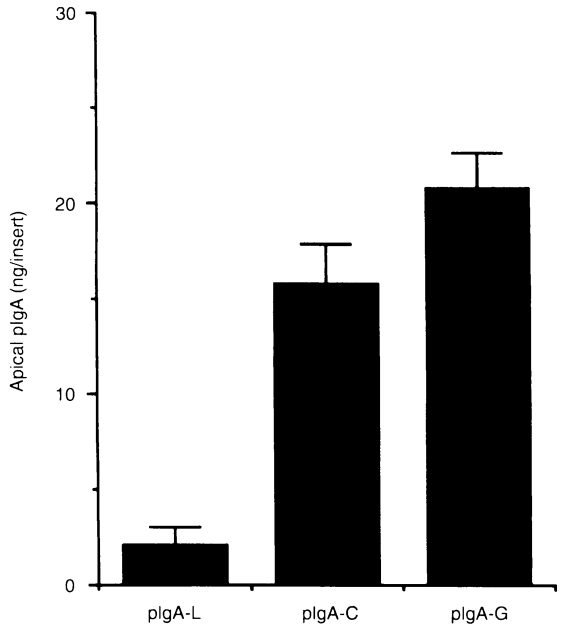

Figure 6. Apical transport of pIgA-L, pIgA-C and pIgA-G, in ng/insert (mean + 1 SD), by polarized MDCK cell monolayers expressing the human pIgR.

layers expressing the human pIgR; and rat hepatocytes *in vivo* with pIgA transfer into bile as read-out system. Again our results unequivocally demonstrated that the presence of J chain in pIgA correlated with active epithelial transport both *in vitro* and *in vivo*. Together with the recently reported blocking effect of two different antibodies to J chain on such transport,[14] our data unanimously support the suggested key role of J chain in secretory immunity.[9,12]

Contradictory results have been published in this respect, however. Thus, two J chain-deficient human pIgA myeloma proteins were claimed to bind covalently bovine ^{125}I-labelled FSC,[26] but no data were provided to show the biological relevance of the interactions, which were weaker than those of J-chain-containing pIgA. In another report, the fraction of a J-chain-containing myeloma, ^{125}I-labelled pIgA that did not bind to FSC–Sepharose, nevertheless was claimed to contain labelled J chain (not confirmed immunologically), although it was poorly transported into rat bile.[27] One possibility is that this fraction was denatured, because we and others have shown that pIgA, when exposed to dissociating agents such as concentrated urea or propionic acid, still contains J chain[20,21] but is poorly transported into rat bile.[14] Notably, our method to isolate J chain-deficient pIgA-L did not involve denaturing agents, and our method for ^{125}I-labelling[23] was chosen because it did not induce a faster catabolism of ^{125}I-labelled IgG compared with unlabelled IgG, representing a test quite sensitive even to minor immunoglobulin denaturation.

It has also been reported that human FSC could bind covalently to, and prevent dissociation of, both polymers and monomers of human IgA2m(1) a less stable IgA variant that dissociates into heavy-chain dimers or larger polymers and light-chain dimers under the sole influence of non-reducing dissociating solvents.[28,29] Because such FSC binding required a large excess of FSC, and also 'stabilized' monomers and the $F(ab')_2$ fragments of IgA2m(1) J chain was clearly not involved in the interactions. In addition, monomeric IgA2m(1) has neither been shown to combine *in vivo* with SC/pIgR, nor to be actively transported by SC/pIgR-expressing epithelial cells. Conversely, we have shown that only J-chain-containing polymeric Fcα fragments, and not Fabα, were actively transported into rat bile when these two fragments of a pIgA1 myeloma protein were produced by IgA protease treatment.[24]

The data from the elegantly constructed J-chain KO mice[15,30] are difficult to interpret, providing arguments both for and against the requirement of J chain for IgA transport into external secretions. In support of this notion was the low content of IgA in bile and faeces of the J-chain KO mice, the lack of associated SC in IgA from all examined secretions (milk, bile, nasal wash, bronchoalveolar lavage, intestinal surface, faeces) and the absent apical transport of their serum IgA by MDCK cells expressing the rabbit pIgR.[15] Apparently in contrast to this, however, were the equal or even higher IgA concentrations in milk, nasal wash, bronchoalveolar lavage and intestinal surface fluid from the same animals compared with samples from normal mice.[30] The authors[30] suggested that there may exist a non-pIgR-mediated,

alternative epithelial transport mechanism for IgA, because similar levels of both IgA and IgG were found in small and large intestinal fluid (but not in faeces) of J-chain KO and normal mice. The surprisingly high level of intestinal IgA in J-chain KO mice was not believed to be explained by passive transfer from their elevated serum IgA content (>30-fold increased over normal). However, the size and structure of their IgA both in secretions and serum remained virtually undefined. In particular, several bands reacting with anti-α-heavy chains in Western blots of unreduced IgA were poorly defined with regard to size and/or composition. Moreover, a possible influence of the size of IgA on the ELISA used to quantify IgA in body fluids and their MOPC-315 IgA standard was not considered.[31] Finally, it is unknown whether the lack of J chain in these animals results in mucosal production of unusually large amounts of mIgA that easily may leak into the lumen. Altogether therefore additional data are needed before a putative new epithelial transport mechanism for J-chain-deficient IgA can be accepted.

It remains elusive why rare myeloma patients apparently synthesize J-chain-deficient pIgA. We do not believe that J chain, although sensitive to proteolysis in its isolated form,[32] could have been lost during storage of our serum samples; we have never observed this artefact for pIgA isolated from sera that have been stored frozen for over 15 years. Selective proteolysis of J chain in human pentameric IgM by subtilisin-like enzymes has been well documented, but the J chain in pIgA appeared less susceptible, though not completely resistant, to such degradation.[33] What we occasionally have observed is that purified pIgA stored without antiproteases, produced less sharp J-chain bands in Western blot (SDS–PAGE with reduction) than when the same pIgA samples were freshly tested (unpublished data). This result suggests that some J-chain degradation may also occur in native isolated pIgA, rendering quantification of J chain in pIg preparations quite problematic. It would have been of interest to examine if the patient producing the J-chain-deficient monoclonal pIgA-L in addition produced normal J-chain-containing polyclonal pIgA, but he unfortunately died before samples were obtained for sIgA measurements in secretions.

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