

SECRETORY COMPONENT AS THE RECEPTOR
FOR POLYMERIC IgA ON RAT HEPATOCYTES

BY EVA ORLANS, JANE PEPPARD, JEFFREY F. FRY, RICHARD H. HINTON, AND
BARBARA M. MULLOCK

*From the Chester Beatty Research Institute, Royal Marsden Hospital, Sutton, Surrey, and the Institute for
Industrial and Environmental Health and Safety, University of Surrey, Guildford, Surrey, England*

The rapid and active transport of polymeric IgA from the blood to the bile (1, 2) is selective (3, 4), which suggests the existence of a specific receptor for this molecule. The liver cells responsible for the transfer have been identified as the hepatocytes by autoradiography and electron microscopy of the livers of rats killed 5, 30, and 60 min after the injection of radiolabeled IgA (5). 5 min after being injected, the radiolabeled IgA appeared to be bound to the sinusoidal membrane of the hepatocytes. We have found that intravenously injected radiolabeled IgM, IgG1, and monomeric IgA did not appear in the bile at all, and that the transport into bile of s-IgA (i.e., polymeric IgA bound to secretory component [SC]) was both considerably slower and quantitatively less than that of polymeric IgA devoid of SC. Studies of the transport into the bile of IgG2a, IgE, IgM, monomeric and polymeric IgA, and s-IgA with isolated perfused rat livers gave similar results: only polymeric IgA that lacked SC was actively transported (6). These facts suggest that SC may be the receptor for IgA on the surface of rat hepatocytes as it is on the epithelial cells from human gut (7). The availability of hepatocytes in short-term monolayer culture (8), together with that of specific antibodies to rat SC, has enabled us to test this hypothesis by experiments in vitro.

Materials and Methods

Preparation of Immunoglobulins, Free SC, and Specifically Purified Antibody to SC. The preparation and properties of the polymeric rat IgA formed by the IR 461 plasmacytoma have been described (1). Rat IgG was the fraction of normal rat serum eluted from DEAE cellulose with 0.01 M phosphate buffer at pH 7.2. Normal rat bile is a good source of both s-IgA and free SC (9). Pooled bile was first treated with 15–20 mg/ml of cholestyramine resin (Cuemid; Merck, Sharpe and Dohme, Ltd., Hoddesdon, Herts., U. K.), to reduce the amount of pigment. After 10-fold concentration by ultrafiltration, followed by centrifugation, the bile was fractionated on DEAE cellulose. All protein, reactive with antibody to either α -chain or to SC, eluted together with a phosphate buffer (0.05 M, pH 6.2) that contained 0.2–0.3 M NaCl. Gel filtration of this material on a column of Ultrogel AcA34 (LKB Instruments, Croydon, Surrey, U. K.) gave an excluded peak that contained IgA and a second peak that contained free SC and albumin. Further gel filtration of these two fractions on Ultrogel AcA22 and AcA34, respectively, yielded a preparation of pure polymeric s-IgA and one of free SC that still contained some serum albumin; the antigenic properties of the biliary SC are shown in Fig. 1 a.

Rabbit antiserum obtained after three injections of a crude SC preparation was absorbed first on an immunoadsorbent column of normal rat serum proteins coupled to AH-Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) and then on a similar column of IR 461 myeloma IgA. The specificity of the anti-SC serum is shown in Fig. 1 b. Specifically purified antibody was made from this antiserum by absorption onto a column of biliary s-IgA coupled to AH-Sepharose, and elution of the washed, bound antibody with 3 M ammonium thiocyanate.

