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Biliary transport of IgA: Role of secretory component

(secretory IgA/protein transport/hepatocyte)

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ABSTRACT **Biliary transport of rat immunoglobulin was studied by perfusion of isolated rat liver with blood containing radiolabeled immunoglobulin. Transport to bile was selective for polymeric IgA. Between 15 and 27% of polymeric IgA was transported from blood to bile during a 210-min perfusion period, and approximately 60% of the IgA transported to bile bore secretory component. Small quantities of IgM (0.12%) were transported; transport of IgG_{2a}, IgE, or monomeric IgA was not detected. Purification of radiolabeled polymeric IgA by affinity chromatography on human secretory component-Sepharose yielded a fraction that was transported more efficiently (i.e., up to 40% transported). In contrast, secretory IgA (colostral or biliary) was transported 1/25th to 1/12th as well as polymeric IgA myeloma protein. Complexes of ^{125}I-labeled secretory component and polymeric IgA formed *in vitro* were transported poorly (0.1%) compared to polymeric IgA (26%). It was concluded that biliary transport of polymeric IgA requires combination of it with secretory component in the liver. In support of this hypothesis, rabbit IgG anti-rat secretory component antibodies were also transported to bile but normal rabbit IgG was not.**

In the rat, IgA is selectively transported from blood to bile in a manner that may be analogous to the mechanism of transport of IgA to all external secretions (1). Transport of IgA to the external secretions is thought to be mediated by secretory component (SC), a glycoprotein synthesized by mucosal epithelial cells. This hypothesis was based initially on immunohistological evidence that indicated that polymeric IgA (pIgA) and polymeric IgM (pIgM) were complexed to SC within the mucosal epithelial cell (2, 3). Recently, it was reported that SC occurs on the surface of human epithelial cells and colon carcinoma cells (4–6). pIgA binds to the surface of these cells but prior incubation of pIgA with SC or incubation of the cells with antibodies to SC prevents binding of the IgA to the epithelial cell (5, 6). *In vitro* binding studies with isolated SC and human monoclonal proteins indicated that SC binds with high affinity to pIgA and pIgM but not to monomeric immunoglobulins (7, 8), and SC is found associated with pIgA and pIgM in the external secretions (9). In the rat, SC binds only to pIgA with high affinity (10).

The isolated, perfused rat liver provides a means to measure the transport of immunoglobulins quantitatively. This paper describes a series of experiments designed to elucidate the molecular properties required for transport of immunoglobulin by the rat liver and, in particular, the role of SC in this process.

MATERIALS AND METHODS

Proteins. Rat monoclonal proteins were obtained from the serum of rats bearing immunocytomas (11). Rat IgA monoclonal protein (IR699) was isolated from serum by methods similar to those used for human monoclonal proteins (12). After DEAE-cellulose chromatography, rat pIgA was purified by successive gel filtration on Sephadex G-200 and Sepharose 6B in 0.01 M potassium phosphate/0.15 M NaCl, pH 7.4 (P_i/NaCl). The monomeric form was purified by rechromatography on Sephadex G-200 in P_i/NaCl. Rat pIgA (IR22) was isolated by successive gel filtration on Sephadex G-200 and Sepharose 6B. The rat pIgA proteins eluted from a Sepharose 6B column in a position similar to that of human IgA dimer; the rat monomeric IgA (mIgA) eluted from Sephadex G-200 in a position identical to that of human IgA monomer.

When tested in immunoelectrophoresis at 5 mg/ml, the rat IgA proteins gave a strong arc with anti-rat IgA and were found to contain two additional contaminants when tested with an antiserum to whole rat serum. Between 65 and 85% of the radiolabeled (^{125}I or ^{131}I) purified rat IgA was precipitable with a specific anti-rat IgA antiserum. Rat secretory IgA was purified from colostrum or bile in a manner similar to IgA protein IR22. Immunoelectrophoresis with anti-rat serum antiserum revealed only one contaminant, and the preparations of radiolabeled secretory IgA were 85% precipitable with anti-rat IgA and 75% precipitable with a specific antiserum to rat SC. Rat and human SC were prepared as described (10), as were human immunoglobulins (12).

