Demonstration of IgA and secretory component in human hepatocytes

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SUMMARY By the immunoperoxidase technique, immunoglobulin A (IgA) was demonstrated in 50% of human hepatocytes. The positively stained cells showed a tendency towards periportal clustering. Very few plasma cells were identified in the liver. The intrahepatocyte IgA is most probably derived from serum and on its way to the bile ducts. Secretory component (SC) had the same distribution as that of IgA. The presence of SC in the hepatocytes reflects either the synthesis of SC or the transport of pre-assembled SC-IgA by liver cells. The significance of transfer of IgA from serum into bile is as yet unknown. However, the transhepatic passage of IgA may represent a reinforcement of local intestinal immunity, as part of the gut-originated IgA antibodies, which fail to go through the intestinal mucosa, may still gain access to the lumen of the gut.

The predominant immunoglobulin (Ig) in the bile of several species, including human, is immunoglobulin A (IgA) of the secretory type.¹⁻⁵ Several recent studies have suggested that the biliary IgA is derived from the serum.^{6,7}

The circulating Igs, before going into the bile, must cross two successive cell layers: (1) the endothelial cells between the blood and the intestinal fluid, and (2) the cells between the fluid and the lumen of the bile ducts, which consist of either hepatocytes or biliary epithelium, depending on whether protein transfer takes place within the liver lobules or in the portal spaces.

The present work was therefore undertaken to verify the role of liver cells or biliary epithelium in the transport of IgA. Using the highly sensitive immunoperoxidase technique, IgA was demonstrated mainly in the liver cells.

Methods

TISSUES

A total of 10 liver specimens were selected for immunoperoxidase studies. Seven specimens were obtained from necropsy cases within two hours of post mortem examination, and another three were

obtained as paraffin blocks of wedge biopsies which were done for staging of Hodgkin's disease. None of these cases had a history of liver disease, abnormal liver function test, or alcoholism. The specimens were immediately fixed in 10% buffered formalin for four hours at room temperature and then dehydrated and embedded in paraffin wax as in routine processing.

All the selected specimens were interpreted as normal by histological examination.

IMMUNOPEROXIDASE STAINING

The present studies were carried out using the unlabelled antibody method described previously.^{8,9} Rabbit antisera (IgG fraction) to human IgG, IgM, IgA (specific for heavy chains) or secretory component (Dakopatts, Accurate, NY) were used as the primary antisera.

Serial sections were cut (5 µm) from paraffin blocks. The sections were deparaffinised in xylene and processed to alcohol. Trypsinisation was performed to enhance the staining intensity.¹⁰ Endogenous peroxidase activity was blocked in methanolic H₂O₂ for 30 minutes. To reduce the non-specific background staining, the sections were pretreated with normal, non-immune, swine serum (1:10) for 10 minutes before the application of the primary antiserum (1:400). Swine anti-rabbit IgG serum (1:50) (Dakopatts) was then applied, which was followed by rabbit anti-peroxidase-peroxidase complex (1:200) (Dakopatts).

Each stage lasted 30 minutes and was followed by

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washing with three changes of tris-buffered saline, pH 7.6 for 30 minutes. The diaminobenzidine (DAB) (Sigma) reaction was used to localise the site of peroxidase. After development in DAB solution, the sections were rinsed in distilled water, osmicated,¹¹ and counterstained with methylgreen (1% in methanol).

SPECIFICITY OF ANTISERA AND CONTROLS

The specificity of antiserum was confirmed by two criteria: (1) no cross-reactivity was found among different antiserum preparations by double immunodiffusion in agar gel; (2) after absorption of each antiserum with the corresponding antigen on Sepharose 4B beads (prepared as described by Streefkerk *et al.*¹²), the staining activity of the antiserum was completely abolished. No diminution or blocking of staining was noted when the antiserum was absorbed with heterologous antigens.

Additional controls for the staining procedure were also carried out as described by DeLellis *et al.*¹³ To test for the non-specific binding of antiserum, tris buffered saline or non-immune serum

were used as substitutes for each step and the results were consistently negative.

Results

HEPATOCYTES

In all sections studied, the hepatocytes reacted positively with anti-IgA antibody, but the intensity of staining varied considerably from cell to cell. Positively stained cells occurred in groups or as isolated units with some tendency towards periportal clustering (Fig. 1). It was estimated that about 50% of the liver cells showed an appreciably positive reaction for IgA. The same was true for the secretory component (SC), although the staining intensity was much lower (Fig. 2A, B).

Positive staining with less intensity for IgG and IgM was also observed in about 20% and 10% of the hepatocytes respectively, which were scattered singly or in groups (Table).

Because of the difficulty of identifying the luminal borders of the bile canaliculi under light microscopy,

Fig. 1 Hepatic tissue stained for IgA. Note the anatomical distribution with a tendency towards periportal clustering of the IgA-positive hepatocytes. There are only a few positive stained cells scattered singly in the region of central lobule (C). $\times 25$.

Fig. 2 *A. High magnification of IgA-positive hepatocytes shown in Fig. 1, $\times 600$. B. Secretory component positive hepatocytes. $\times 600$. (Note: This photomicrograph has been overdeveloped to enhance morphological detail, the staining intensity of SC, therefore, was weaker than that for IgA).*

Table Immunoenzymatic localisation of immunoglobulins and secretory component in livers

% of cells stained with staining intensity	Hepatocytes	Epithelial cells of intrahepatic bile duct
SC and IgA	50% (+++) * for IgA (+) for SC	10% (+ or ++)
IgG	20% (+)	None
IgM	10% (+ or $\pm$)	None

*Staining intensity was assigned semiquantitatively, as +++, strong positive (brown-black); ++, positive (brown); +, weak positive (light brown); $\pm$, equivocal; -, negative.

