Low level transport of IgA to bile via the asialoglycoprotein receptor

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The rat and rabbit transport IgA from blood to bile by a highly efficient transcellular pathway mediated by secretory component (SC). Other mammals do not express SC on liver hepatocytes, but they do transport a small amount of IgA to bile. In the first part of this study, human polymeric IgA was radiolabeled and depleted of SC binding activity by successive affinity adsorption. Transport of this preparation intact to rat bile was 4%, but was reduced to 2% when 50 mg unlabeled asialoglycoprotein was preadministered. The 2% decline corresponds to the percent of asialo-orosomucoid diverted to bile from the lysosomal pathway. In guinea-pigs, missorting of asialo-orosomucoid intact to bile was 10% of the injected dose. Transport of normal human IgA to bile was 1–2%, even though guinea-pigs do not express SC in the liver. Excess unlabeled asialofetuin reduced the transport of asialo-orosomucoid by 10-fold and IgA by 6-fold. This demonstrates that the asialoglycoprotein receptor can mediate transport of IgA to bile in small amounts, but that this transport may be only a biological artifact resulting from limited fidelity of intracellular protein sorting.

Immunoglobulin A *Asialoglycoprotein receptor* *Secretory component* *Receptor-mediated endocytosis* *Transport to bile* *Protein sorting*

1. INTRODUCTION

The liver hepatocyte takes up many proteins from blood by receptor-mediated endocytosis. Most proteins, such as desialylated glycoproteins (ASG) [1–3], are processed primarily by degradation in lysosomes. There is only one known example for quantitative delivery of the ligand intact to bile: the IgA transport system, originally described in the rat [4–6]. Polymeric IgA is cleared from blood within a few minutes, and essentially all of the receptor-bound ligand appears in bile about 30 min later [7]. The receptor for this pathway is the membrane analogue of secretory component [8–11]. SC is specific for polymeric immunoglobulins: other proteins (including ASG) do not bind.

A remarkable feature of the IgA pathway in the liver is that its expression is restricted to only a few mammalian species. Little SC is expressed on normal human hepatocytes [12]; only ~2% of intravenously injected IgA is transported to human bile in a 24-h period [13,14]. Part of this transport occurs through bile duct cells [15]; the remainder may occur through hepatocytes via a pathway not mediated by SC [16]. Biochemical features of this alternative pathway, and complete assessment of its physiological importance, have remained elusive.

Recently, Stockert et al. [17] provided the important observation that human IgA can bind to the ASG receptor in vitro. This is relevant, because a small proportion of specifically endocytosed ASG is missorted and delivered instead to bile [7]. These two observations prompt the question of

Abbreviations: ASFet, asialofetuin; ASG, asialoglycoprotein; ASOr, asialo-orosomucoid; BH, Bolton and Hunter (labeled); HPLC, high pressure liquid chromatography; IgA, immunoglobulin A; SC, secretory component

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whether IgA can use the ASG receptor to get to bile.

2. METHODS

Human polymeric IgA_1 was isolated from myeloma serum by ammonium sulfate and octanoic acid precipitation [18], followed by chromatography on DEAE cellulose, and then Sepharose 6B. Rat polymeric IgA was isolated using a similar protocol, from the ascites fluid of plasmacytoma line IR22, kindly provided by H. Bazin of the University of Louvain, Brussels. Human SC was prepared by affinity chromatography of colostral whey on IgM Sepharose [19]. Human orosomucoid was the generous gift of the American Red Cross Blood Services Laboratory, Bethesda, MD. It was desialylated by mild acid hydrolysis [20]. Fetuin was prepared from fetal calf serum by the method of Spiro [20], or purchased from Sigma, St. Louis. Fetuin was desialylated by incubation overnight at 37°C with agarose-linked neuraminidase [7].

IgA and ASOr were radioiodinated using iodine monochloride [21] or the Bolton and Hunter (BH) reagent [22]. Details of the labeling protocols, removal of free radioisotope, and characterization have been given previously [7]. To generate preparations of IgA devoid of SC-binding activity, labeled IgA was incubated in 1 ml of phosphate-buffered saline (pH 7.4), containing 1 mg human albumin and 0.15 ml SC-Sepharose (1 mg SC/ml Sepharose 4B) for 12 h at room temperature on a rotator. Supernatants were passaged to fresh SC-Sepharose for further adsorption. Final products were redialysed against 0.9% saline before use in transport studies. Fractional SC binding activities were determined in vitro by incubating trace amounts of IgA preparations with 15 μl SC-Sepharose under the same conditions. SC-non-adherent human IgA was $>90\%$ precipitable with anti-human IgA Sepharose.

