Invited Review

Ground-glass hepatocytes: light and electron microscopy. Characterization of the different types

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Summary. Morphological observations of paraffin-embedded histological sections stained with H&E led to the discovery of some cytoplasmic changes which occur in different conditions, although they look alike under the light microscope. These hepatocytic changes consisted basically of homogeneous areas which are weakly eosinophilic in H&E-stained sections. They are frequently referred to as «inclusion» bodies, even when they are not true inclusions. The hepatocytic changes observed in HBsAg carriers, in chronic alcoholic patients treated with cyanamide to discourage them from drinking alcohol, in Lafora’s disease, and in glycogenosis type IV, look very similar in paraffin sections stained with H&E. Nevertheless, they can be differentiated using ancillary techniques. On electron microscopy they do not look alike. Of particular interest are the «inclusion» bodies induced by cyanamide, a predictable and reproducible lesion, which in man eventually leads to cirrhosis.

Other types of hepatocytic changes also giving a rather vague «ground-glass» appearance to the cytoplasm are those resulting from intracytoplasmic accumulation of proteins, particularly fibrinogen, and those observed in patients treated with different drugs.

Key words: Ground-glass hepatocytes, Liver-cell inclusions, HbsAg, Cyanamide, Lafora’s disease, Glycogenosis type IV, Fibrinogen inclusions

Introduction

Morphological observations of paraffin-embedded histological sections stained with haematoxylin and eosin led to the discovery of some cytoplasmic changes which appear in different conditions, although they look alike under the light microscope. The exact interpretation of these changes depends on the degree of expertise of the pathologist and particularly on the results of special stainings and ultrastructural studies. These hepatocytic changes consist basically of more or less large, homogeneous areas which are weakly eosinophilic in haematoxylin and eosin-stained sections. When these areas are well-demarcated they are referred to as inclusion bodies, even when they are not true inclusions, since most of them are not membrane-bound, and correspond to transformed or altered cytoplasm or, being membrane-bound, contain a material produced by the cell itself.

Ground-glass hepatocytes in HBsAg carriers

The best known type of ground-glass hepatocytes is that observed in Australia antigen (HBsAg) carriers in relation to the hepatitis B viral infection (Fig. 1). Hadziyannis et al. (1973) described the presence of hepatocytes with ground-glass appearance in HBsAg-seropositive patients. These hepatocytes were associated with bright HBsAg-specific cytoplasmic immunofluorescence. These ground-glass cytoplasmic areas can be stained with orcein, aldehyde-fuchsin and aldehyde-thionin and give a strong reactivity when treated with antibodies raised against HBsAg (Hadziyannis et al., 1973). In addition, they give a PAS (periodic-acid-Schiff)-positive reaction.

On electron microscopy the ground-glass areas show hyperplasia of smooth endoplasmic reticulum which displaces other organelles to the cell periphery. Within the smooth endoplasmic reticulum profiles there are long filaments, 20-26 nm in diameter and of variable length up to 1 μm, which are similar to the HBsAg filaments found in the serum of these patients (Huang and Groh, 1973; Gerber et al., 1974; Winckler et al., 1976). This filamentous material corresponds to virus-induced coat protein synthesized by the endoplasmic reticulum of the infected liver cells (Huang and Groh, 1973). The ground-glass hepatocytes are found mainly in chronic hepatitis B.
and particularly in carriers without evidence of hepatitis. They are observed with a random distribution through the lobule.

**Cyanamide-induced «inclusion» bodies**

The liver cells bearing «inclusion» bodies induced by cyanamide in chronic alcoholic patients, treated to discourage them from drinking alcohol, have a close resemblance to the «ground-glass» hepatocytes found in the HBsAg carriers.

The «inclusion» bodies induced by cyanamide consist of distinctive round or kidney-shaped cytoplasmic areas, which are homogeneous or partly granular in haematoxylin- and eosin-stained sections (Fig. 2). These bodies appear slightly eosinophilic and are very often surrounded by an artifactual halo. They frequently displace the nucleus to the cell periphery and often occupy most of the cell body sparing only a thin rim of normal looking cytoplasm, giving the cells a signet-ring appearance (Vázquez and Pardo-Mindán, 1979).

In PAS-stained preparation the «inclusions» give a strongly positive reaction. This PAS-positive material is completely hydrolized by incubation with saliva, but the time required for its total digestion is often longer than that required for hydrolysis of glycogen in a normal liver. «Inclusion» bodies stained also give a positive colloidal iron reaction, are deeply stained with methanamine silver, giving a light to moderate positive Prussian blue reaction. On the contrary, Shikata stain for HBsAg is negative.

In semi-thin sections of plastic-embedded material the «inclusion» bodies frequently show dense granules and fine vacuoles. In these semi-thin sections the artifactual halo around the «inclusions» is not seen.

