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HLA-A,B,C, HLA-D/DR and HLA-D/DQ expression on unfixed liver biopsy sections from patients with chronic liver disease

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SUMMARY

The distribution of HLA-A,B,C, HLA-D/DR and HLA-D/DQ molecules was studied by indirect immunofluorescence with an avidin–biotin technique and monoclonal antibodies, in unfixed cryostat sections of liver biopsies from 76 patients with chronic liver diseases of various aetiologies and five normal liver biopsy specimens. In pathological liver, strong cytoplasmic or membrane-like positivity for HLA-A,B,C of hepatocytes was observed in piecemeal necrosis areas in all groups. Cytoplasmic staining was mainly seen in lobular areas in autoimmune, cryptogenic and HBV-related cases with viral replication, while membrane-like positivity was more frequently observed in primary biliary cirrhosis, alcoholic and HBV-related cases without viral replication. A weak cytoplasmic staining for HLA-D/DR was observed in piecemeal necrosis and lobular areas mainly in HBV-related cases with viral replication. While bile duct cells were positive for both HLA-D/DR and HLA-D/DQ, hepatocytes were consistently HLA-D/DQ negative. The increased HLA-A,B,C expression on hepatocytes should allow T cytotoxic cell aggression. Hepatocellular HLA-D/DR expression is definite but weak and probably does not allow direct autoantigen presentation and induction of autoimmunity. Negativity for HLA-D/DQ further supports this hypothesis. Since cytoplasmic staining for Class I and II molecules is greatly lowered by fixing cryostat liver sections, prestaining conditions should be taken into account when comparing different studies.

Keywords liver immunology histocompatibility antigens liver disease HLA-A,B,C HLA-D/DR HLA-D/DQ

INTRODUCTION

Class I molecules (HLA-A,B,C) of major histocompatibility complex (MHC) are displayed by most nucleated cells of the body, although their expression varies in different cells and is dependent on their functional state. Contradictory reports have appeared questioning whether normal hepatocytes may express HLA-A,B,C molecules (Barbatis *et al.*, 1981; Fleming *et al.*, 1981; Montano *et al.*, 1982; Daar *et al.*, 1984; Lautenschlager *et al.*, 1984; Nagafuchi *et al.*, 1985; Nagafuchi & Scheuer,

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1986a,b; Fukusato *et al.*, 1986), while in various pathological conditions, such as alcoholic hepatitis, primary biliary cirrhosis (PBC), chronic autoimmune and HBV-related hepatitis without viral replication and liver rejection, a membrane-like positivity has been described (Barbatis *et al.*, 1981; Montano *et al.*, 1982; Nagafuchi *et al.*, 1985; Nagafuchi & Scheuer, 1986a,b; Fukusato *et al.*, 1986).

Class II histocompatibility molecules (HLA-D/DR and DQ) are normally restricted to specialized cells: HLA-D/DR is detectable on B lymphocytes, macrophages, dendritic and other antigen presenting cells, epithelial cells lining body cavities (Natali *et al.*, 1981) and activated T lymphocytes (Ko *et al.*, 1979); HLA-D/DQ expression is more restricted (Natali *et al.*, 1984). Sinusoidal cells, but not bile duct cells and hepatocytes, are positive for Class II molecules in normal liver (Natali *et al.*, 1981). The evidence from experimental models suggests that T cytotoxic lymphocytes need to recognize HLA-A,B,C products in order to kill virally infected cells efficiently (Zinkernagel & Doherty, 1979), whereas Class II molecules are responsible for the antigen presentation and the initiation of the immune response; moreover, evidence is coming out that the contemporary expression of HLA-D/DR and DQ is mandatory for efficient antigen presentation in certain conditions (Gonwa *et al.*, 1983).

It has been shown that in a number of autoimmune disorders (thyroiditis, Type I diabetes and PBC) epithelial cells (thyrocytes, pancreatic Beta cells and bile duct cells) can express aberrantly HLA-D/DR (Hanafusa *et al.*, 1983; Bottazzo *et al.*, 1985; Ballardini *et al.*, 1984) and HLA-D/DQ molecules (Most, Knapp & Wick, 1986) and this event could be relevant in triggering or perpetuating autoimmune processes (Bottazzo *et al.*, 1983; Most *et al.*, 1986).

Available data on the expression of HLA-A,B,C and HLA-D/DR molecules in the liver are partially contrasting and hardly comparable since they have been obtained with different techniques. Information on HLA-D/DQ expression is lacking. We have therefore decided to investigate the expression of Class I and II molecules by indirect immunofluorescence with a sensitive avidin–biotin technique on a large series of liver biopsies including patients affected by chronic liver diseases of various aetiologies.

