Brief Definitive Report

HIGH LEVELS OF SECRETORY IgA AND FREE SECRETORY COMPONENT IN THE SERUM OF RATS WITH BILE DUCT OBSTRUCTION*

By ISABEL LEMAÎTRE-COELHO, GRAHAM DOUGLAS FISHER JACKSON, and JEAN-PIERRE VAERMAN

(From the Unit of Experimental Medicine, International Institute of Cellular and Molecular Pathology, Catholic University of Louvain, B-1200 Brussels, Belgium)

Secretory IgA (SIgA) is in majority confined to exocrine secretions, where it represents the predominant immunoglobulin in most mammalian species (1, 2). In humans, SIgA occurs at very low levels in normal serum (≅1–2% of total IgA), and is increased in a variety of conditions not always clearly involving glandular mucosae (3). The highest levels of SIgA in human serum were observed in lactating women, where the SIgA represented as much as 7% of the total serum IgA. Although usually undetectable in animals, SIgA has been found in serum in a few instances (2, 4, 5), including serum from rats (unpublished observations) with transplantable IgA immunocytoma (6). In contrast, free secretory component (FSC) was never detected in mammalian serum.

It was recently shown that cannulated rat bile contained three major proteins; albumin, SIgA, and FSC (7). The absolute IgA levels in rat bile were higher than were serum levels. Roughly, 10–15 mg each of SIgA and FSC are poured daily into the rat intestine via the bile (7). In another study,[1] we demonstrated that the perfused rat liver is able to actively transfer a rat monoclonal IgA from the circulation into the cannulated bile, against a strong concentration gradient. These findings suggest that obstruction of the bile duct of the rat (which has no gall bladder) may modify the IgA levels of rat serum.

The present report deals with the consequences of bile duct ligation on the immunoglobulin concentrations in rat serum. The study was performed with particular emphasis on IgA.

Materials and Methods

Animals. Male OFA rats (200 ± 20 g) were used throughout.

Antisera. Monospecific antisera against rat IgA, IgM, IgG, albumin, and FSC have been described previously (8, 9).

Sera. Sera, obtained from tail-vein blood, were either kept frozen or at 4°C with 0.1% NaN_3.

Surgery. The bile ducts of rats anesthesized with ether were occluded according to the method of Lambert (10). For temporary obstruction, the bile duct was compressed against a 5-mm long plastic tube by two silk knots; 24 h later, the knots were cut and the bile flow was reestablished. The sham operation consisted of a laparotomy and mild manipulation of liver and intestine for about 10 min.

Immunochemical Methods. The presence of IgA, SIgA, and FSC in serum was detected by conventional immunoelectrophoresis and Ouchterlony analyses. Quantitative estimations of IgA, IgM, IgG, and albumin in sera were performed by single radial immunodiffusion (11).

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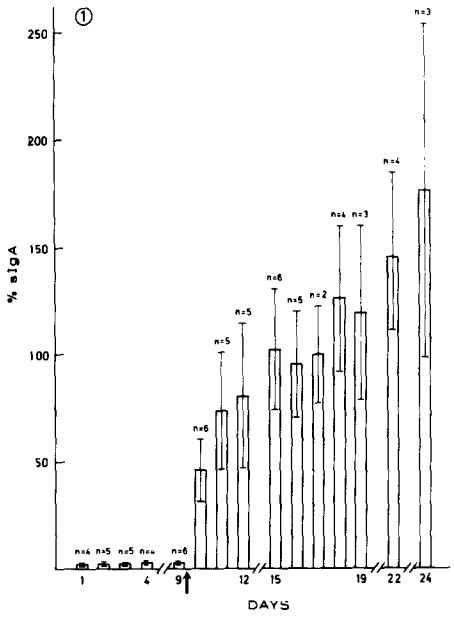

FIG. 1. IgA levels (means ± SD) in sera of n rats at various times before and after ligature (arrow) of the bile duct: 100% = 5.1 mg of IgA/ml (see Materials and Methods).

Levels were expressed as percentages of pooled normal rat serum for IgM, IgG, and albumin, and as percentages of the IgA content of a sample of semi-purified milk SIgA (9) for IgA (100% = 5.1 mg/ml).

Gel Filtration. 7 ml of serum from a normal rat, or from a rat under bile duct occlusion for 2 wk, was applied to a 2.5 × 90-cm column of Ultrogel AcA 22, eluted with 2% NaCl, buffered with 0.02 M Tris-HCl, pH 8.0, and containing 0.1% NaN_3. Eluates were pooled in several fractions, according to their optical density at 280 nm, which were then concentrated to 1 ml before analysis by agarose-gel electrophoresis (12) and immunoelectrophoresis. The column was calibrated with samples of human IgM, milk SIgA, IgG, and albumin.