Rat monoclonal proteins IgE (IR162), IgG_{2a} (S207), and IgM (IR202) were isolated by methods similar to those described (11, 12). The rat IgM was filtered on Sepharose 6B to remove aggregates. Each of these proteins showed a single band on immunoelectrophoresis.

Antisera. Antisera to rat IgG, rat IgA, and rat IgM were purchased from Miles or prepared in our own laboratories by multiple immunization of rabbits or goats with purified monoclonal proteins. The antisera prepared in our own laboratory to rat IgA were rendered class specific by absorption with a Sepharose-normal rat serum immunosorbent. The anti-rat IgA gave a pattern of identity when tested in immunodiffusion tests with purified IgA monoclonal proteins and secretory IgA. Antisera specific for both "fast" and "slow" rat SC (13) was prepared as described (10).

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Abbreviations: pIgA, polymeric IgA; pIgM, polymeric IgM; mIgA, monomeric IgA; SC, secretory component; P_i/NaCl, 0.01 M potassium phosphate/0.15 M NaCl, pH 7.4.

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Purification of Rabbit Anti-Rat SC IgG Antibodies. Purified rabbit antibodies to rat SC were prepared by passing 3 ml of specific anti-rat SC antiserum over an immunosorbent consisting of Sepharose coupled to 3 mg of rat SC and 3 mg of rat secretory IgA. After the unbound protein was removed by elution with P_i/NaCl, the bound antibody was eluted with 1 M acetic acid and subjected to exhaustive dialysis against P_i/NaCl. The isolated antibody (1 mg/ml) was tested in immunodiffusion with specific antisera to rabbit IgG, IgA, and IgM and only rabbit IgG was detected. The specifically purified IgG anti-rat SC antibodies reacted in immunodiffusion tests with isolated rat SC and secretory IgA but not with purified rat myeloma proteins. Prior to use in perfusion experiments, the anti-rat SC antibodies (labeled with ^{125}I) were filtered on Sephadex G-200 and only the 7S fraction was used. This preparation was 63% adsorbable by Sepharose-rat SC. Normal rabbit IgG was prepared by DEAE-cellulose chromatography (12).

Perfusion of Isolated Rat Liver. Rat livers (7–9 g) were removed from 250- to 350-g male Wistar rats and continuously perfused with 100 ml of heparinized rat blood as described (14). After a stabilization period of 2 hr, radiolabeled proteins were added to the perfusion fluid and serial samples of bile and perfusion fluid were collected for a period of 210 min. The quantity of protein added was less than 1% of the total pool of the particular class in the perfusion fluid. In most cases, two proteins were added simultaneously, one labeled with ^{125}I and the other labeled with ^{131}I. This double label technique facilitated comparison and normalized the variation that might have occurred between different experiments.

Radioimmunoprecipitation Analysis. The quantity of radiolabeled IgA precipitable with either anti-IgA or anti-SC was analyzed in two ways. In the first method, radiolabeled protein was added to 20 μg of purified secretory IgA followed by 100 μl of anti-rat IgA or anti-SC (previously determined to be in antibody excess). The precipitates formed after incubation at 4°C for 18 hr, were washed (three times with cold P_i/NaCl), isolated by centrifugation, and assayed for radioactivity in a gamma counter. In the second method, radiolabeled IgA was added to 5 μl of normal rabbit serum, 5 μl of rabbit anti-rat SC, or 5 μl of rabbit anti-rat IgA. After 1 hr, 200 μl (antibody excess) of sheep anti-rabbit IgG was added and the mixture was incubated at 4°C for 18 hr. The resulting precipitates were isolated and assayed as above. Prior to its use in the radioimmunoassay, the sheep anti-rabbit IgG was absorbed with excess normal rat serum to neutralize crossreacting antibody and the 7S fraction was isolated, free of antigen–antibody complexes, by Sephadex G-200 gel filtration.

