Human IgA as a Heterovalent Ligand: Switching from the Asialoglycoprotein Receptor to Secretory Component during Transport across the Rat Hepatocyte

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Abstract. Asialoglycoproteins are taken up by the rat liver for degradation; rat polymeric IgA is taken up via a separate receptor, secretory component (SC), for quantitative delivery to bile. There is negligible uptake of these ligands by the converse receptor, and only a low level of missorting of ligands to opposite destinations. The two pathways are not cross-inhibitable and operate independently (Schiff, J. M., M. M. Fisher, and B. J. Underdown, 1984, *J. Cell Biol.*, 98:79–89). We report here that when human IgA is presented as a ligand in the rat, it is processed using elements of both pathways. To study this in detail, different IgA fractions were prepared using two radiolabeling methods that provide separate probes for degradation or re-secretion.

Behavior of intravenously injected human polymeric IgA in the rat depended on its binding properties. If deprived of SC binding activity by affinity adsorption or by reduction and alkylation, >80% of human IgA was degraded in hepatic lysosomes; radioactive catabolites were released into bile by a leupeptin-inhibitable process. If prevented from binding to the asialoglycoprotein receptor by competition or by treatment with galactose oxidase, human IgA was cleared and transported to bile directly via SC, but its uptake was about fivefold slower than rat IgA. Untreated human IgA was taken up rapidly by the asialoglycoprotein receptor, but depended on SC binding to get to bile: the proportion secreted correlated 1:1 with SC binding activity determined in vitro, and the IgA was released into bile with SC still attached. These results demonstrate that human IgA is normally heterovalent: it is first captured from blood by the asialoglycoprotein receptor, but escapes the usual fate of asialoglycoproteins by switching to SC during transport. Since the biliary transit times of native human and rat IgA are the same, it is probable that the receptor switching event occurs en route. This implies that the two receptors briefly share a common intracellular compartment.

During its transport across the liver hepatocyte, immunoglobulin A (IgA)[1] must be separated from other ligands being brought into the cell for degradation in lysosomes. Entrance into the transcellular and degradative pathways is mediated by distinct receptors. The sorting step occurs either at the cell membrane or in an early intracellular compartment (39). This gives rise to an intriguing question: what would be the fate of a ligand able to bind to receptors for both pathways? One might predict a priori that the division of the ligand population would depend simply on the relative surface concentration of the two receptors and their relative efficiency in capturing the ligand from the circulation. However, for the example studied in this paper, this turns out not to be the case.

[1] *Abbreviations used in this paper:* ASFet, asialo-fetuin; ASG, asialoglycoprotein(s); ASOr, asialo-orosomucoid; BH, Bolton and Hunter (reagent); IgA, immunoglobulin A; HBP, hepatic binding protein (the ASG receptor); HPLC, high pressure liquid chromatography; SC, secretory component.

In the rat, transport of autologous polymeric IgA intact to bile is a highly efficient process. Its receptor is the transmembrane form of secretory component (SC) (28) present on the surface of the hepatocyte at ~200,000 molecules per cell (42). Intravenously injected rat polymeric IgA is cleared rapidly from blood and appears in bile ~30 min later (11, 19, 31). Transport across the cell occurs primarily via small vesicles (30, 36, 50); there is conflicting morphological evidence regarding the possible participation of other intracellular organelles (14, 21). IgA binds to its receptor with high affinity: the cell must therefore sacrifice the receptor by polypeptide cleavage in order to jettison the ligand at the canalicular face (23). To our knowledge, IgA is the only protein endocytosed by the liver that is primarily disposed by re-secretion across the cell.

Since the liver plays an important physiological role in the regulation of protein balance in blood, many circulating pro-

teins are taken up by the hepatocyte for degradation. These include lipoproteins, polypeptide hormones, and senescent plasma proteins. Perhaps the best studied model ligand for this pathway is asialoglycoprotein (ASG) (reviewed in references 1 and 49). Its receptor is composed of 41,000-mol-wt subunits, which assemble into polymers in the plasma membrane (46). These receptors are at least as frequent on the cell surface as SC (41, 59, 60). Specific endocytosis is accelerated by ligand binding (6): it initiates a pathway that takes ASG through an acidic compartment and multivesicular bodies to secondary lysosomes, the terminal organelle where digestion occurs (7, 13, 47, 55).

Because human IgA binds to rat SC in vitro, it is sometimes used as a ligand in the rat hepatobiliary transport model. Unlike rat IgA, the natural substrate, human IgA has been detected by electron microscopic autoradiography in multivesicular bodies and lysosomes (20). This prompted us to examine the possibility that human IgA could optionally use an alternative receptor to gain entry into the cell, and thereby into a different pathway.

Our strategy was to perturb the ability of radiolabeled IgA to interact with hepatic protein receptors by affinity separation, chemical alteration, or competition with unlabeled ligands. We found that two different receptors, which normally mediate separate events, can both bind human IgA. These receptors participate cooperatively in the efficient transport of human IgA to bile.

Materials and Methods

Proteins

Human monoclonal polymeric IgA was isolated from the plasma of a patient with IgA1 myeloma by ammonium sulfate and octanoic acid precipitation (10). IgG and minor impurities were removed by two rounds of chromatography on DEAE (diethylaminoethyl) cellulose using a step gradient of NaCl in Tris buffer, pH 8.0. Aggregates were removed by gel filtration on Sepharose 6-B (Pharmacia Fine Chemicals, Uppsala, Sweden; 4.4 × 140 cm in phosphate-buffered saline [PBS]: 0.15 M NaCl, 0.01 M phosphate buffer pH 7.4, 0.02% azide). Rat monoclonal IgA was derived from the ascites fluid of plasmacytoma line IR22, kindly provided by H. Bazin of the University of Louvain, Brussels. It was isolated in a similar fashion, except that only one round of DEAE chromatography was performed. The polymeric fraction was recovered on a 2.6 × 90-cm Sepharose 6-B column. Human SC was isolated from colostral whey by affinity chromatography on IgM Sepharose (52). Rat SC was isolated by a similar procedure, from the Sephadex G-50 excluded fraction of pooled rat bile (manuscript in preparation).

For the competition experiments shown in Figs. 5–7, fetuin was isolated from fetal calf serum according to the method described by Spiro (44). It was desialylated in 10 mM phosphate buffer, pH 6.1, by incubating on a rotator for 2 d at 37°C with agarose-linked *Clostridium perfringens* neuraminidase (type X-A, purchased from Sigma Chemical Co., St. Louis, MO). As detected by the thiobarbituric assay (56), this resulted in the release of 54% of the total hydrolyzable sialic acid. Parallel transport experiments were conducted with fetuin purified by ammonium sulfate fractionation (32) (Sigma type III) and desialylated by acid hydrolysis (44). Competition experiments using the different Fet and ASFet preparations gave compatible results. Purified human orosomucoid was generously donated by the American Red Cross Blood Products Laboratory, Bethesda, MD, and was desialylated by acid hydrolysis (44).

Radiolabeling

[^{125}I]Bolton and Hunter (BH) reagent was purchased from Amersham Corp., Arlington Heights, IL. Alternatively, [^{125}I]BH reagent and [^{131}I]BH reagent were prepared by labeling *N*-hydroxysuccinimidyl 3(4-hydroxyphenyl) propionic acid (Tagit; Calbiochem-Behring Corp., La Jolla, CA) using the chloramine-T method (4). The procedure was adapted for the ^{131}I isotope by maintaining the same ratio of iodide/Tagit in terms of milliCuries/micrograms as described for ^{125}I (4). Labeling was carried out by drying 20–200 μCi of the reagent in the bottom of a small test tube, adding 100 μg of protein in 200 μl of 0.1 M Tris buffer (pH 8.0), and incubating at 0°C for 30 min. Unconjugated reagent was removed from labeled IgA by dialysis against 0.85% saline that contained 0.1% glycine overnight at room temperature, then against saline alone. The IgA was stored with 1 mg/ml human albumin in PBS, and dialyzed or redialyzed within 3 wk before use in a transport experiment. BH–asialo-orosomucoid (ASOr) was prepared as described previously (39). BH–protein conjugate preparations contained <4% free radiolabel, as determined by high pressure liquid chromatography (HPLC) analysis.