PATIENTS

Seventy-six patients with chronic liver disease were studied. Details of number and histological diagnosis are given in Table 1. The diagnosis was made on the basis of accepted clinical, biochemical, histological and immunological criteria (Leevy, Popper & Sherlock, 1976). Fourteen out of the 15 PBC patients had antimitochondrial antibodies and the autoimmune group included 13 patients with high titre ($\geqslant 1:40$) nuclear and/or smooth muscle antibodies. The HBV group was divided into two subgroups on the basis of the absence (R^-, 18 cases) or the presence (R^+, 13 cases) of viral replication as judged by the detection of tissue HBc (in nine cases) or Delta antigens (in four cases) or serum HBe antigen (in 11 cases).

In the alcoholic group all the 12 patients were negative for HBV markers and autoantibodies. The cryptogenic group (five cases) was defined by the negativity of all the viral and immunological markers mentioned above. Four autoimmune and three cryptogenic cases were under steroid treatment at maintenance doses (metilprednisolone 4–10 mg day). As controls, five surgical liver biopsies from patients operated upon for extrahepatic disorders were studied.

Liver biopsies. Liver biopsy specimens were obtained during peritoneoscopy (Franklin–Silverman needle) or percutaneously with a 1·6 Hepafix needle. Samples, with a length ranging from 15 to 30 mm, were divided into two fragments: one was fixed in formalin and embedded in paraffin for histological examination, the other was immediately embedded in Tissue Tek OCT compound (Miles), mounted on a piece of cork, snap-frozen in N-methylbutane precooled in liquid nitrogen, and kept at −70°C.

Indirect immunofluorescence technique (IFL). To discover the best staining conditions preliminary studies were carried out on unfixed and fixed cryostat sections from one case of PBC stage IV, one case of cryptogenic, autoimmune and alcoholic cirrhosis, two cases of chronic active hepatitis (one with and one without detectable HBc antigen) and one normal liver. Sections were air

Table 1. Patients

Condition	Number	Details	R^+	R^-
Controls	5	Surgical biopsies from patients operated for extrahepatic disorders		
PBC	15	10 stage 1–2		
		5 stage 3–4		
Autoimmune	13	1 chronic persistent hepatitis		
		4 chronic active hepatitis		
		8 cirrhosis		
Cryptogenic	5	5 cirrhosis		
Alcoholic	12	5 fibrosteatosis		
		7 cirrhosis		
HBV related	31	3 prolonged hepatitis	2	1
		7 chronic persistent hepatitis	2	5
		6 chronic active hepatitis	4	2
		15 cirrhosis	5	10
			13	18

R^+, with features of viral replication as positivity for tissue HBcAg or Delta antigen and/or serum HBeAg.

dried for 6 h and tested unfixed and after treatment with acetone or acetone-chloroform (1:1) at room temperature for 5 and 10 min with five antibodies: W6 32 (Sera Lab), beta-2-microglobulin (Dako) (anti-Class I related antigens), Mid 3 (G. Guarnotta, P. Lydiard, Middlesex Hospital, London, UK), and DK22 (Dakopatts) (HLA-D/DR antigens), Leu 10 (Becton & Dickinson) (HLA-D/DQ).

Unfixed serial 4 μm cryostat sections from at least two different biopsy levels were stained with monoclonal antibodies (MoAb) to the non-polymorphic region of HLA-A,B,C (W6 32) and HLA-D/DR (Mid 3) molecules by IFL with an avidin–biotin technique previously described (Ballardini *et al.*, 1984). Sections of five PBC biopsies and of three biopsies from each other group of patients were also stained with a MoAb to HLA-D/DQ (Leu 10).

Double IFL technique. Double IFL staining was carried out by applying, as the first layer, MoAb to Pan T (Leu 4), helper-inducer (Leu 3a), suppressor-cyytotoxic (Leu 2a) T cell phenotypes (all from Becton & Dickinson), followed by anti HLA-A,B,C as the second layer, on selected biopsies showing both hepatocellular cytoplasmic and membrane enhanced Class I molecules expression.

A similar double IFL staining technique was used to compare the distribution of viral antigens (HBsAg, HBcAg, Delta) and HLA molecules. Anti HBsAg rabbit antibodies were purchased by Beheringwerke AG (Marburg, West Germany) and directly FITC conjugated human anti-HBc and anti-Delta antibodies were kindly supplied by Dr M. Rizzetto (Turin, Italy).