Fig. 3 *A. Intrahepatic bile duct. The plasma cells (short arrows) and scattered epithelial cells (long arrow) are positive for IgA. $\times 600$. B. Stained for secretory component. Few biliary epithelial cells are definitely stained (long arrow). Note the non-stained plasma cells (short arrow). $\times 600$.*

the presence or absence of Igs in the bile canaliculi could not be ascertained.

BILIARY EPITHELIUM

Both IgA and SC could be demonstrated in the epithelium of intrahepatic bile ducts (Fig. 3A, B). However, the percentage of positively stained epithelial cells varied from one duct to another, being less than 10%. In the cells stained, the staining was generally more prominent in the supranuclear cytoplasm.

Very few plasma cells were identified in the adjacent interstitium. It was noted that such plasma cells contained IgA but not SC. In most of the bile ducts examined, the presence of IgA in the biliary epithelium appeared to be associated with the presence of local plasma cells. Yet, as the number of plasma cells was very low, the precise origin of intraepithelial IgA remained unclear.

The staining for IgG and IgM was not identifiable in the epithelium of the intrahepatic bile ducts.

Discussion

Using the immunoperoxidase technique, IgA, IgG, and IgM were demonstrated in a large population of human hepatocytes, although the staining intensity varied considerably from cell to cell. As the staining reagents used were considered to be specific by several criteria, it seemed unlikely that the observed staining results were due to the non-specific binding of the antisera preparation. Particularly noteworthy was the high percentage of liver cells that were stained with IgA, and the presence of SC in the liver cells.

For many years, it has been known that liver cells are capable of transferring serum proteins, such as albumin, orosomucoid, transferrin, immunoglobulins, and hormones into the bile.^{1,2} In most cases, the passage of serum proteins into the bile is influenced by their molecular sizes and is directly proportional to their serum concentrations.^{1,2} It is also noted that glycoproteins, in general, are cleared by passing through the liver.¹⁴ Thus, as all Ig molecules are glycoprotein in nature, it is likely that the liver mediates their transfer in a manner analogous to the transfer of non-Ig molecules. In this respect, biliary IgA is unique in that its relative concentration to other proteins in the bile greatly exceeds that in the serum.^{1,3-5}

Orlans *et al.*⁶ has shown that injected oligomeric ¹²⁵I-IgA disappears from the serum much more rapidly than IgG₂. This rapid disappearance of injected IgA is not seen in rats with ligated bile ducts. Also, in rats with cannulated bile ducts, the labelled IgA

appears rapidly in the bile with about 25% of the injected radioactivity being recovered in three hours.⁶ Lemaitre-Coelho *et al.*⁵ reported that the ligation of the bile ducts in rats induced a rapid and progressive rise of the secretory IgA in the serum. The rise was reversible if the bile duct was reopened. These studies support the theory that IgA molecules are selectively transported from serum into the bile.

In theory, the passage of IgA from serum into bile can occur either through the hepatocytes or *via* the bile duct epithelium. Recently, using autoradiography and electromicroscopy, Birbeck *et al.*⁷ showed that, shortly after the injection of ¹²⁵I-polymeric IgA into rats, the IgA molecules became bound to the plasma membrane of hepatocytes, and, after 30 minutes, much of it was transported across the cytoplasm and became localised around the bile canaliculi. This result leads to the conclusion that rat hepatocytes do provide a route for the passage of IgA. Our studies demonstrating the presence of IgA in human hepatocytes is consistent with the above mechanism. Although our immunoperoxidase study did demonstrate the presence of IgA and SC in some biliary epithelial cells, the majority of the epithelial cells remained unstained by anti-IgA and anti-SC antisera. Moreover, the presence of IgA in the biliary epithelium tends to be associated with the presence of local plasma cells which occur rather infrequently. At the present time, we are unable to determine whether the intrabiliary epithelial IgA is derived from the serum or is synthesised by local plasma cells, or both.

Since the beginning of this study, there has been one report which contradicts our data. Using peroxidase labelled antibody and electromicroscopy, Smith *et al.*¹⁵ conducted a study from one liver specimen and concluded that IgA was not present in human hepatocytes and that serum IgA appeared to be transported by the biliary epithelium rather than the hepatocytes. The discrepancy between their result and the present study may be due to the differences in the amplification of the antigen-antibody reaction, the method of detection, and the areas of liver examined.

The mechanism of passage of IgA through human hepatocytes is not completely understood. In the rat, it was found that monomeric IgA and secretory IgA (SC-IgA) were transported poorly when compared with polymeric IgA without SC.¹⁴ Moreover, the majority of IgA in the bile is of secretory type,^{5,14,15} it appears that the trans-hepatocyte transport of IgA requires prior combination with SC.¹⁴ Similar studies have not been done in humans. However, in this study, the presence of SC in human hepatocytes is consistent with the idea that SC might act as the liver cell receptor for polymeric

IgA and mediate its transfer into the bile spaces.^{16 17} Alternatively, some of the SC detected in the liver cells may also come from preassembled IgA (or IgM).

The transfer of small amounts of IgG and IgM through hepatocytes is likely to occur through a mechanism common to all macro-molecules.^{1 2} The higher number and greater intensity of IgG positive hepatocytes compared with IgM-positive hepatocytes probably reflects the higher serum concentration and lower molecular weight of IgG. However, as IgM also has the ability to bind SC, it may also be transported through an SC-mediated mechanism.¹⁷ In any event, the ability of liver cells to transfer all classes of Igs indicates that liver cells may play an important role in regulating serum Ig concentrations. Failure to excrete Igs into bile may contribute, in part, to the hypergammaglobulinaemia and raised serum IgA and SC observed in many patients with micronodular cirrhosis or extrahepatic biliary obstruction.¹⁸

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