To generate probes for the transcellular pathway alone, proteins were directly radioiodinated by the iodine monochloride method, using ICl prepared in our laboratory (16). Labeling 100 μg protein gave specific activities of 10^8–10^{10} cpm/mg at a substitution ratio of less than one atom of ^{125}I or ^{131}I per molecule. Free radioiodide was removed by exhaustive dialysis against PBS that contained 0.075% NaI, and then PBS alone. Radioactivity was >98% precipitable in 15% trichloroacetic acid.

Affinity Separation of IgA Fractions

Isolated human SC was coupled directly to BrCN-activated Sepharose (33, 40) at 1 mg SC/ml resin. Unreacted coupling groups were blocked with 40 mg/ml bovine albumin in PBS. SC-binding activity was removed from purified, unlabeled human IgA by several passages over a 3-ml column of SC-Sepharose. After washing, the adherent fraction was recovered by elution with 1 M KSCN in 0.01 M potassium phosphate, pH 7.4, and then dialyzed. Labeled human or rat IgA preparations were fractionated by incubating in 1 ml PBS with 100 μl SC-Sepharose on a rotator in the presence of 1 mg unlabeled human albumin as carrier. The adherent fractions were recovered from the washed adsorbant after a 1 h incubation, while the activity in the supernatant was passaged 2–4 times over fresh aliquots of SC-Sepharose for further depletion of binding activity over 12-h periods. Each BH- and ICl-labeled fraction was prepared at least twice for use in the hepatobiliary transport experiments.

Size fractionation of non-adherent human IgA was performed on an SW-3000 gel filtration HPLC column with a GSWP precolumn (LKB-Produckter AB, Bromma, Sweden). Samples were run at 1 ml/min in 0.85% saline, and 1-ml aliquots were collected for counting. 2 ml from the ascending limb of the first peak or from the descending limb of the second peak constituted the polymer and monomer fractions, respectively. Reduction and alkylation of the affinity purified fraction were performed in PBS at room temperature by incubating the radiolabeled protein for 1 h with 10 mM dithiothreitol, and then with 2.5 equivalents of iodoacetamide for 2 h. Treatment with galactose oxidase was done in azide-free PBS by the addition of 5 U of the enzyme (Sigma type V, from *Dactylium dendroides*) to a 0.5-ml solution of labeled IgA, and incubation at room temperature overnight. This procedure has been shown elsewhere to prevent human IgA from inhibiting the ASG-receptor-induced cross-linking of galactose-bearing red cells (48). Radiolabeled IgA modified by either method was redialyzed against 0.85% saline before use in transport studies.

Characterization of the IgA Fractions

Heavy and light chain content was determined by polyacrylamide gel electrophoresis in sodium lauryl sulfate (25). J chain content was determined by gel electrophoresis in an alkaline–urea buffer system (35). Polymer content was determined by HPLC gel filtration on SW-3000 and SW-4000 columns standardized with homogeneous IgA polymers of known composition. Immunoadsorbants were prepared by coupling the IgG fraction of polyclonal monospecific sera to BrCN-activated Sepharose. Analytical immunoprecipitation and SC binding experiments were conducted by adding a trace amount of labeled IgA to 10–60 μl adsorbant in PBS with 1 mg/ml bovine albumin as carrier. After incubation at room temperature overnight on a rotator, the adsorbant was centrifuged out and washed. Each preparation was tested in triplicate. Within the range used, the percentage of label recovered in the bound fraction was independent of the concentration of protein or adsorbant.

Transport Studies

Male Wistar rats (250–350 g: Charles River Canada, St. Contant, Quebec) were maintained on lab chow (Purina, Mississauga, Ontario) and fasted 8–12 h before surgery. Under Nembutal anesthesia, cannulas were inserted in the bile duct, right femoral vein, and left iliac artery as described in detail in a previous publication (39). Use of PE-20 polyethylene tubing (Clay-Adams, Parsippany, NJ) for the iliac artery cannula enabled collection of individual blood samples within 5 s. When not in use, the cannula was attached to a syringe that contained 150 USP units heparin (preservative-free Hepalean; Organon Canada Ltd., Toronto) per ml 0.875% saline.

Transport studies were conducted as follows: Samples of two IgA preparations, labeled with opposite radioisotopes, were mixed and injected into the femoral vein, and bile was collected into preweighed tubes. The bile flow rate ranged from 7 to 21 μg/min. At appropriate time points, ~75 μl of blood was withdrawn through the iliac cannula into a 1-ml syringe with directly attached 27-gauge needle (Monoject, St. Louis, MO). The blood was transferred immediately to a plastic tube, and 50 μl was measured out accurately for counting. Leupeptin inhibition studies were performed by injecting 5 mg of drug (9) (Sigma Chemical Co.) in 0.5 ml saline into the femoral vein 1 h before the radioactive sample. Protein competitors (Fet, ASFet, human albumin, or unfractionated human polymeric IgA; 40–50 mg/ml in saline solution) were injected in two 0.5-ml boluses at 6 and 3 min preceding the radioactive sample.

Disposition of radiolabel in bile was determined by gel filtration HPLC on an SW-3000 column. The proportion bound to intact IgA eluted at a position that corresponded to 100,000 mol wt or greater; radioactive catabolites eluted at the total column volume.

Data were corrected by computer for isotopic overlap and radioactive decay. First, clearance half-times (Table I and Figs. 5 and 7) were calculated by measuring the activity present in the 1-min blood sample, and logarithmically interpolating between subsequent samples for the time required to reach half this level. For unfractionated rat IgA preparations, galactose oxidase–treated preparations, and in the competition experiments, a correction has been made in this calculation for the radioactivity that remained in blood at the end of the experiment (39). The post-injection time for each 10-min bile aliquot (Fig. 5) has been plotted at the middle of the interval during which the collection was made, after a 2–5 min correction for the cannula dead volume. To generate the relative transport rate (%V_{peak}: Figs. 6–7), the 150-min rate was compared with the peak rate by computer in units of percent injected radioactivity transported per minute. Standard errors for %V_{peak} were calculated using logarithmic values. P, the probability of equal means, was calculated using the one-tailed Wilcoxon nonparametric rank sum test.

Results

Affinity Fractionation of Human IgA on the Basis of SC Binding Activity

IgA preparations contain a proportion of molecules that lack an ability to bind the receptor that mediates direct transport to bile (SC). The human IgA preparation used for this study possessed 0.5–0.7 SC binding sites per IgA dimer, although labeling with ICl was found to diminish this slightly. To examine the role of SC binding in IgA processing in the hepatobiliary transport model, human IgA was fractionated on SC Sepharose according to the scheme shown in Fig. 1. Radiolabeling was done before or after an initial affinity purification step with similar results. The adherent phase from the first adsorption step consisted of >90% polymer and was 70–85% enriched for SC binding activity. The non-adherent phase was incubated with fresh SC-Sepharose until the residual binding activity was <10% of the surviving protein. Since monomeric IgA does not bind SC (27, 58), the non-adherent fraction was enriched for monomer (30–70% of the radiolabel). For some experiments, the monomer and polymer components were separated by HPLC, and the polymer fraction was incubated once more over SC-Sepharose to deplete residual binding activity.