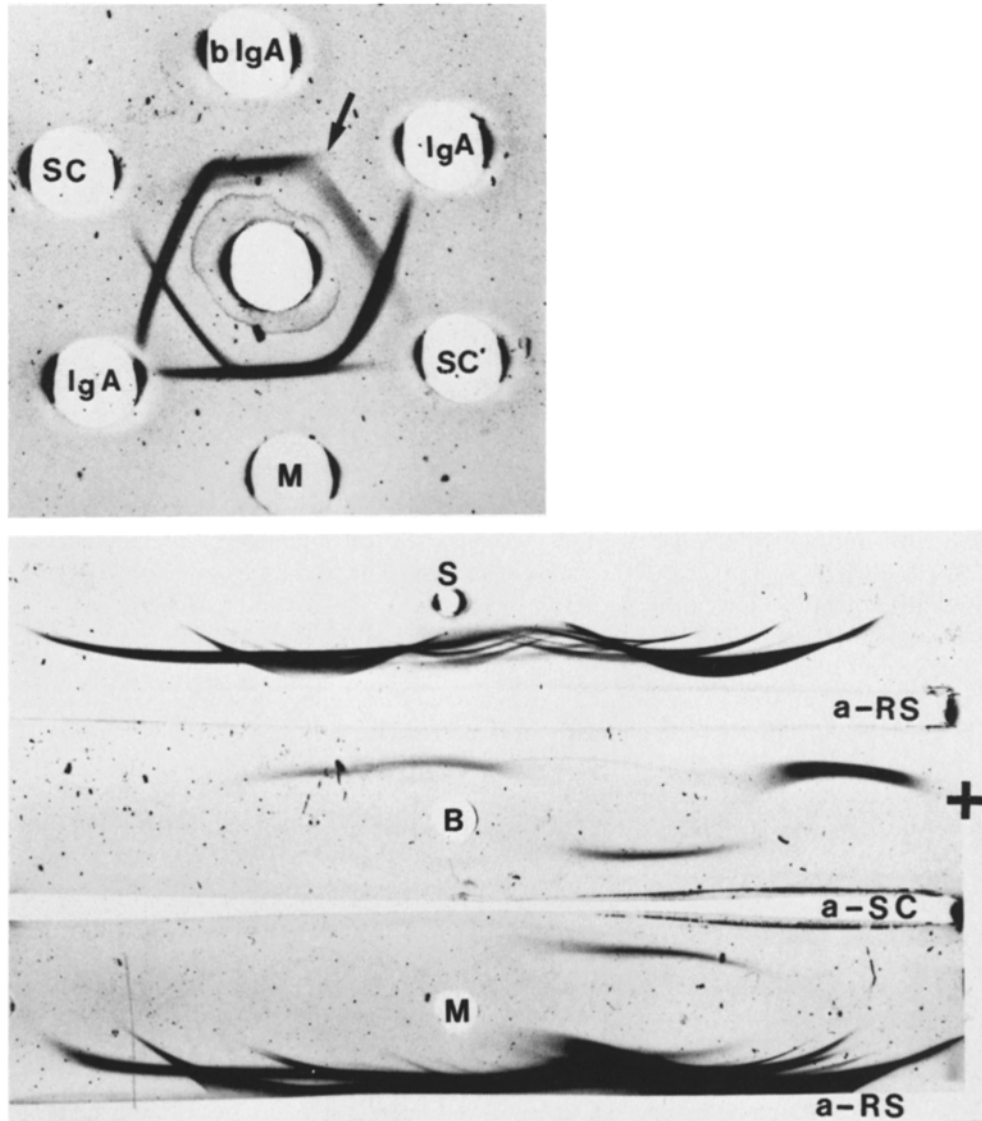


FIG. 1. (a) (top) The precipitation reactions of a rabbit antiserum specific for both rat α -chains and SC (center well) with free SC isolated from bile, myeloma IgA (IgA), purified biliary IgA (bIgA), and whole milk whey (M). The spurring of the biliary IgA band over that formed by myeloma IgA (arrow) shows that biliary IgA is secretory; milk whey contains s-IgA, free SC, and IgA without SC. The bands formed by the free SC characteristically fuse into the s-IgA bands and cross those formed by myeloma IgA. (b) (bottom) Immunoelectrophoretic pattern formed by rat serum (S), bile (B), and milk whey (M) with antisera to whole rat serum (a-RS) and to SC (a-SC). The anti-SC serum, derived from that used in Fig. 1 a by absorption with myeloma IgA, forms a single arc, due to SC, with whole bile or milk whey.

Radiolabeling of Proteins. Proteins were labeled with carrier-free ^{125}I (The Radiochemical Centre, Amersham, U. K.) to an activity not greater than $0.08 \mu\text{Ci}/\mu\text{g}$ by standard methods (10). Radioactivity was measured on a Wallac 8000 scintillation counter (LKB Instruments, Ltd., Croydon, U. K.).

Tissue Culture. Cells were grown in L-15 medium that contained 10% fetal calf serum (FCS), 10% tryptose phosphate broth, and penicillin/streptomycin (all from Gibco-Biocult, Ltd., Paisley, Scotland). The washing buffer (phosphate-buffered saline [PBS]) contained 8.00 g NaCl, 0.20 g KH_2PO_4 , and 1.15 g Na_2HPO_4 in 1 liter of water. The preparation of hepatocytes, their growth as monolayers in tissue culture flasks (25 cm^2 , Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.), and their viability in culture have been described (8, 11). Approximately 2×10^6 cells were plated in each flask. The hepatocytes were used after 18–24 h in culture, when no other cell type could be seen. Kidney cells prepared like the hepatocytes were used after 4 d in culture and consisted of similar numbers of fibroblasts and epithelial cells.

Binding of ^{125}I -Proteins to Cells in Culture. The entire procedure was carried out in a room maintained at 37°C and lasted a total of 35 min for any given flask. A large excess of the radiolabeled reagents was used to ensure that the amount bound depended only upon the number of available sites, and the proportion of bound radioactivity never exceeded 2% of the input.

Just before use the flasks were shaken to detach any dead cells, and the cells were washed with 2 ml of PBS. 2 ml of fresh medium was then added, followed by the reagents, (Table I) the total volume being, at most, 150 μl . Four flasks that contained cells and at least two that contained only medium were used for each set of conditions. At the end of the incubation period, 100 μl of medium was taken from each flask for counting and calculating the input; the remaining medium was discarded, and the flasks were washed three times with 2 ml of PBS. Finally, the cells were scraped from the flasks and transferred to counting vials with 1 ml of distilled water. Control flasks were treated in the same way.