Miscellaneous. Proteins were iodinated with the iodine monochloride technique (15) to a specific activity of 0.2–0.7 mCi (1 Ci = 3.7×10^{10} becquerels)/mg of protein. Complexes of ^{125}I-labeled SC (^{125}I-SC) and rat monoclonal IgA were prepared by adding 10 μg of ^{125}I-SC to 100 μg (excess) of rat IgA, incubating the mixture overnight at 4°C, and separating bound from free ^{125}I-SC on Sephadex G-200 in P_i/NaCl. Unlabeled complexes of rat SC (0.7 mg) to rat IgA (1.0 mg), incubating the mixture overnight at 4°C, and isolating the complex from free SC by Sephadex G-200 gel filtration. A control experiment in which no SC was added was also carried out. The protein eluting in the void volume (i.e, SC–pIgA) and pIgA, respectively, were then radiolabeled.

RESULTS

Kinetics of IgA transport

Transport of rat pIgA by the isolated perfused rat liver was remarkably consistent among different experiments. Radiolabeled IgA was routinely found in the first sample (10 min) of bile collected after addition of IgA to the perfusion fluid. A typical experiment is represented in Fig. 1. The maximal quantity (and concentration) of IgA in bile always occurred 90 min after addition of IgA to the perfusion fluid; the quantity of IgA in the last sample of bile collected was 33–38% of the peak value. The concentration of radiolabeled pIgA in bile at the peak value (90 min) was 20–30 times the concentration in the perfusion fluid at the same time. The radiolabeled IgA contained in bile was filtered on Sephadex G-200 and found to be polymeric. Radioimmunoprecipitation analysis in four separate experiments revealed that the radiolabeled IgA was 80–85% precipitable with anti-rat IgA, of which 40–75% (in

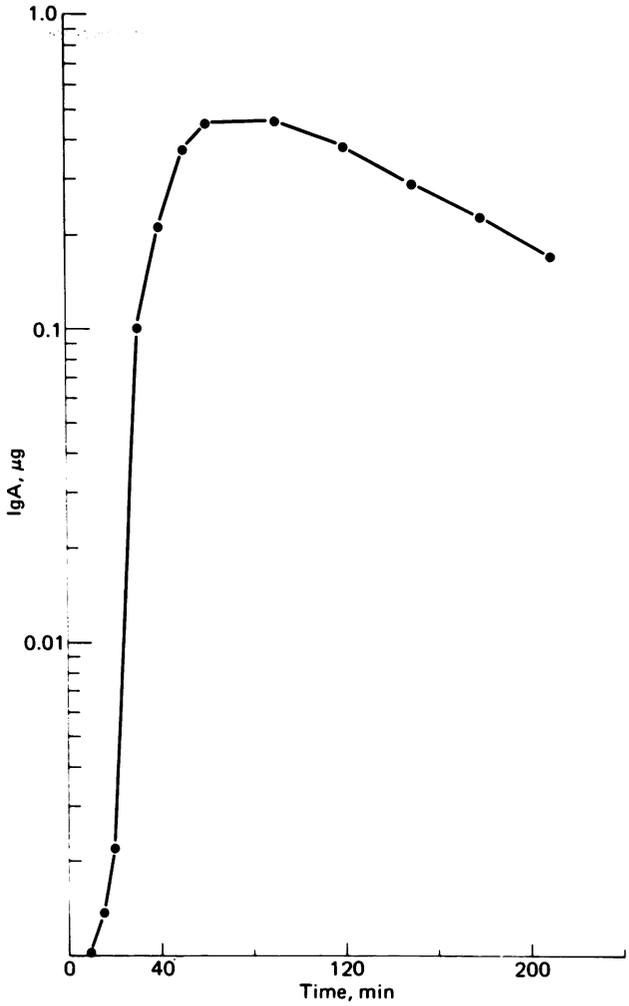

FIG. 1. Kinetics of biliary transport of radiolabeled IgA: 0.01 mg of ^{125}I-labeled pIgA was added to 100 ml of perfusate.