The BH-labeled human IgA fractions were characterized extensively (40). All fractions showed similar heavy and light chain composition, and were 70–95% precipitable with an immunoadsorbent specific for human alpha chain. The affinity purified fraction and non-adherent polymer subfraction consisted primarily of dimer with <10% monomer and no more than 20% higher polymers. They were indistinguishable by all criteria tested, except in the ability to bind SC.

Two Possible Destinies for Human IgA in Rat Liver

We were interested in following the IgA fractions through both endocytic pathways: the direct transcellular pathway to bile and the degradative pathway that results in ligand digestion. With this objective, two radiolabeling methods were used. In some experiments, protein was radioiodinated with ICl as a direct indicator of the transcellular pathway: only protein-bound label is secreted into bile (39), since free radioiodide from degraded ligand is returned to blood (24). Alternatively, protein was conjugated with the BH reagent as a probe for both pathways: alongside any protein-bound label, radiocatabolites are secreted into bile in proportion to the amount of ligand degraded inside the liver (39). Transport studies were initiated in anesthetized rats by injecting the radiolabeled IgA preparations into the femoral vein. Blood was collected from the iliac artery to monitor ligand uptake, and bile was collected through a fistula of the common bile duct to monitor hepatic processing. Radioactivity present in bile was analyzed by gel filtration HPLC to determine the proportion bound to intact protein.

The character of transported radioactivity from these ex-

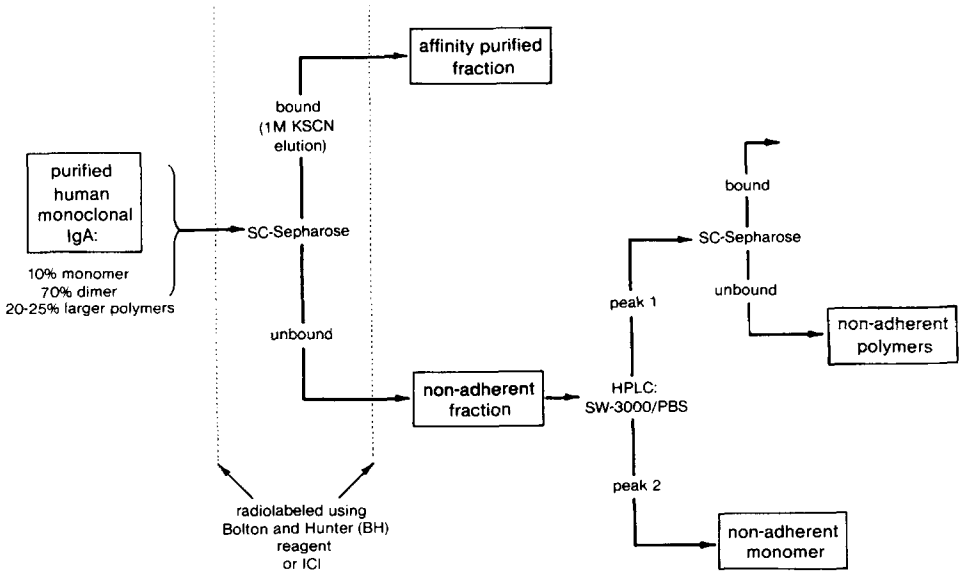

Figure 1. Strategy for fractionation of human IgA. The IgA was separated into affinity purified and non-SC-binding fractions before or after labeling. The non-adherent fraction was subfractionated by gel filtration HPLC, to prepare functionally inactive polymers that were otherwise indistinguishable from the affinity purified fraction.

periments is shown in Fig. 2. The results are compared to similar experiments with ASOr, a functionally homogeneous protein taken up by the liver for degradation. Affinity purified human IgA was transported directly to bile, reflecting the enrichment of SC binding activity in these fractions. Intact transport of human or rat IgA depleted of SC binding activity was limited, but a considerable proportion of the human IgA underwent degradation, as indicated by biliary release of BH-derived degradation products. The HPLC profile of radioactivity in bile showed two peaks: one corresponding to intact IgA, the other eluting at the total column volume. There were no intermediate peaks, which indicates that when IgA is subjected to digestion within the cell, the degradative process goes to completion.

The kinetics of clearance and transport are presented in Table I. All human IgA preparations were cleared rapidly from blood, regardless of SC binding activity. Monomeric preparations cleared significantly faster than did polymeric preparations. The clearance data show why there were no catabolites released into bile from the non-SC-adherent rat IgA: this fraction was taken up poorly by the liver; most of the activity had remained in blood. Apparently, only human IgA can be rapidly taken up into the degradative pathway.

Transport to bile of the intact component (as indicated by the ICl-labeled probes) was equally fast for all human IgA preparations (Table I). The time taken for human IgA to cross the cell was no longer than for rat IgA. However, the radioactivity from the BH-labeled non-adherent human IgA preparations (mostly catabolites) peaked in bile ~20 min after the intact component. This is consistent with an additional time requirement for the ligand to be transported to lysosomes,

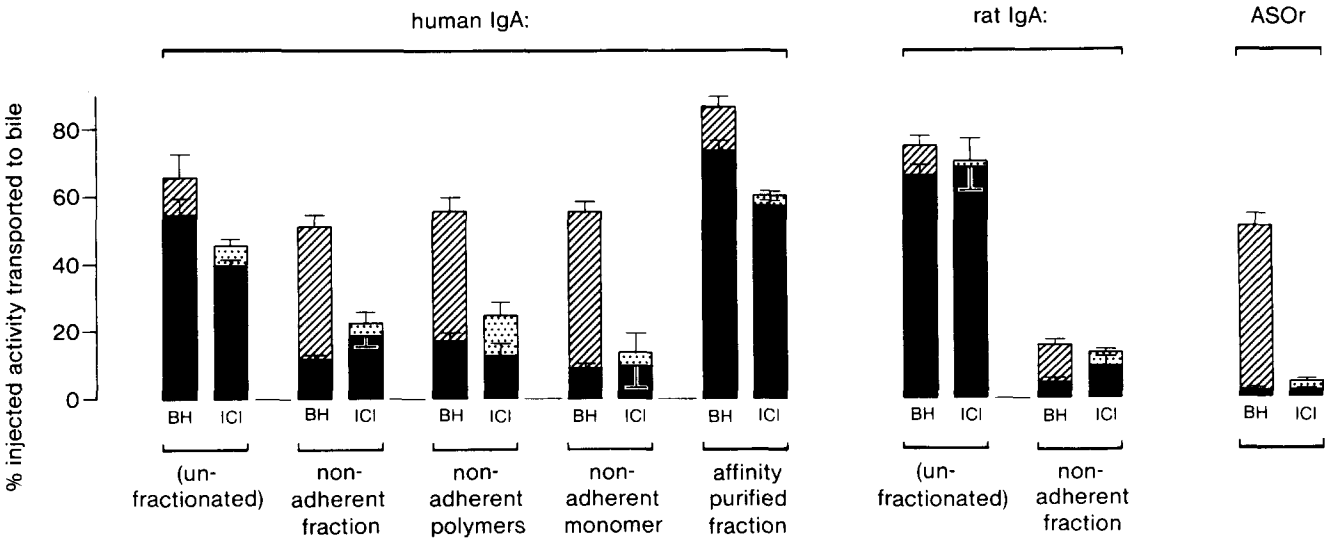

Figure 2. Transport of radiolabel to rat bile within 150 min, as a percentage of the injected dose. The solid area within each bar indicates the amount of radiolabel in bile attached to intact protein, determined by gel filtration HPLC. Release of radioactive catabolites from BH-labeled preparations (striped area) reflects ligand degradation. Unlike rat IgA, SC-non-adherent human IgA (both polymers and monomer) has the ability to enter the degradative pathway. Comparative data showing degradation of ASOr are from a previous publication (39). (Each bar shows mean ± SE for 4–6 experiments). ▨, total radiolabel in bile; ■, protein-bound radiolabel in bile.