The following morphological parameters were evaluated: number and IFL intensity of HLA-D/DR positive sinusoidal cells (normal, increased, decreased), presence of HLA-A,B,C, HLA-D/DR and DQ positive hepatocytes, both in piecemeal necrosis and lobular areas. These parameters were visually scored by two independent observers. In particular, HLA-A,B,C parenchymal membrane and/or cytoplasmic positivity was scored as detectable on more or less than 50% hepatocytes.

RESULTS

Using fixed sections a membrane-like positivity for Class I related antigens was consistently found, although the intensity was lower in normal than in pathological liver (Fig. 1a, 2a). Cytoplasmic positivity for HLA-A,B,C (Fig. 1b, 2b) and HLA-D/DR was greatly lowered by fixation, while it was preserved in unfixed sections.

Unfixed sections were preferred since they allowed us to evaluate a range of staining patterns wider than that obtained on fixed sections. A corresponding staining pattern was observed with the

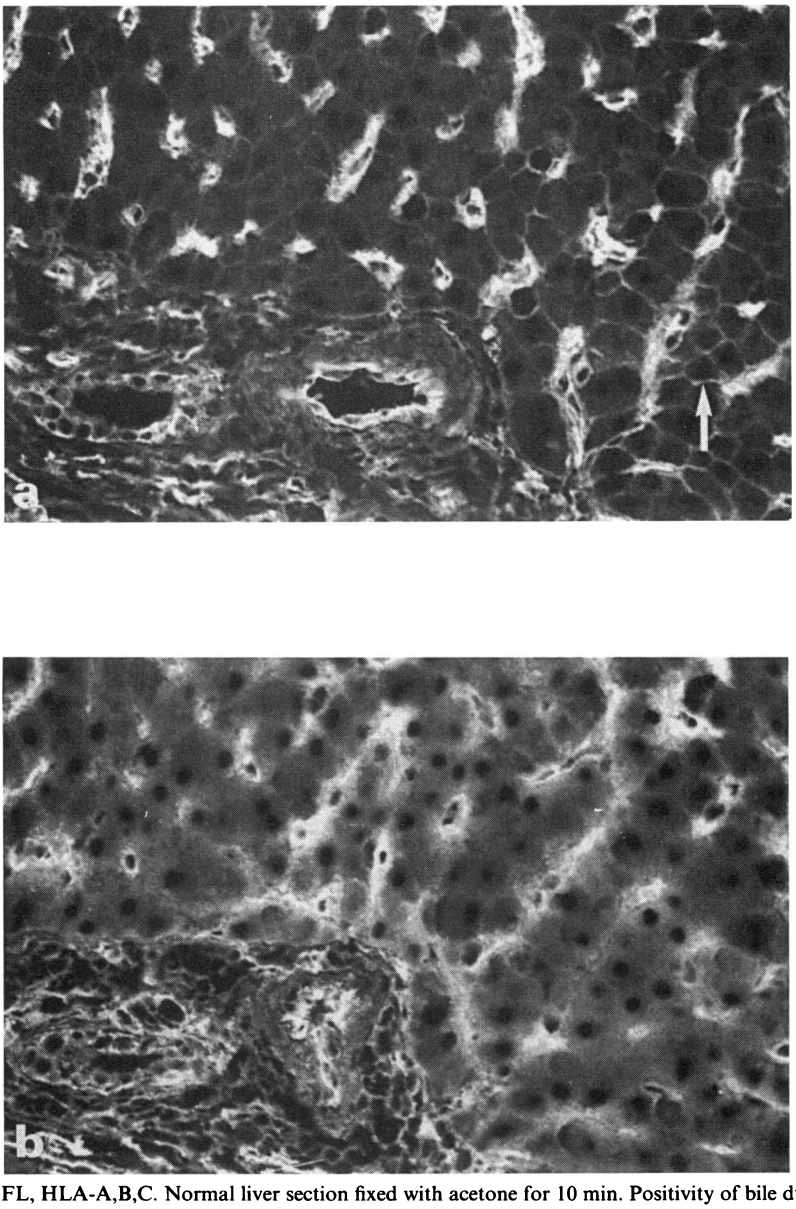

Fig. 1 (a) IFL, HLA-A,B,C. Normal liver section fixed with acetone for 10 min. Positivity of bile duct, artery endothelium, sinusoidal cells. Faint membrane-like staining of hepatocytes (arrow). ×250, (b) IFL, HLA-A,B,C. Unfixed normal liver section. Consecutive to Fig. 1(a). Hepatocellular membrane-like staining is not detectable. Faint hepatocellular cytoplasmic staining. ×250.

two Class I and Class II related MoAb, respectively, both on normal and pathological, fixed and unfixed liver sections.