Table 1. Biliary transport of rat Ig classes

Exp.	Protein	% recovered in bile
1	^{125}I-pIgA	27
	^{131}I-pIgA	27
2	^{131}I-pIgA	24
	^{125}I-IgG2a	<0.05
3	^{131}I-pIgA	22
	^{125}I-IgE	<0.05
4	^{125}I-pIgA	26
	^{131}I-IgM	0.1

Table 2. Biliary transport of pIgA and mIgA

Exp.	Protein (from rat)	Inhibitor (human)	% recovered in bile	% inhibition
5	^{131}I-pIgA	—	25	—
	^{125}I-mIgA	—	<0.05	—
6	^{125}I-pIgA	pIgA*	2.2	92
7	^{125}I-pIgA	mIgA*	25	0

* At 1 mg/ml of perfusate.

Table 3. Biliary transport of secretory and serum IgA

Exp.	Protein	% recovered in bile
8	^{131}I-pIgA	15
	Colostral ^{125}I-pIgA	0.6
9	^{125}I-pIgA	27.0
	Biliary ^{131}I-pIgA	2.1
10*	^{131}I-pIgA (IR699)	17.0
	Biliary ^{125}I-pIgA (IR699)	6.0
11	^{131}I-pIgA	22
	^{125}I-(SC–pIgA)	5

* In this experiment the biliary ^{125}I-pIgA was the monoclonal rat pIgA transported to bile in a preceding rat liver perfusion experiment.

different experiments) was precipitable with anti-SC. In contrast, prior to perfusion, the radiolabeled IgA was 65–85% precipitable with anti-rat IgA and 2–3% precipitable with anti-SC. Both pIgA proteins IR22 and IR699 behaved in a similar fashion.

Comparison of biliary transport of different rat immunoglobulins

The results in Table 1 indicate marked selection for IgA. In 12 different perfusion experiments the mean (± SD) IgA transported was 23.8 ± 3.7%. A small but significant quantity of IgM was transported; no transport of IgE or IgG_{2a} was detected. In each case, collected bile and perfusion fluid was analyzed by gel filtration and the majority (≥75%) of the radiolabeled protein in both fluids was found to undegraded.

pIgA and mIgA. Transport of IgA was exclusive for pIgA (Table 2, Exp. 5). Gel filtration analysis of the mixture of polymeric ^{131}I-pIgA and ^{125}I-mIgA used in Exp. 5 of Table 2 is shown in Fig. 2*a*. The elution profile indicates that the mIgA protein contained a small quantity of polymer. Gel filtration of the perfusion fluid at the end of the experiment showed a decrease of polymer relative to monomer (Fig. 2*b*) and the appearance of polymer exclusively in bile (Fig. 2*c*).

Addition of human IgA dimer or IgA monomer prior to addition of rat ^{125}I-pIgA revealed the specificity of transport for pIgA (Exps. 6 and 7, Table 2). Only human IgA dimer blocked transport. Qualitative immunodiffusion analysis of bile collected in Exps. 6 and 7 revealed that human IgA dimer was transported to bile whereas human IgA monomer was not detected in bile.

Secretory IgA and Serum pIgA. Because only monoclonal pIgA was transported to bile, we compared secretory pIgA with serum monoclonal pIgA (Table 3). Both colostral (Exp. 8) and biliary (Exp. 9) secretory IgA were transported less well than serum (monoclonal) pIgA. Rat monoclonal ^{125}I-pIgA was also isolated from bile (by Sephadex G-200 gel filtration) after it had been transported in a liver perfusion experiment. This preparation was then compared with fresh ^{131}I-pIgA in a second perfusion (Exp. 10, Table 3). Again, the secretory form was transported less well. Radioimmunoprecipitation analysis indicated that the distribution of radiolabeled IgA bearing rat SC was: colostral secretory IgA, 87%; biliary secretory IgA, 87%; biliary ^{125}I-monoclonal pIgA (IR699), 60%. These results suggest that addition of SC to pIgA decreases its ability to be transported to bile.