Table I. Clearance and Transport Kinetics

	BH-labeled preparations			ICl-labeled preparations		
	Blood clearance half-time*	Uncleared after 1 h	Time of peak transport‡	Blood clearance half-time*	Uncleared after 1 h	Time of peak transport‡
	min	*%*	*min*	*min*	*%*	*min*
Human IgA						
Unfractionated	3.6–5.0	16	28 ± 4	4.4–5.6	12	*28 ± 4*
Non-adherent fraction	2.9–4.8	16	*47 ± 3*	2.7–5.8	15	30 ± 2
Non-adherent polymers	6.4–7.5	16	*47 ± 3*			30 ± 2
Non-adherent monomer	1.8–2.7	15	*44 ± 5*			34 ± 4
Affinity purified	2.7–4.2	2	30 ± 2	3.6–4.6	8	*28 ± 4*
Rat IgA						
Unfractionated	5.2–10.5	21	31 ± 6	6.0–16.4	21	*33 ± 3*
Non-adherent fraction		*58*	(103)		*53*	(37)
ASOr	0.6–0.8	6§	*40 ± 4*	0.7–0.8	13§	25 ± 2

Italicized numbers show that ligands entering the liver primarily for degradation take longer to be processed than ligands transported intact to bile.

* Time to clear first 50%; range for 2–3 experiments.

‡ Mean ± SE for 4–6 experiments, based on 10-min collection intervals.

§ Mostly degraded material released back into blood (53).

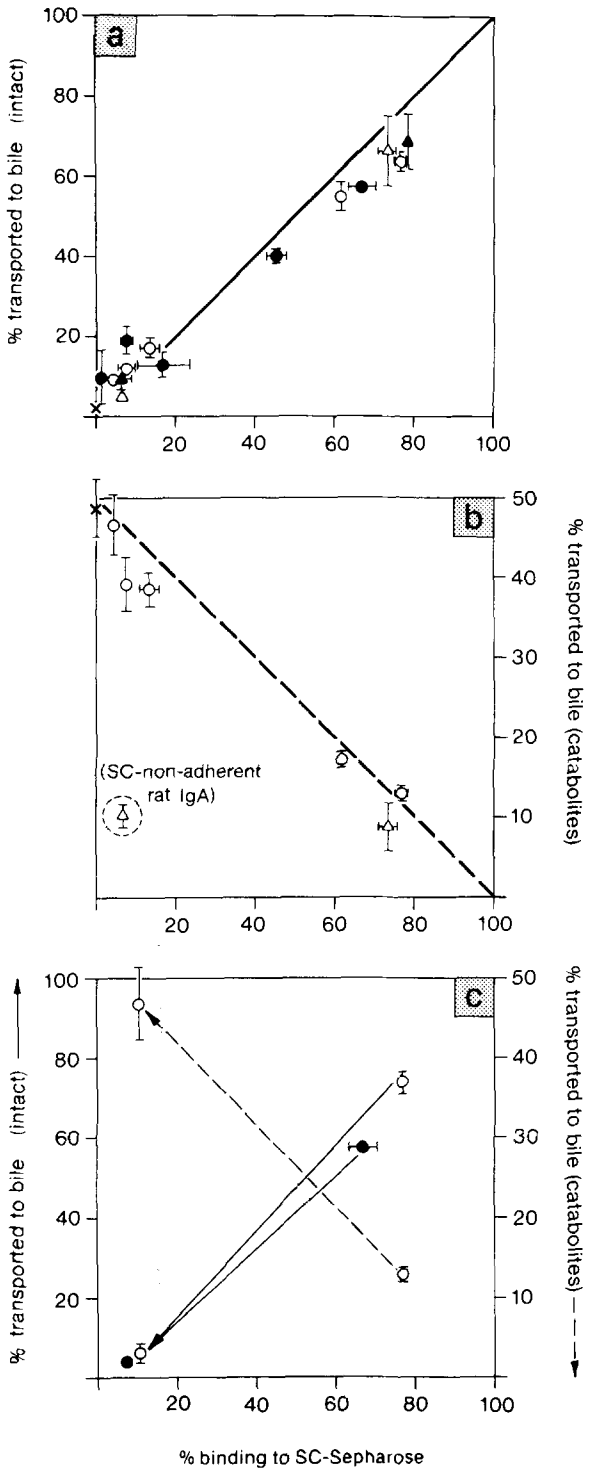

Figure 3. (*a*) Transcellular transport. 1:1 correlation between SC-binding activity measured in vitro (abscissa), and the proportion of human and rat IgA preparations reaching bile intact (ordinate). (●, ○) human IgA; (▲, △) rat IgA; (×) ASOr. Open symbols indicate BH-labeled protein; solid symbols indicate protein labeled with ICl (mean of ≥4 animals). Horizontal and vertical whiskers indicate SE where sizeable in the respective determinations. (*b*) Degradation. Reverse correlation between SC-binding activity, and the proportion of label from BH human IgA that reaches bile as low molecular weight catabolites. (*c*) Effect of reduction and alkylation on the affinity purified human IgA fractions. Subsequent to the loss of SC binding activity, the ligand is no longer transported to bile intact and reverts to processing by degradation.

Table II. Characterization of Human IgA Transported to Bile

	IgA before injection*	IgA transported to bile‡
Binding to immunoadsorbent§		
Anti-human IgA	90.0 ± 0.8%	92.5 ± 2.7%
Anti-rat SC	5.0 ± 0.7%	92.4 ± 3.9%
Normal rabbit IgG	3.3 ± 0.8%	4.7 ± 1.7%
Binding to human SC Sepharose	42.5 ± 2.3%	1.9 ± 0.6%

* Unfractionated ICl-labeled human IgA. Values given are mean ± SE for three determinations.

‡ Bile from transport experiments, dialyzed against two changes of PBS. Values given are mean ± SE for three determinations on bile from each of two transport experiments.

§ Sepharose-linked monospecific antibody or IgG control.

degraded, and then for the catabolites to be taken to the bile canaliculus.

Relationship between SC Binding and the Fate of IgA in the Hepatocyte

A comparison between SC-binding activity determined in vitro, and the fate of the IgA fractions in the liver is made in Fig. 3. There was a direct correspondence between SC binding of either human or rat IgA and transport to bile via the transcellular pathway (Fig. 3*a*). On the other hand, degradation of the human IgA, detected by release of catabolites from BH-labeled preparations, was reversely correlated with SC-binding activity (Fig. 3*b*). The graph extrapolates back through SC non-adherent IgA preparations to the amount of catabolites released from BH-ASOr. This indicates that under certain conditions, almost all of the human IgA can be degraded. The transported and degraded proportions are complementary, i.e., those IgA molecules that cannot bind SC enter the degradative pathway by default, apparently by an independent receptor.

Another way of demonstrating the dependence of the transcellular pathway on SC binding is to show that the IgA actually becomes linked to SC during transport. This was tested by immunoprecipitation of the nondialyzable radioactivity in bile (Table II): After transport, human IgA had no unoccupied SC binding sites (shown by loss of binding to SC-Sepharose), but became precipitable by immunoadsorbent specific for rat SC.

Human IgA as a Heterovalent Ligand

The fact that degradation of human IgA occurs only in the absence of SC-binding activity provokes the question of whether the SC-binding fraction has the ability to bind receptors for both pathways. An experimental approach to this question is to abolish SC-binding activity from affinity purified human IgA, to see if it will then revert to processing through the degradative pathway. In polymeric IgA, intramonomer stabilization relies partly on noncovalent forces, but the only inter-monomer linkages are disulfide bonds (17). Mild reduction and alkylation thus provides a convenient method for disassembling the polymer and destroying the SC binding site, while leaving the monomeric substructure relatively intact.