Results

The results of the binding studies are summarized in Table I. The initial experiments (first two lines of Table I) showed that hepatocytes but not kidney cells bound ^{125}I -IgA. As the amount of ^{125}I -IgA bound to flasks that contained kidney cells was no were used as the controls in subsequent experiments.

The failure of ^{125}I -IgG to bind to hepatocytes shows that binding is not a property of immunoglobulins in general; it also shows that the binding of ^{125}I -IgA is not a result of nonspecific adhesion of protein, which in any case should have been obviated by the presence of 10% FCS. The fourfold reduction in the binding of ^{125}I -IgA seen in the presence of unlabeled IgA demonstrates competition for specific binding sites, and

TABLE I
Radioactivity Recovered

Cell type	Pretreatment of cells	^{125}I -labeled protein	cpm bound per flask $\times 10^{-2}$	
			With cells	Without cells
Kidney	None	IgA*	4.8 \pm 1.0	6.0 \pm 1.3
Hepatocyte	None	IgA*	39 \pm 4.3	
Hepatocyte	None	IgG	5.5 \pm 0.8	3.8
Hepatocyte	None	IgA	40 \pm 2.3	4.0
Hepatocyte	10-fold excess cold IgA for 5 min	IgA	10 \pm 2.5	—
Hepatocyte	Anti-SC serum for 1 min	IgA	8.5 \pm 0.9	—
Hepatocyte	Anti-SC serum for 30 min	IgA	6.2 \pm 0.6	—
Hepatocyte	Normal rabbit serum for 5 min	IgA	56 \pm 2.7	—
Hepatocyte	None	anti-SC (50 μl)	120 \pm 12	6.0
Hepatocyte	None	anti-SC (150 μl)	160 \pm 27	8.5

Input counts (cpm): IgG: $4.5 \times 10^5 \pm 0.4$; IgA: $7.0 \times 10^5 \pm 0.7$ or $*1.7 \times 10^5 \pm 0.1$; anti-SC (50 μl): $1.0 \times 10^7 \pm 0.03$; anti-SC (150 μl): $2.6 \times 10^7 \pm 0.03$.

The radioactivity (cpm \pm SE) recovered from Falcon flasks that contained $\approx 2 \times 10^6$ cultured hepatocytes or, in one experiment, kidney cells, and from control flasks that contained only medium, after the addition of various unlabeled and radiolabeled proteins, incubation, and three washes. The values shown both for added and bound cpm are the means \pm SE from quadruplicate estimations.

also that the labeled IgA was not damaged by radioiodination. Similar inhibition of the binding of ^{125}I -IgA was obtained after a 1- or 30-min preincubation of the cells with rabbit antiserum to SC diluted 1:100; no such inhibition was seen with normal rabbit serum used at the same dilution.

To demonstrate that the inhibition by anti-SC serum was a result of the blocking of a site on the cell surface rather than on the IgA molecule, the anti-SC antibody from this antiserum was specifically purified and radiolabeled. Because the amount required for saturation had not been previously determined, two concentrations of anti-SC antibody were used in experiments. Direct binding to the hepatocytes by the anti-SC antibody was obtained, and no increase in binding, when compared with the background levels, was achieved with the higher antibody concentration.

Discussion

The inhibition by antiserum to SC of the binding of rat IgA to cultured rat hepatocytes, and the ability of specifically purified antibody to SC to bind to these cells, indicate that SC is the receptor for IgA on hepatocytes. The IgA found in bile is, in the main, combined with SC (Fig. 1 a), which it has acquired during its transit through the liver (2). It seems, therefore, that the mechanism of the transport of IgA across hepatocytes is like that proposed by Brown (12) for transport across enterocytes: the polymeric IgA combines with the SC exposed on the sinusoidal surface of the cell; the combined molecules are endocytosed and transported through the cytoplasm before being discharged into the bile canaliculus, or the lumen of the gut in the case of enterocytes. The association of IgA with small endocytic vesicles in the cytoplasm of rat hepatocytes has been recently demonstrated (13).

Our identification of the membrane receptor for IgA as being SC depends upon common antigenic determinants in the soluble and membrane-bound forms of SC; further characterization of the latter is difficult because it can only be solubilized with detergents (Orlans et al. Unpublished results.).

The specific binding *in vitro* of polymeric IgA to hepatocytes is not restricted to the rat system. The binding of some preparations of human polymeric IgA to suspensions of human (14) and rabbit (15) hepatocytes has been demonstrated by immunofluorescence, but SC could not be detected on the human hepatocytes. This may be a result of the species difference, but could also be explained by the relative insensitivity of immunofluorescence or the different method of cell preparation.

Summary

Rat hepatocytes in short-term monolayer cultures bound radiolabeled polymeric rat IgA but not IgG. The binding of ^{125}I -IgA was inhibited equally well by unlabeled polymeric IgA and by antiserum to rat secretory component (SC). The antibody to SC, after specific purification and radiolabeling, was bound to hepatocytes as effectively as the IgA. These results indicate that SC acts as the receptor for polymeric IgA on rat hepatocytes as it does on human gut epithelia, and that the transport of IgA from blood to bile in rats across the liver is analogous to that of IgA across human enterocytes.

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