Because it was possible that another process common to the transport process could also be responsible for the decreased transport of secretory IgA, we examined the biliary transport

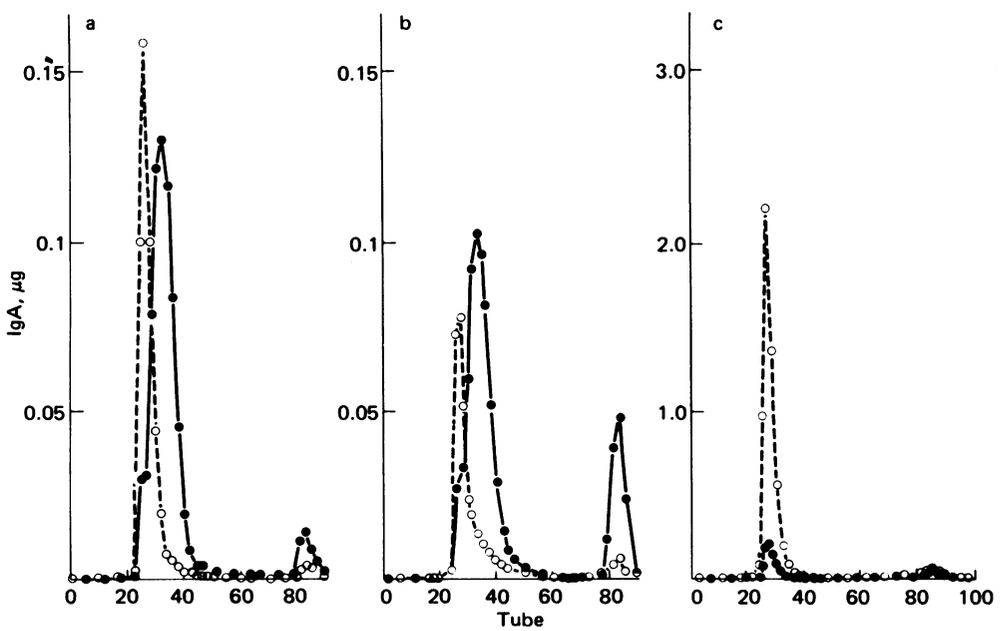

FIG. 2. Gel filtration (Sephadex G-200; P_i/NaCl) analysis of material used in Exp. 5 of Table 2. ○, ^{131}I-pIgA; ●, ^{125}I-mIgA. (*a*) Input mixture added to perfusion fluid; (*b*) sample of blood perfusate taken after liver perfusion; (*c*) sample of bile collected after liver perfusion.

Table 4. Biliary transport of SC–IgA complexes

Exp.	Protein	% recovered in bile
12	^{131}I-pIgA	23
	^{125}I-SC–pIgA	0.07
13	^{125}I-SC–pIgA	0.1
14	^{125}I-SC–pIgA	0.1

of complexes formed *in vitro* between SC and pIgA. In this experiment, immunoprecipitation analysis indicated that 70% of the complex was associated with SC. The complex formed *in vitro* between SC and monoclonal pIgA was transported less well than was the uncomplexed pIgA (Exp. 11, Table 3).

^{125}I-(SC–pIgA) Complexes. The above experiments suggested that complexes of SC with pIgA could not be transported to bile. Consequently the fate of ^{125}I-SC complexed to pIgA was followed as a means of studying SC-containing complexes directly. In each experiment, transport of radiolabeled complexed SC was much less than that of pIgA (Table 4).

pIgA Isolated by Affinity Chromatography on SC-Sepharose. Affinity chromatography of radiolabeled rat pIgA on human SC-Sepharose yielded two fractions, one that could bind rat SC (80% of total protein) and one that could not. Both fractions had similar elution profiles on Sepharose 6B but the radiolabeled pIgA fraction that did not bind to the SC-Sepharose column was not precipitable by anti-rat IgA, indicating it contained either denatured IgA or contaminating protein. In two liver perfusion experiments with the affinity-purified fraction, 30 and 40% of the radiolabeled IgA was transported. The fraction that did not bind to the SC-Sepharose column was not transported.