The effect of reduction and alkylation on hepatic handling of the affinity purified fractions is shown in Fig. 3*c*. Reduced human IgA was still taken up efficiently by the rat liver: the

half-time was 2.1–2.6 min, and only 13% remained in blood 1 h after injection. However, associated with the loss of SC binding activity was a concomitant decrease in the proportion of IgA transported directly to bile. The affected molecules had been successfully converted into ligands for the degradative pathway.

This indicates that before reduction, the affinity purified polymers had the potential to bind both SC and the receptor that mediated uptake for degradation. The ability to bind this alternative receptor is thus almost universally shared by all human IgA molecules in all fractions. In other words, the molecules that possess an SC binding site are actually heterovalent. When SC binding activity is present, it is dominant in deciding the fate of the ligand.

Degradation of Human IgA in Lysosomes

Lysosomal function can be blocked by administering the cathespin inhibitor, leupeptin (9). The fate of human IgA in animals pretreated with leupeptin is shown in Fig. 4. In proportion to the non-SC-binding IgA present in each preparation, BH radiocatabolites were secreted into bile of the untreated control animals: 40–60% for non-adherent fractions, and 10–20% for the affinity purified fraction. Impairment of lysosomal function largely abolished catabolite secretion from these fractions, with a compensatory accumulation of radioactivity in the liver. In contrast, there was no statistically significant decrease in the amount of IgA that reached bile intact. This implicates lysosomes as the site of degradation of the human IgA that cannot bind SC. Human IgA that can bind SC must bypass lysosomes in its pathway to bile.

The ASG Receptor Mediates Uptake of Human IgA into the Lysosomal Pathway

The next task was to identify the receptor that gave non-SC-binding human IgA access to the degradative pathway. Recently, Stockert et al. (48) showed that human IgA1 could bind to the ASG receptor in vitro. We therefore designed a series of inhibition experiments to test the involvement of the ASG receptor in human IgA processing by the rat liver.

To study the kinetics of the two pathways separately, we prepared two radioligands with opposite isotopes, each of which primarily probes a single process: non-adherent BH human IgA for the ligand degradation pathway, and affinity purified ICl human IgA for biliary transport via the transcellular pathway. These ligands were injected together into experimental animals after prior infusion of a potential protein competitor. Recovery from peak transport rate ($\%V_{peak}$) at the end of the collection period was calculated as a sensitive kinetic indicator of inhibition, since the ability of the liver to complete secretion of radioactivity within the allotted time is impaired when transport events are delayed (39). Clearance and transport curves from individual experiments are shown in Fig. 5; the total amount transported and the corresponding kinetic data are reported in Fig. 6.

Results from the competition experiments demonstrate that the ASG receptor is responsible for allowing human IgA access to the degradative route: asialofetuin (ASFet), but not native fetuin, blocked the uptake and processing of the SC-non-adherent fraction.

Surprisingly, the presence of competing ASFet even affected the processing of SC-binding human IgA: clearance time from

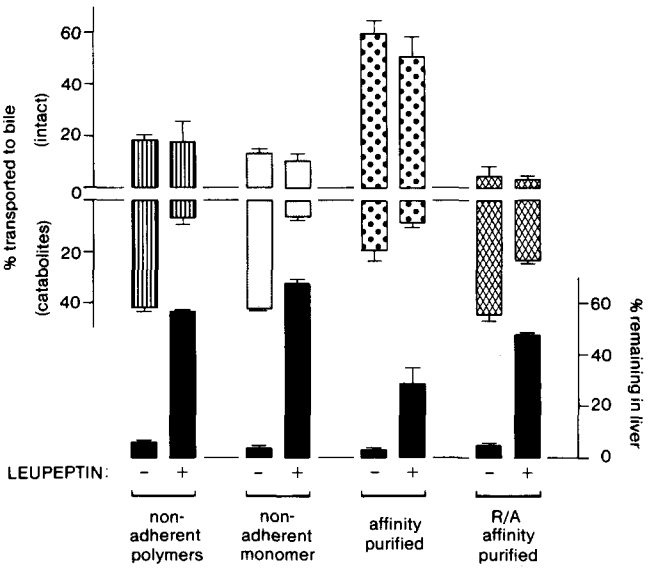

Figure 4. Effect of leupeptin, the lysosomal protease inhibitor, on the transport and degradation of BH human IgA. (−) Saline-treated controls; (+) animals pretreated 1 h before administration of the radiolabeled sample with 5 mg leupeptin, injected intravenously. After 3 h of bile collection, the livers were perfused with cold saline solution and removed for counting. The proportion of radiolabel bound to intact protein in bile was determined by HPLC. There is no statistically significant reduction in intact IgA transport, but degradation of IgA is inhibited in all cases at $P < 0.03$. *R/A*, reduced and alkylated. (Mean ± SE; $n = 3$–5).

blood of the affinity purified fraction was increased by 3.5–7.5-fold (Fig. 5). Even though the SC-binding activity did reach bile eventually, there was a corresponding delay in the median re-secretion time of 15 min (Figs. 5–6). This represents a distinct departure from the behavior of rat IgA, which is not affected by the presence of excess ASFet. Thus, inhibition by ASFet is highly specific for human IgA; it is not just due to long-range interdependence of the two receptor systems.

The ASG Receptor Also Participates in the Transport of Human IgA to Bile

Even though binding to SC is still essential for the intact transport of IgA to bile, the ability of competing ASFet to delay the transport of heterovalent human IgA implicates the ASG receptor in this pathway as well. The most immediate way to account for the observed delay in both uptake and secretion is to suppose that the ASG receptor is directly responsible for capturing human IgA from blood. This can be tested a second way, by enzymatically treating the IgA, rendering it ineligible as a ligand for the ASG receptor. If the ASG receptor is not involved in IgA processing directly (i.e., if binding to SC is sufficient for efficient bile transport), then the modified IgA would be decoupled from inhibition effects of competing ASFet, but would still be transported to bile rapidly.

To diminish binding activity for the ASG receptor, labeled IgA preparations were treated with galactose oxidase (48). Results of the subsequent transport experiments are shown in Fig. 7. Treated non-adherent human IgA had only limited access to either the transcellular or the degradative pathway: this was expected, since binding activity for both receptors