Results

After the ligature of the bile duct of rats, the IgA level in their sera increased rapidly and very strongly (Fig. 1). Within 24 h, the IgA in serum had risen 10- to 20-fold. Thereafter, the IgA concentration in serum continued to increase progressively, until maximal values of 30–100 times those of the preoperative period were reached, just before the animal's death (10–30 days).

The selectivity of these raised levels of IgA in serum was demonstrated by the lack of corresponding changes in the serum levels of IgG, IgM, and albumin (Fig. 2). Also, none of the sham-operated rats displayed any significant alteration in their serum levels of IgA, IgG, IgM, and albumin.

The kinetics of the increase and decrease of IgA in serum after ligature and reopening of the bile duct, respectively, are illustrated in Figs. 3 and 4. When the ligature was maintained for only 24 h, the increased level of IgA persisted for only 1–5 days (Fig. 3). 1 h after ligation, there was already a noticeable increase of the IgA level in serum, which became obvious upon immunoelectrophoresis at 3 h after obstruction (Fig. 4). Antibodies against rat secretory component (SC) revealed that, at all times, increased levels of IgA in serum seemed entirely due to SIgA, as indicated by the parallel intensities of the IgA

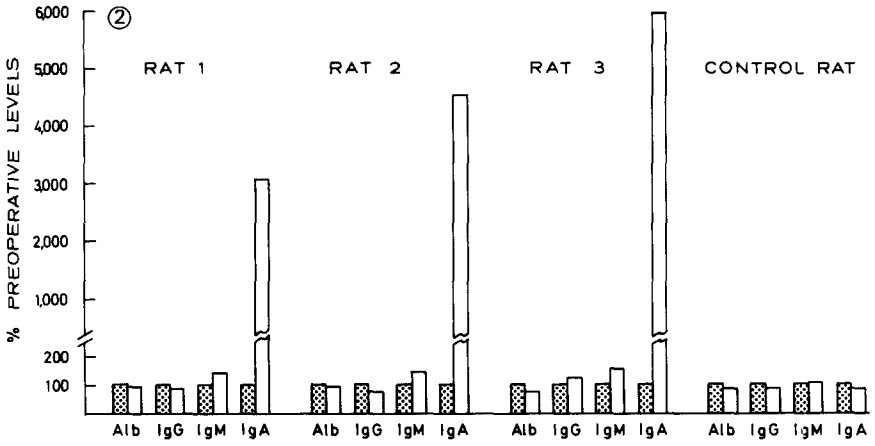

FIG. 2. Serum levels of albumin, IgG, IgM, and IgA in three rats before (dotted columns) and after (open columns) bile duct ligation or sham operation (control). The bile ducts of rats 1, 2, and 3 were ligated for 6, 14, and 28 days, respectively. The control serum was taken 6 days after sham operation.

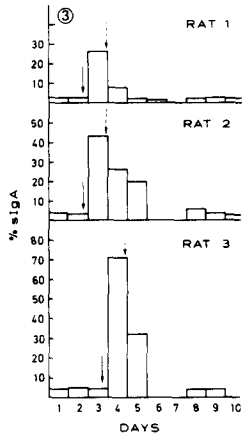

FIG. 3. Levels of IgA in serum of three rats at various times before and after bile obstruction (solid arrow) and reopening (dashed arrow).

and SIgA precipitin lines developed by anti-α-chain and anti-SC antibodies, respectively (Fig. 4). At 3 h after ligature (and occasionally earlier), FSC, easily identified by its rapid anodal mobility, appeared in the serum. The FSC level increased with time after ligature (Fig. 4), but after 2–4 wk of jaundice, the level in serum decreased without the apparent drop of SIgA in serum (data not shown). When the bile duct was reopened after 24 h of obstruction, FSC disappeared from serum within 24 h (Fig. 4).