Transport studies were conducted in male Wistar rats (250–350 g) and albino guinea-pigs (500–650 g). Each rat was anesthetized with sodium pentobarbital, the right femoral vein was cannulated for sample injection using PE-10 Intramedic polyethylene tubing (Clay Adams, Parsippany, NJ), and the bile duct was cannulated through an abdominal mid-line incision using PE-50 tubing. Each guinea-pig was anesthetized with the volatile anesthetic methoxyflurane ('Pentrane', Abbott Laboratories, Montreal). The left jugular vein was cannulated with PE-10 tubing, the bile duct was cannulated with PE-50 tubing so that the tip of the cannula was within 0.5 cm of the liver, and the gall bladder was ligated. 50 mg of potential competitor (Fet or ASFet) in 1 ml 0.9% saline was infused over 10 min through the venous cannula, and was followed immediately by the radioactive sample. 10 min bile aliquots were collected for 3 h. Activity transported was corrected by computer for isotopic overlap and radioactive decay. Each determination in the kinetic profiles is plotted at the median of the interval over which the sample was collected, after correction for the volume of the cannula.

To determine the proportion of transported activity present in bile as protein-bound label, 0.5 ml of pooled bile from each animal was passed over an LKB (Uppsala) SW-3000 gel filtration HPLC column in Tris-buffered saline (pH 7.1). 2-ml fractions were collected for counting. Because of the high bile flow rate and low level of IgA transport in guinea-pigs, the proportion of protein-bound label in bile aliquots from these experiments was determined by precipitation with an equal volume of 0.5% phosphotungstic acid in 1 N HCl. The absolute percentage of injected material transported to bile as intact protein was thus defined as the product of the total activity transported and the proportion which was protein-bound in the analysed sample.

3. RESULTS AND DISCUSSION

We have shown previously that ASFet does not compete with the SC-mediated transport of rat IgA to bile [7]. However, the presence of galactose-terminated oligosaccharides in the hinge region of human IgA_1 [23] could give the human protein better access to transport pathways mediated by the ASG receptor. To determine whether binding to this alternative receptor could provide a substitute mechanism for IgA transport to bile, we isolated the subfraction of human IgA deficient in SC binding. Labeled human IgA polymers were adsorbed serially over 5 fresh aliquots of SC-Sepharose. Specific SC binding activity of the product, compared with the starting preparation, had been

reduced 10-fold. When injected into rats, >75% of the SC-non-adherent human IgA was cleared from blood within 10 min, and ~4% was transported to bile as intact protein.

To determine whether the ASG receptor played a role in the hepatic processing of this preparation, experiments were conducted in which the injection of the test ligand was preceded by 50 mg of an unlabeled potential competing glycoprotein: either native fetuin, or ASFet (fig.1). Native fetuin provided a negative control for inhibition: it has no detectable effect on any of the pathways tested in this study [7]. Compared to the control, excess ASFet had little relative impact on the SC-mediated pathway: the total proportion of SC-binding human or rat IgA transported to bile was unaffected. In contrast, ASFet was able to reduce by half the small amount of intact protein reaching bile from injections of labeled ASOr (another desialylated glycoprotein, otherwise unrelated). Most importantly, excess ASFet also reduced by half the low-level transport of the IgA preparation that had been depleted of SC-binding activity. The

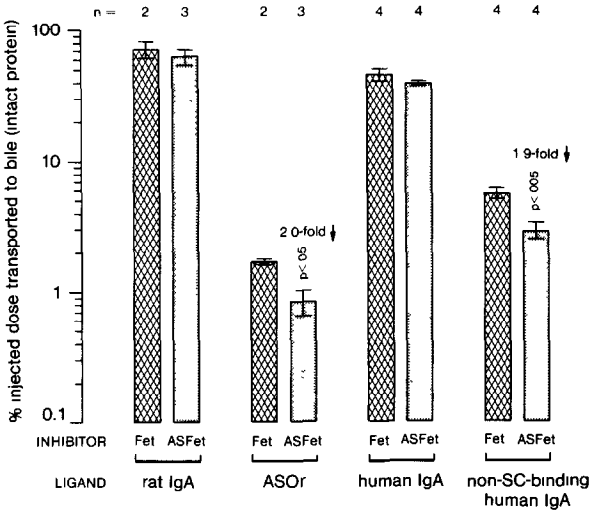

Fig.1. Intact protein transported to rat bile. Experiments in which the radioligand was preceded by 50 mg unlabeled ASFet are compared to parallel control experiments with native fetuin (Fet). Note the use of the geometric scale. Bile was collected for 150 min after injection of the radioactive probe, and analysed by HPLC for protein-bound label. Results are mean of 2–4 animals in each group (uncertainty shown is SE, or range where $n = 2$). p values were calculated using the 1-sided Student's t-test for equal means.

IgA still transported presumably reflects residual SC-binding activity that had survived the adsorption procedure.

These results indicate that circulating human IgA can be captured efficiently by the ASG receptor in the rat, and that 2% of an injected dose can use this receptor to get to bile. This is about the same proportion that appears in bile intact following a trace injection of ASG, and presumably occurs by the same mechanism: missorting of protein entering the lysosomal pathway [7]. The proportion transported is small: IgA without an SC binding site has no special capacity to avoid en masse the fate of other proteins entering the degradative route. In order to achieve substantial transcellular transport, the ability to bind SC is apparently essential.