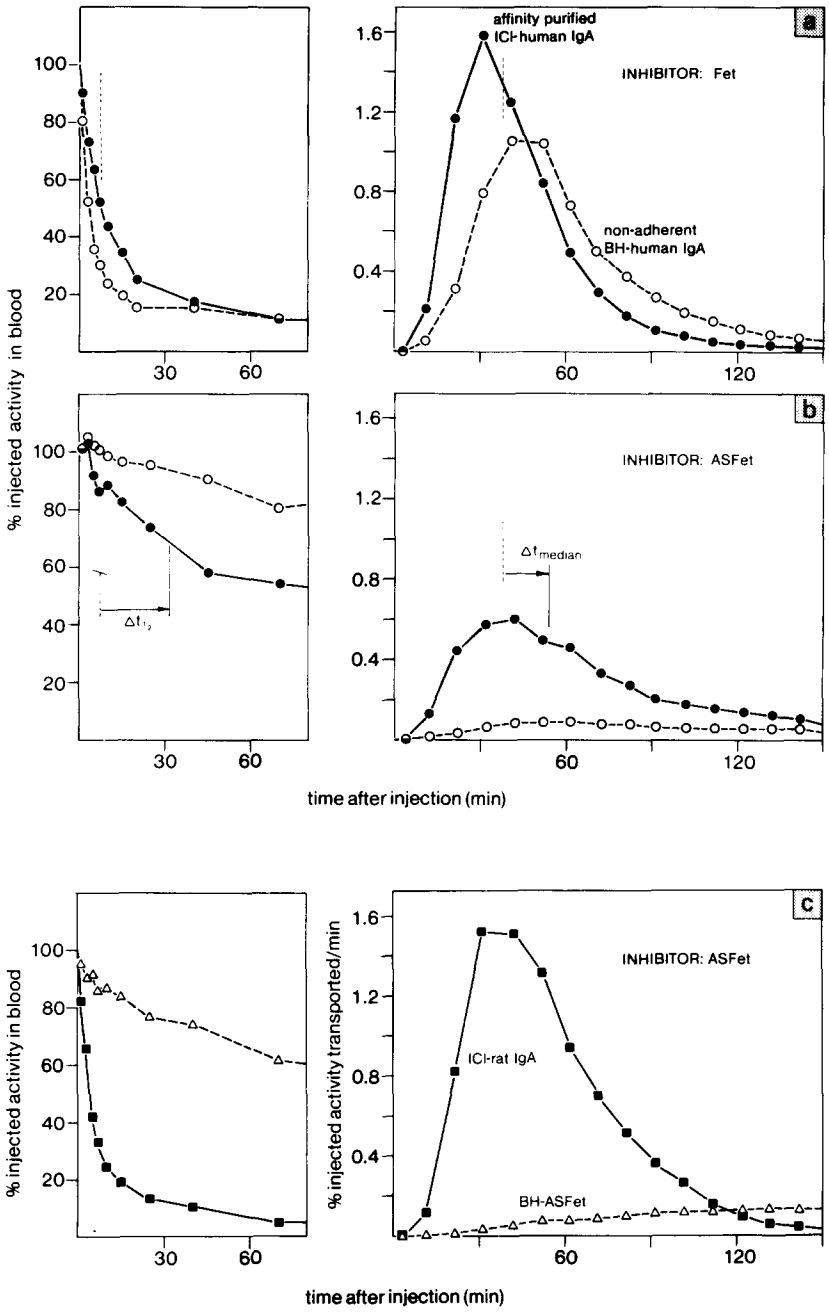

Figure 5. Effect of competing proteins on the clearance and transport of IgA in the rat. These are representative curves from individual animals injected with 40 mg of unlabeled potential competitor in 1 ml saline, 3–6 min before two radioligands with alternative isotopes. Non-adherent [^{125}I]BH human IgA probes the degradative pathway, while affinity purified ^{131}ICl human IgA probes the transcellular pathway delivering human IgA directly to bile. In the control experiment (*a*), the non-adherent fraction cleared slightly faster than the affinity purified IgA, due to enrichment for IgA monomers, which have more direct access to cell-surface receptors. Excess unlabeled ASFet (*b*) blocks uptake into the degradative pathway and delays processing of human IgA in the transcellular pathway (increasing the uptake and median transport times relative to the control, as indicated). There is no effect of ASFet on rat IgA processing (*c*).

had been depleted. Treated rat IgA is the other control in this experiment: it was cleared and transported to bile rapidly, showing that treatment with galactose oxidase did not affect functions mediated solely by SC.

The effect of galactose oxidase on the affinity purified human IgA fraction was twofold. First, it decoupled human IgA transport from inhibition effects of ASFet. Most importantly, it resulted in slower uptake and subsequent biliary release of human IgA, similar to the delay induced by ASFet inhibition of the untreated IgA described in the previous section. Nearly the entire complement of SC-binding activity could still reach bile using cell-surface SC directly, but this process was evidently much less efficient. To be cleared and transported to bile as rapidly as its rat counterpart, human IgA has an absolute requirement for binding to the ASG receptor.

Discussion

Competition for a Heterovalent Ligand between Two Receptors: Human IgA Switches Receptors Midstream

Our observations show that the ASG receptor is intimately involved in the capture of human IgA from blood, regardless of its final disposition in the liver (Figs. 5–7). Competition with unlabeled ASG or treatment of the IgA with galactose oxidase delayed clearance of each human IgA fraction from the circulation by at least fourfold. This indicates that irrespective of other receptor binding capacities borne by the ligand, it is an initial association with the ASG receptor that is responsible for rapid uptake by the liver cell. The ASG receptor provides access of human IgA to the hepatocyte's lysosomal pathway; or alternatively, it facilitates entry into the transcellular pathway, enhancing the kinetics of ligand secretion into bile.

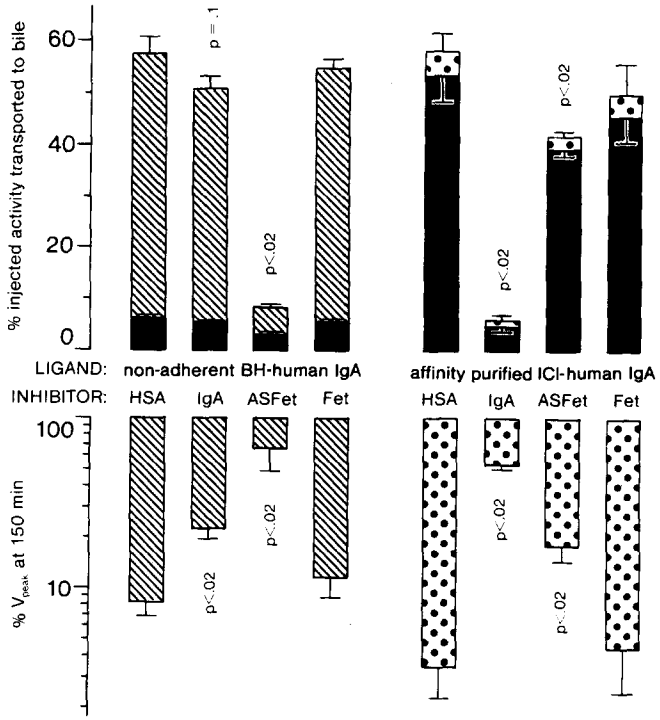

Figure 6. Effect of competing proteins on the amount of radiolabel that reaches bile, and the kinetics of transport. Radiolabeled probes and inhibitors were administered as in Fig. 5. (40 mg is ~0.1 μmol of human IgA dimer or ~1 μmol of ASFet). Upper bars show the amount of radiolabel that reaches bile within 150 min of injection. The solid area within each bar indicates the amount of radiolabel in bile attached to intact protein, determined by HPLC. Lower bars indicate the ability of the liver to complete transport of the label to bile by the end of the experiment: $\%V_{peak}$ (the transport rate at 150 min relative to the peak rate) enables comparisons between experiments with the same radiolabeled probe, to detect kinetic inhibition. (Mean ± SE; $n = 4$).

On the other hand, the fate of human IgA once captured by the cell still ultimately depends on its SC binding capacity. The evidence for this is twofold. First, the proportion of IgA in each fraction transported to bile intact always reflects the SC-binding capacity determined in vitro on a 1:1 basis (Fig. 3*a*). Human IgA is degraded in the liver only by default. Second, virtually all IgA reaching bile acquires an ability to precipitate with anti-rat SC (Table II), i.e., it retains this receptor even after release from the cell. The reduction and alkylation experiments showed that heterovalent human IgA is a perfectly good substrate for the degradative pathway, but by binding to SC, it can escape the usual fate of ASG.

In summary, most of the human IgA reaching bile is captured initially by the ASG receptor, but essentially all of it ends up bound to SC. Taken together, these results provide the conclusion that the predominant mechanism for human IgA transport through the transcellular pathway requires switching of the ligand from one receptor to the other (Fig. 8). There may be other occurrences in which receptor switching plays a critical role: for example, between the ASG and transferrin receptors in the diacytosis pathway that returns asialotransferrin to blood (34). As discussed below, the ability of receptors to cooperate in this fashion has implications for the ligand sorting process that occurs within the hepatocyte.