To confirm that increased IgA levels were due to SIgA, normal rat serum and the serum of a rat whose bile duct had been occluded for 2 wk were submitted to gel filtration on Ultrogel AcA 22 (data not shown). After concentration of the absorbance peaks, two major IgA-containing fractions were found only in the eluates of the jaundiced serum. As for bile, the second of these fractions eluted at the same volume as did 11S human SIgA. The two IgA

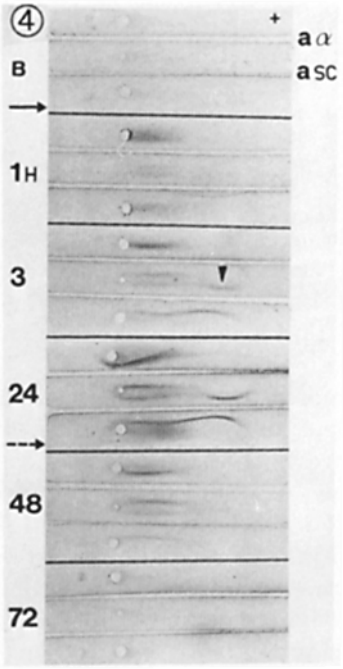

FIG. 4. Immunoelectrophoreses of sera from a rat taken before (B) and at various times (1, 3, 24, 48, and 72 h) after ligature (solid arrow) and reopening (dashed arrow) of the bile duct. Each plate was developed by anti-α-chain (aα) and anti-SC (aSC) antisera, respectively, in the upper and lower troughs. Note that the concentration of IgA in serum before ligature is so small that no precipitin line is visible, even using a large antigen reservoir. Arrowhead shows FSC.

fractions reacted equally well with anti-α-chain and anti-SC antisera. In addition, FSC was immunoelectrophoretically detected in a fraction from the jaundiced serum eluted between IgG and albumin, as expected. The normal serum fractions showed only weak reactivity with anti-α-chains and no reactivity with anti-SC.

Discussion

These results demonstrate that, in the rat, ligation of the bile duct leads rapidly to a large and selective rise in the concentration of IgA in serum. This additional IgA is predominantly, if not entirely, of the secretory type. FSC also appears in serum after ligation. At 24 h after obstruction, these changes are completely reversible within a few days by reopening the duct, whereby FSC first disappears from serum, followed by the slower decline of SIgA in the serum.

An explanation for these findings may reside in our observation (7) that rat bile is a rich source of SIgA and FSC. These proteins could simply regurgitate in serum after ligature of the bile duct. The amounts of SIgA and FSC found in serum at 24 h after ligature are compatible with this hypothesis. Furthermore, we have found in rats (unpublished observations) and mice (13), a very rapid initial serum clearance of intravenously injected monoclonal IgA; this initial clearance was so rapid that it seemed improbable that extravascular equilibration and catabolism could be the sole factors involved. Some IgA may be

secreted into exocrine secretions. In mice, the injected IgA was easily detected in several exocrine secretions, with a surprisingly high level in bile, where it was entirely bound to SC (14). In rats, liver perfusion experiments showed an active transfer of rat monoclonal IgA from the perfusion medium into bile, where it was also bound to SC.[1] These data imply that in rats and mice, the liver actively removes IgA from serum and transfers it into bile in the form of SIgA.

Why does the liver do this? IgA antibodies in serum (15), mainly IgA polymers (16), have been claimed to have potentially adverse effects against defense mechanisms, and the liver could prevent their accumulation in serum. In addition, it was shown that a significant contribution to rat serum IgA was coming from the intestinal mucosa plasma cells via the mesenteric and thoracic duct lymph (8). It is attractive to think that some IgA molecules which were synthesized in the intestine mucosa plasma cells, but which did not directly find their way to the intestinal secretions, could still become SIgA in the duodenum via the lymph, general circulation, liver, and bile. This could represent a reinforcement mechanism of the local intestinal immunity. It is desirable to know whether this additional SIgA is of minor or major importance.

It remains to be determined if all the IgA in rat bile (and in serum after bile duct obstruction) is of circulatory origin, or if a substantial fraction is synthesized locally as is reported to be the case in humans and dogs (17–20). Also, it should be examined whether the mechanism of transfer of IgA in bile is the same as those in intestinal (21–23) and mammary (24) secretions.

The relevance to the human SIgA system of these studies on rats may be the high IgA levels in human serum detected in all patients with liver disease, and in particular in those with primary biliary cirrhosis and obstructive jaundice (25).

Summary

In the rat, ligation of the bile duct induces a rapid and progressive elevation of the IgA levels in serum. The increase is about 4-fold at 1 h, 15-fold at 1 day, and 30-fold at 1 wk after ligation. The additional IgA is of the secretory type. Free secretory component also appears in serum after bile duct obstruction; it does not continue to increase and occasionally disappears from serum after prolonged ligation. The increase in serum IgA levels is selective. These changes are totally reversible if the bile duct is reopened at 1 day after ligature. These findings confirm the role of the rat liver in the transfer of circulating IgA into the bile.

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