On electron microscopy the «inclusions» contain a large amount of glycogen disposed in beta-granules, secondary lysosomes containing whorled lamellar structures, occasional lipid droplets, a filamentous matrix, and residues of degenerating organelles: rough and smooth endoplasmic reticulum and mitochondria (Fig. 3). Smooth endoplasmic reticulum is almost completely absent. Glycogen is the predominant component of «inclusions» in some patients, while debris resulting from organelle breakdown predominates in others.
Ground-glass hepatocytes

Table 1. Differential diagnosis

<table>
<thead>
<tr>
<th>Type</th>
<th>PAS stain</th>
<th>Shikata stain\textsuperscript{a}</th>
<th>Acid fuchs\textsuperscript{b}</th>
<th>Immunocytochemistry</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HBsAg</td>
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<tr>
<td>HBsAg</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>Cyanamide</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Lafora's</td>
<td>+</td>
<td>-</td>
<td>-/+</td>
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<tr>
<td>Glucogenosis type IV</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Fibrinogen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Oncocytic reaction</td>
<td>-</td>
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<td>+</td>
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\textsuperscript{a} orcein;  
\textsuperscript{b} Cowdry stain for mitochondria, Masson's trichrome stain.

Fig. 3. Electron micrograph of a cholangiolar made up of two biliary cells, upper right, and one binucleate liver cell, which bears an inclusion body containing abundant glycogen and degenerating organelles. × 3,000

Fig. 4. Needle biopsy from a patient suffering from myoclonic epilepsy (Lafora's disease). Some hepatocytes contain inclusion bodies. Note the characteristic halo around the ground-glass areas. H & E, × 400
Fig. 5. Lafora's disease. Hepatocyte bearing inclusion body (dotted line). Note the abundant glycogen. Some mitochondria and smooth endoplasmic reticulum profiles are seen inside the inclusion. × 14,400

The peripheral rim of cytoplasm disposed around the «inclusion» contains numerous mitochondria and rough endoplasmic reticulum profiles, as usually seen in normal hepatocytes. Strikingly, this rim usually gives a PAS-negative reaction because it does not contain any glycogen.

The distribution of «inclusion» bodies through the lobule varies from case to case, according to the duration of treatment and the existence of pauses in the treatment. The lesion starts at the periphery of the lobule and progresses towards the centre. Therefore, in patients treated recently and for a short time only the periportal liver cells contain «inclusions», whereas in those treated for long periods and particularly having had some pauses in the treatment, the affected hepatocytes have a more random distribution throughout the lobule.

Patients treated with cyanamide develop a progressive fibrosis (Moreno et al., 1984). In cases with intense fibroblastic activation, the hepatocytic plates in the periportal area undergo a tubular transformation; «inclusion» bodies are also seen in the liver cells forming part in the lining of the cholangioles (Vázquez et al., 1983a).

Fig. 6. Biopsy taken from a patient on antiepileptic drugs. The cytoplasm is clearer than normal because of the smooth endoplasmic reticulum increase. H & E, × 620

The lesion induced by cyanamide is predictable; that is, it appears in all patients treated with this drug, and is reproducible in the rat liver (Vázquez et al., 1983b; Guillén and Vázquez, 1984).

Two events are particularly important in the development of «inclusion» bodies induced by cyanamide: the storage of a rather abnormal glycogen and the breakdown of organelles. In normal liver cells glycogen is arranged in alfa-granules. On the contrary, in these «inclusions» it is disposed in beta-granules and in some filaments, giving a colloidal iron-positive reaction. The accumulation of such glycogen could be explained by the inhibition of certain hepatic enzymes by cyanamide. In addition, the almost complete disappearance of smooth endoplasmic reticulum is accompanied by the loss of its membrane-bound enzymes. The breakdown of smooth endoplasmic reticulum and mitochondrial membranes inside lysosomes may well result in the whorled lamellar, structures seen by the electron microscopy (Vázquez and Pardo-Mindán, 1979).

The cyanamide-induced «inclusions» eventually
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disappear after discontinuing the drug consumption, mainly through a process of removal of the inclusion-bearing liver cells; an event which has been studied in human beings (Thomsen and Reinicke, 1981; Vázquez et al., 1983b; Idoate, 1987) and in rats (Idoate, 1987). The disappearance of «inclusions» in human beings may be expected three years after withdrawal of the drug. In rats it takes place much sooner.

«Inclusion» bodies in Lafora’s disease

«Inclusion» bodies described in the liver tissue in cases of myoclonic epilepsy (Austin and Sakai, 1976; Ramón y Cajal et al., 1974) are quite similar to the cyanamide-induced «inclusions» on observation by light microscopy. The affected hepatocytes are also perportal in location. Hepatic «inclusion» bodies in Lafora’s disease are usually less well demarcated and are rarely numerous (Fig. 4). They are found in groups of hepatocytes close to the portal space, but never in all perportal hepatocytes as seen in cyanamide-treated patients at the beginning of the treatment. They give a PAS-positive reaction, although weaker than that of cyanamide bodies, which also disappears after saliva digestion. On electron microscopy the hepatocytes consist of glycogen disposed in beta-granules and some mitochondria that look normal (Fig. 5).

Similar bodies may be found in other organs, particularly in the secretory portion of sweat glands, which makes an easy diagnosis available by means of a skin biopsy.