Structural and Functional Differences between Human and Rat IgA

The ASG receptor plays no role in the uptake and transport of rat polymeric IgA; this ligand binds directly to SC. In contrast, human IgA is almost homogeneous in being able to bind the ASG receptor, but it cannot bind directly to cell surface SC nearly as fast as its rat counterpart. Thus, both receptors detect important structural differences between human and rat IgA.

What we have referred to as the ASG receptor is undoubtedly the hepatic binding protein (HBP), originally described by Hudgin et al. (18). In a hemagglutination inhibition assay, Stockert et al. (48) showed that human IgA1 could bind the isolated HBP, but IgA2 could not. A notable difference between the IgA subclasses is the hinge region of IgA1, which bears short serine-linked oligosaccharides terminated with galactose (2). There is no homologous region in human IgA2. If this provides the site for HBP binding, then the failure of HBP to recognize rat IgA is understandable, since serine-linked carbohydrate is absent from the hinge region of IgA from lower mammals (37).

We did not anticipate that SC on the hepatocyte surface would show a kinetic preference for rat IgA. This is because (*a*) SC plays an important physiological role in man by mediating efficient transport of locally produced IgA across the mucosal epithelium (5); and (*b*) in vitro assays have indicated that human IgA binds human and rat SC equally well (43). Yet, we have found that when human IgA is prevented from interacting with the ASG receptor, it enters the rat liver via SC much more slowly than does rat IgA. This newly described ligand preference may be a previously undetected property intrinsic to rat SC, or an effect of receptor presentation in the plasma membrane.

Rapid Culture of Human IgA by the ASG Receptor

An important finding in this study is that heterovalent human IgA (which switches receptors) takes no longer to get to bile than does rat IgA (which does not). This was reported in Table I. For the kinetics of these two ligands to match, there are two requirements: first, the ASG receptor must capture human IgA as rapidly as SC captures rat IgA; second, the receptor switching event must be too rapid to contribute significantly to the observed transit time.

We have previously demonstrated a molecular weight sieving effect that kinetically impairs access of molecules >100,000 mol wt to receptors on the hepatocyte surface in a size-dependent fashion (38). It is the sieving effect, not the direct rate of receptor coupling, that limits the rate at which both human and rat IgA clear from the circulation. Even treating human IgA with neuraminidase does not enhance the rate of hepatic uptake (unpublished data). Once human IgA bypasses the sieve, the association rate with the ASG receptor is rapid, perhaps just as rapid as other galactose-bearing proteins such as ASOr.

Baenziger and Fiete (3) have pointed out that the isolated human IgA1 hinge fragment binds to the HBP in vitro with only moderate affinity. In a dynamic physiological context, the forward rate is probably more important than binding properties measured at equilibrium: Several hinge peptides in the whole IgA molecule (4 per dimer) may provide an avidity effect that is sufficient for maintaining the ligand–receptor

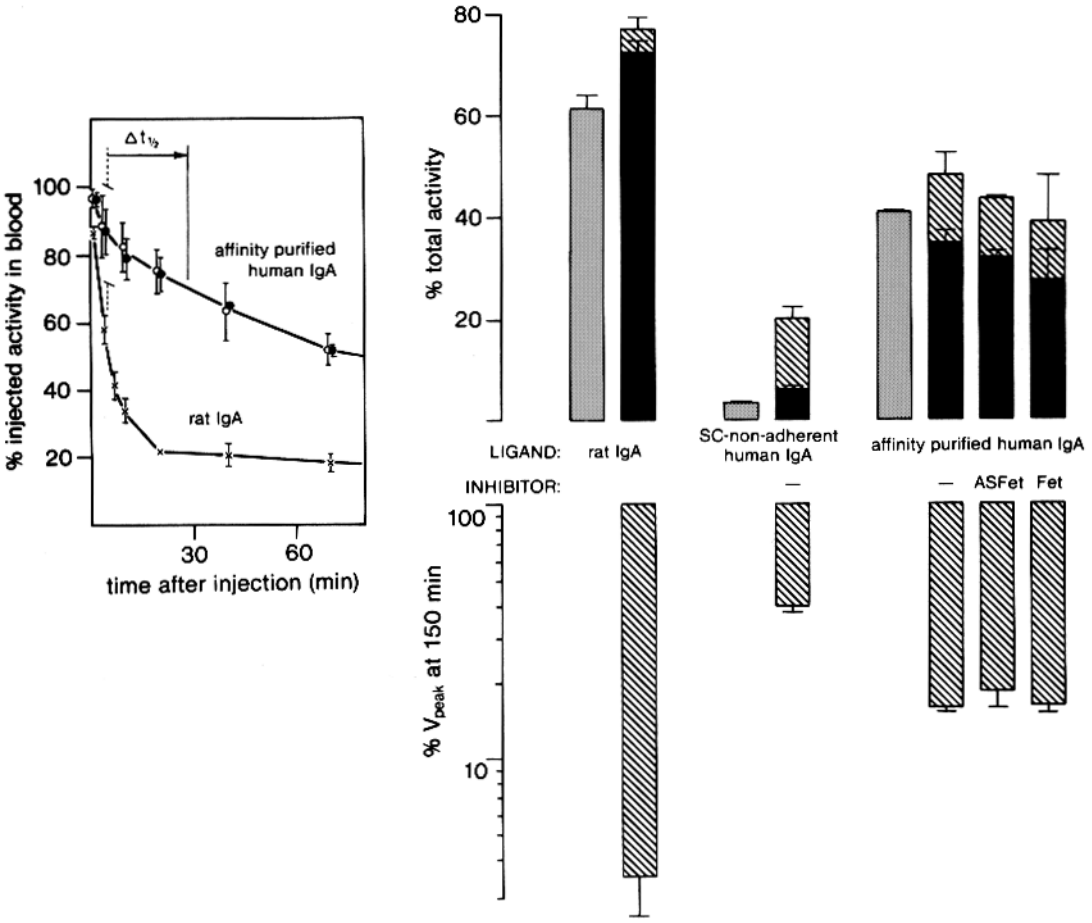

Figure 7. Role of galactose residues in the processing of IgA by the liver. BH-labeled IgA fractions were modified by incubating overnight with 5 U of galactose oxidase, to diminish binding activity for the ASG receptor. Unlabeled competing proteins were preadministered as described in Fig. 5. (*Left*) Clearance from blood of affinity purified human IgA in the presence of competing Fet (●) or ASFet (○), is compared with the clearance of rat IgA (×). (Mean ± range; $n = 2$). (*Right*) The amount of label transported to bile, and the kinetics of transport. (Mean ± SE; $n = 2$–3). ▩, SC binding; ▨, transport to bile; ■, protein-bound radiolabel in bile.

complex on the hepatocyte surface until the next endocytic event occurs. Even though primates also have a highly active HBP, the circulating half-time of the same human IgA protein used here is >5h in the monkey (manuscript submitted for publication). The rapid capture of human IgA by the rat liver implies a fine specificity difference between the rat and human HBP in their recognition of the responsible determinant.

Where Does Sorting between the Transcellular and Degradative Pathways Take Place?

By using affinity techniques, we have been able to target certain human IgA fractions to hepatic lysosomes. These fractions were excellent substrates for lysosomal enzymes, undergoing rapid and complete degradation. Blocking lysosomal enzymes with leupeptin did not augment transport of intact IgA to bile. Thus, the notion that IgA reaches bile simply by surviving digestion (26) can be safely discarded. SC must help sort IgA away from other ligands before most proteolytic enzymes are introduced into the lysosomal pathway.