The next objective was to evaluate the ability of the ASG receptor to mediate IgA transport in a species that did not express SC on liver parenchymal cells. We chose to use guinea-pigs, which are capable of transporting only ~2% of injected IgA to bile [24,25]. To demonstrate the efficacy of the inhibition protocol, experiments were conducted using ICl-labeled ASOr, which is transported to bile primarily as intact protein, and BH-ASOr, which releases catabolites into bile following lysosomal degradation [7]. Results are shown in fig.2. Both the major pathway leading to lysosomal degradation, and the minor pathway releasing intact ASOr into bile, were impaired substantially by prior ASFet administration. Both pathways are thus mediated by a receptor specific for ASG.

In the experiment of primary interest, preadministration of ASFet successfully inhibited the biliary transport of unfractionated human IgA (fig.2): both in terms of total radiolabel, and label that was protein bound. The total proportion transported over the course of the experiment was reduced 6-fold (fig.3), almost as much as the relative reduction of intact ASOr transport. Thus, the ASG receptor provides access of IgA to bile, irrespective of the absence of the SC-mediated transcellular pathway. The amount transported is small, and does not exceed that of other glycoproteins with terminal galactose residues. However, transport via this receptor does account for most of the IgA that is transported from blood to bile in this species.

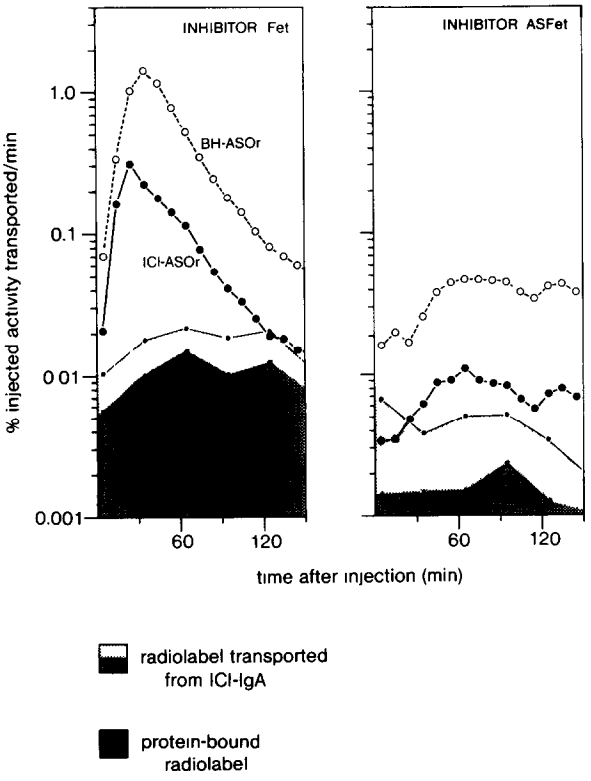

Fig.2. Kinetic profiles of appearance of radiolabel in the bile of individual guinea-pigs. 50 mg Fet or ASFet was administered intravenously, followed immediately by the radiolabeled probe: ICI-ASOr (mainly reflecting transport of intact ASOr), BH-ASOr (reflecting lysosomal degradation of ASOr by biliary release of catabolites), or ICI-IgA. Radiolabel in bile bound to intact IgA was determined for individual 10-min aliquots by acid precipitation.

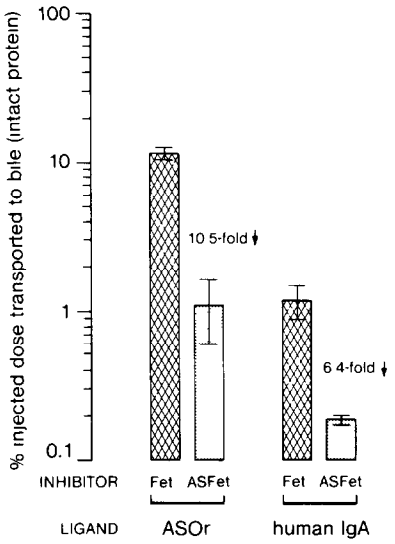

Fig.3. Intact protein transported to guinea-pig bile. The radioligand was injected just following a 50 mg dose of unlabeled Fet or ASFet. Results are the mean (± range) of 150-min collections of bile from 2 animals in each group. Differences within each pair are significant at $p < 0.05$.

It is obviously difficult to obtain direct experimental proof that the ASG receptor mediates IgA transport to bile in man. However, it is evident from this study that binding to a receptor primarily mediating degradation, followed by the apparently universal occurrence of intracellular missorting, may provide the hitherto undescribed mechanism for IgA to get to bile. The low-level diversion from lysosomes to the bile canaliculus provides important clues to the steps involved in ligand sorting, and is therefore of continuing interest. However, in our opinion, this explanation dininishes the likelihood that plasma to bile transport of antibody provides a bone fide contribution to mucosal immunity in man. Low-level appearance of other proteins in bile - insulin [26], epidermal growth factor [27], hemoglobin [28,29], enzymes [30–34], and apolipoproteins [35,36] - may also be the fortuitous result of protein missorting: intriguing, but of minimal physiological impact.

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