«Inclusion» bodies in glycogenosis type IV

Quite similar to the previously described bodies are the «inclusions» observed in type IV glycogenosis which is due to branching enzyme deficiency. They are also located predominantly in the acinar zone 1 (Schochet et al., 1970), and in haematoxylin- and eosin-stained sections are faintly eosinophilic or amphophilic and rather granular. As in cyanamide bodies, a clear artifactual halo often surrounds the inclusions. The
abnormal glycogen which makes up the «inclusion» bodies stains with PAS and colloidal iron. It is only partially digested with diastase, but can be completely digested by pectinase (Reed et al., 1968; Schochet et al., 1970). The lesion is followed by a progressive fibrosis which eventually progresses to cirrhosis. On electron microscopy the inclusions are not membrane-bound and contain fibrillar material.

In addition to the liver «inclusions», basophilic deposits of polysaccharides may be found in muscle fibers, particularly from the tongue.

Although the type IV glycogen storage disease typically manifests itself in infants, with death occurring between the second and fourth year of life (Greene et al., 1988), cases occurring in children and even in adults have been reported (Ferguson et al., 1983; Greene et al., 1987).

**Hepatocytes with ground-glass appearance induced by other drugs**

Many drugs produce liver cell changes which vaguely resemble the ground-glass hepatocytes (Fig. 6). In this way, they may be considered the so-called adaptative changes observed after long-term treatment with anticonvulsant drugs (Jezequel et al., 1984; Pamperl et al., 1984), phenobarbital or phenytoin (Klinge and Bannash, 1988) rifampicin (Jezequel, 1971; Scheuer, 1974) chlorpromazine (Popper, 1973) and in patients receiving more than one drug (Winkler, 1976). In the cases in which an ultrastructural study was carried out a pronounced increase of smooth endoplasmic reticulum was observed (Fig. 7).

Ground-glass hepatocytes bearing inclusions of different types, induced by two or more drugs may be found in the same patient (Alonso-Marti et al., 1990).

**Fibrinogen «inclusions»**

Other types of «inclusions» also giving a rather vague «ground-glass» appearance to the cytoplasm are those resulting from intracytoplasmic accumulation of proteins (Pfeifer and Klinge, 1974; Porte et al., 1977; NG et al., 1989), particularly fibrinogen whose deposit was...
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described both in non-neoplastic hepatocytes (Callea et al., 1986; NG et al., 1989) and in carcinoma cells (Stromeyer et al., 1980). On haematoxin- and eosin-stained sections the fibrinogen inclusions are round or kidney-shaped, homogeneous and weakly eosinophilic. They give negative reactions with orcein, PAS and silver methenamine. In PAS staining, a rim of glycogen is sometimes observed in the ring of cytoplasm around the inclusions. Immunocytochemically they react for fibrinogen very strongly, giving a granular appearance. The inclusions vary in size and more than one of them may be seen in the same hepatocyte (Fig. 8).

Ultrastructurally, the fibrinogen inclusions described by Porte et al. (1977) and Callea et al. (1986) in non-neoplastic hepatocytes are bound by a membrane which bears ribosomes, considered then as being dilated cisterna of the rough endoplasmic reticulum. The cisterna bears ribosomes, considered then as being dilated cisterna of the rough endoplasmic reticulum. The alteration in carcinoma cells in three cases of fibrolamellar hepatocarcinoma, but they described the fibrinogen deposits as non-membrane-bound. Stromeyer et al. (1980) described a similar alteration in carcinoma cells in three cases of fibrolamellar hepatocarcinoma, but they described the fibrinogen deposits as non-membrane-bound. Nevertheless, the material they studied was previously fixed in formalin and although the ultrastructure was fairly well preserved, the cytoplasmic membranes do not show up well; they appear fragmented. Craig et al. (1980) and Goodman et al. (1985) also described the presence of eosinophilic, hyaline, cytoplasmic PAS-negative globules in cases of lamellar hepatocarcinoma; they did not carry out ultrastructural study.

The intracellular retention of fibrinogen may be the result of a molecular defect which interferes with its transportation towards the smooth endoplasmic reticulum (Callea et al., 1986).

Oncocytic change of hepatocytes

Among other changes in the cytoplasm of liver cells resembling the ground-glass hepatocytes, there is also the oncocytic change sometimes observed in patients having cirrhosis. The cytoplasm has a granular appearance and stains intensively with eosin and acid fuchsin.

Ultrastructurally, large amounts of mitochondria are seen. The hepatocytes with oncocytic reaction are arranged in small groups, very often close to connective tissue septa (Fig. 10).

Differential diagnosis of ground-glass hepatocyte types

The hepatocytic changes observed in HBsAg carriers, in patients on cyanamide, in Laforta’s disease and in glycogenesis type IV look very similar in paraffin sections stained with haematoxylin and eosin. Nevertheless, they can be differentiated using ancillary techniques (Table 1). On electron microscopy they look Unlike.

References


Ground-glass hepatocytes