In a previous paper (39), we considered in detail the subcellular location where this sorting takes place. We demonstrated that the transcellular and degradative pathways diverge either at the cell surface or in an early intracellular compartment. The failure of ASFet to inhibit rat IgA transport led us to predict that the receptors for the two pathways were rarely in juxtaposition on the cell surface. This was consistent with the morphological data published by Jones and his colleagues (21, 36). By electron microscopic autoradiography, they tracked radiolabeled rat IgA through endocytic vesicles that bore different structural features from those involved in the degradative pathway. They concluded that ligands with different destinations are endocytosed at the cell surface directly into separate compartments. However, using a colloidal gold immune overlay technique, Geuze et al. (14) recently identified IgA and ASG receptors together in tubular compartments subjacent to the membrane. In apparent contradiction to the work of Jones et al., this group concluded that IgA and ASG are brought into the cell together, and then sorted from each other in an intracellular organelle.

The study presented here provides the ultimate resolution of where ligand sorting occurs. The observation that bears on this issue is that the receptor switching event undertaken by human IgA is too rapid to delay its transport to bile by a detectable extent. This prohibits the possibility that human IgA takes a more complicated pathway than rat IgA: for example, endocytosis via the ASG receptor and recycling to the plasma membrane for subsequent transfer to SC. Human

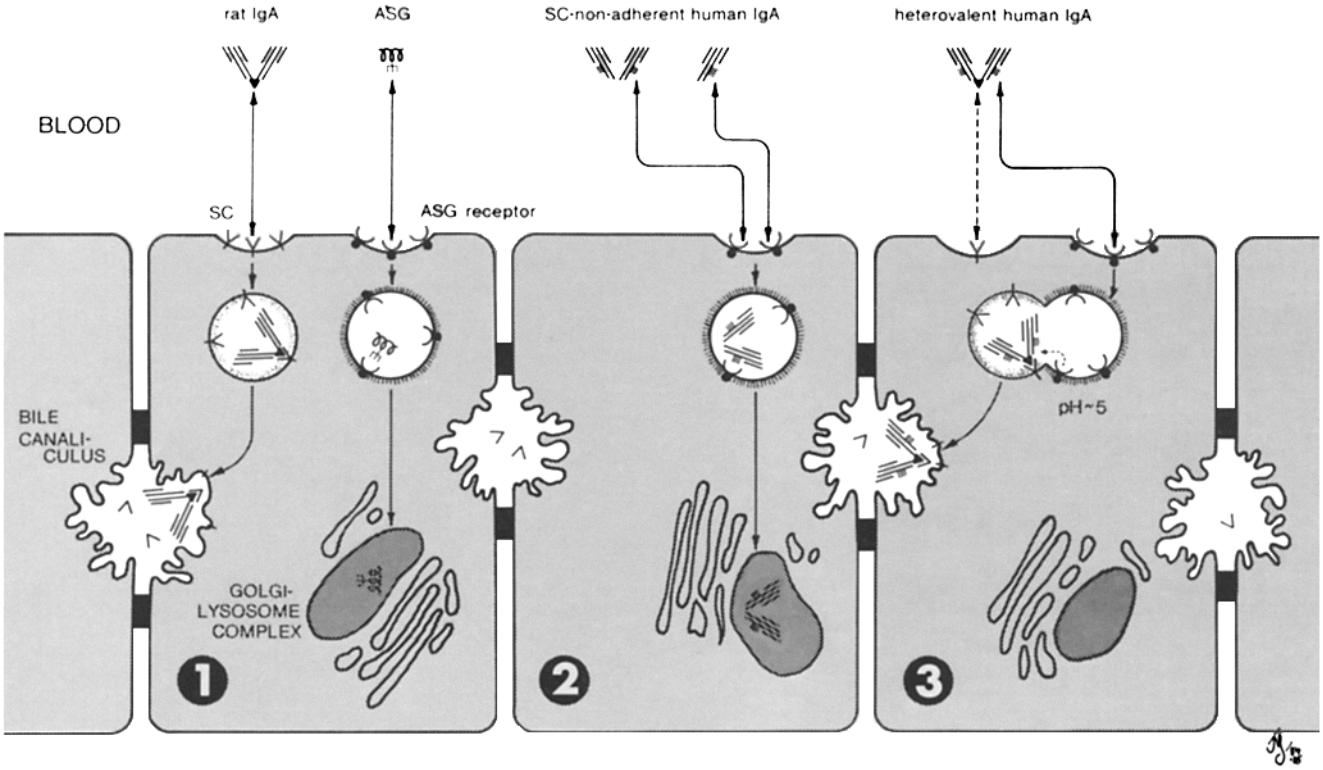

Figure 8. Diagrammatic summary of observations. (*1*) Asialoglycoproteins are cleared by a specific receptor (the hepatic binding protein) and enter the lysosomal pathway for degradation. Rat IgA is recognized through its SC binding site (▼), and enters an independent pathway for direct transport to bile. Human polymeric IgA behaves in a similar fashion to rat IgA when binding to the ASG receptor is blocked (not shown), but the capture of human IgA by SC is much less efficient. (*2*) Human IgA monomers and polymers that have lost their SC binding site bind exclusively through carbohydrate determinants to the ASG receptor and enter the lysosomal pathway. When the ASG receptor is blocked, these ligands remain in blood. (*3*) Normally, most untreated human IgA polymers are heterovalent: they can bind both receptors. Their efficient clearance from blood relies on binding to the ASG receptor, but ligand destination still ultimately depends on an ability to bind SC. The fact that human IgA gets to bile as rapidly as its rat counterpart suggests that the receptor switching event occurs en route. Vesicular acidification induces a confirmational change in the ASG receptor, and would promote switching of human IgA to SC for transport to the bile canaliculus. This explanation requires that the two receptors briefly share an intracellular compartment, after the acidification step but before the two ligand pathways diverge.

IgA on its way to bile undoubtedly takes the same route as rat IgA, but switches receptors before the transcellular and degradative pathways diverge.

Under this constraint, let us consider the hypothesis that SC and the ASG receptor are endocytosed from the plasma membrane into completely separate compartments. This would mean that human IgA would have to switch receptors at the cell surface in order to get to bile. The relative association rates with the two receptors imply that human IgA would spend most of its time with the ASG receptor, for which it is a potent inducer of endocytosis. It could only be transported quantitatively to the bile canaliculus if a specific mechanism existed to transfer the ligand to SC before this occurred. But the existence of a dedicated switching mechanism is highly unlikely, because there is no physiological requirement for such a mechanism: rat IgA, the natural substrate for biliary transport, binds to cell-surface SC directly. In short, rapid transport of human IgA to bile is an unexplainable finding in the context of receptor separation at the plasma membrane.

In the alternative hypothesis, that the transcellular and degradative pathways diverge shortly after ligands enter the cell, these difficulties vanish. ASG is released from its receptor in an acidic compartment underneath the membrane (12, 15, 51). The release is irreversible, because binding activity of the ASG receptor is acutely pH dependent (8, 54), and the receptor is recycled to the sinusoidal membrane after decoupling (45, 57). In contrast, IgA–SC binding is stable down to pH 5 (22, 58), and unoccupied receptors are continually shuttling from the sinusoidal membrane all the way to the bile canaliculus (29). Under this hypothesis, then, IgA could transfer between receptors in the acidic compartment (Fig. 8). After decoupling from the ASG receptor, binding to SC would be facilitated by the high local SC concentration inside this compartment. If the limiting factor was the transit time of SC across the cell, then the time from endocytosis to secretion for human and rat IgA should match exactly. The morphological data of Jones et al. (21) can probably be reconciled with this sequence of events, providing the residency of IgA in the shared compartment is transient.

The receptor switching phenomenon can evidently be accomodated in the subcellular sorting hypothesis, but is irreconcilable with the cell surface sorting hypothesis. For this reason, our observations provide the first biochemical evidence for sorting between the transcellular and degradative pathways inside the cell.

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