Anti-SC Antibodies. The above experiments suggested that pIgA was transported to bile by virtue of its affinity for SC, presumably located in the rat liver. Accordingly, radiolabeled purified anti-rat SC antibodies were added to the perfusion fluid to determine whether an IgG molecule with affinity for SC would be transported. The results (Table 5) clearly show that transport of rabbit IgG anti-SC did occur, although less efficiently than transport of rat pIgA. Gel filtration of collected bile on Sephadex G-200 revealed ^{125}I-labeled anti-SC in both the excluded fraction (presumably complexed to SC) and in the 7S fraction. Gel filtration on Sepharose 4B in 6 M guanidine·HCl established that all the radioactivity eluted in a single peak consistent with IgG and ruled out the possibility that the radioactivity transported in the ^{125}I-labeled anti-SC preparation was due to contaminating rabbit pIgA.

DISCUSSION

Biliary transport of immunoglobulin by the isolated, perfused rat liver was selective for pIgA, a result that is consistent with previous qualitative analysis of rat hepatic bile (1). The quantity of radiolabeled IgA added was <1% of the total IgA pool and the percentage of radiolabeled IgA transported appeared to be independent of the amount (0.4–40 μg) of IgA added. Thus, the transportable fraction of the radiolabeled IgA behaved in a manner similar to that of the unlabeled IgA in the blood perfusate.

Table 5. Biliary transport of purified IgG anti-SC antibodies

Exp.	Protein	% recovered in bile
15	^{125}I-anti-SC	3.9
	^{131}I-IgG	<0.05
16	^{125}I-anti-SC	2.6
	^{131}I-IgG	<0.05

Transport of IgA was specific for the polymeric form, and the bulk of the evidence presented suggests that SC acts as a liver cell receptor for pIgA and mediates its transport into the bile space. SC binds with high affinity only to pIgA and only this form was transported. Secretory IgA was not transported, presumably because it would be unable to bind to the putative SC receptor in the liver. Finally, although IgG was not transported, IgG that could bind SC (i.e., anti-SC antibodies) was transported.

Hall and associates (16) have recently demonstrated radiolabeled IgA in hepatocytes of rats injected intraveneously with this protein. Thus, SC may be located in the plasma membrane of the rat hepatocyte. IgA passing through the venous sinusoid would bind SC and be transported to the bile space. Alternatively, IgA may be transported from blood via the bile duct epithelium.

In considering mechanisms of biliary transport, it should be noted that Thomas and Summers (17) reported that glycoproteins in general are cleared by the rat liver. In their experiments, somewhat less glycoprotein (≤10%) was transported to bile than reported here for radiolabeled IgA (up to 40%). Because mIgA (both native and reduced) as well as IgE [a glycoprotein (18)] were not transported, we believe it unlikely that transport of pIgA is mediated by a carbohydrate receptor in the liver. Indeed, transport of IgG anti-SC antibodies but not of normal IgG argues strongly against carbohydrate determinants being important for biliary transport of immunoglobulin anti-SC.

Radioimmunoprecipitation analysis indicated that only a portion of the radiolabeled IgA transported to bile bore rat SC (i.e, 50–70% in the case of IgA myeloma and 80% in the case of native biliary IgA). It is possible that, during isolation, some of the SC dissociates from the complex. We have assumed that the fraction of radiolabeled secretory IgA transported to bile was the material which did not contain SC. Indeed, it was demonstrated that SC complexed to IgA was transported poorly and that addition of SC to IgA *in vitro* decreased the fraction of IgA transported. The simplest assumption therefore is that binding of SC to pIgA prevents the pIgA from binding to cell-bound SC and thus from being transported. In the rat, SC binds IgM weakly (ref. 10; unpublished data), and in fact relatively little IgM was transported by the perfused liver (Table 1). It will be of interest to carry out similar experiments in an animal in which SC binds IgM with high affinity [e.g., man (7, 8)] to determine whether biliary transport of IgM occurs as efficiently as transport of pIgA.

The possibility that SC might function as a transport protein was first raised by South *et al.* (19) who suggested the term "transport" or "T piece" for this protein. It has also been demonstrated that infusion of canine pIgA into dogs results in the appearance of the IgA in saliva (20) whereas secretory IgA injected intravenously in humans fails to enter the secretions (21). These experiments and those cited earlier suggest that the secretion of pIgA into bile and the external secretions is accomplished by the same mechanism and suggest that SC may be the primary receptor for IgA in this process.

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