Unless differently stated, the following data refer to unfixed material.

MHC expression on normal liver tissue. In the five control biopsies HLA-A,B,C molecules showed a wide cellular distribution. They were observed on macrophage like and spindle cells of

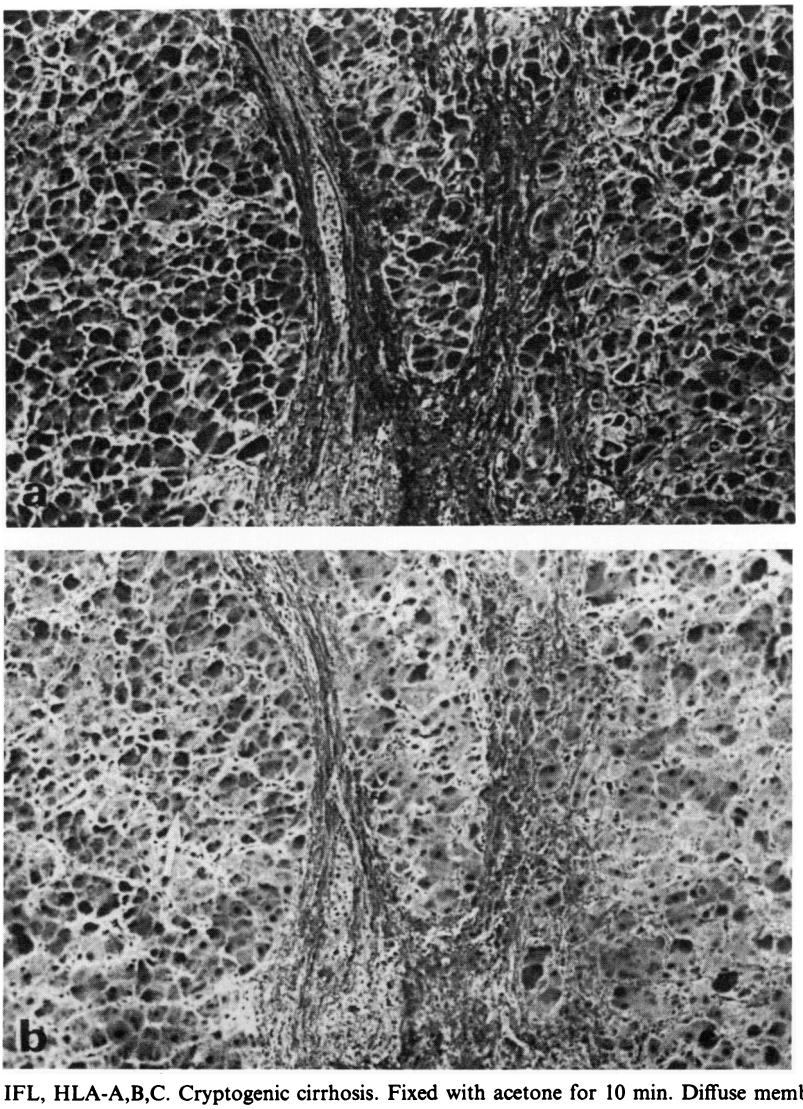

Fig. 2. (a) IFL, HLA-A,B,C. Cryptogenic cirrhosis. Fixed with acetone for 10 min. Diffuse membrane-like positivity of hepatocytes. × 100. (b) IFL, HLA-A,B,C. Cryptogenic cirrhosis. Unfixed section. Consecutive to Fig. 2(a). Prevalence of hepatocellular cytoplasmic positivity, associated with membrane-like staining in some areas (arrow). × 100.

portal tracts, bile ducts, sinusoidal lining cells and all vascular endothelia. In addition, although weak, a definite reactivity was observed on hepatocytes (Fig. 1b).

HLA-D/DR molecules were detected only on macrophage-like and spindle cells in portal tracts, mononuclear and single sinusoidal cells (probably Kupffer cells) in the parenchyma. An occasional weak reactivity was seen on vascular endothelium but bile ducts and hepatocytes were consistently negative (Fig. 3). HLA-D/DQ molecules were displayed with a similar pattern of positivity, but there were fewer sinusoidal HLA-D/DQ positive cells than HLA-D/DR positive cells and endothelial positivity was not observed.

Comparing the IFL pattern of the antibodies stained by conventional indirect IFL and the avidin–biotin technique, a clear-cut increase in both intensity and number of positive cells was

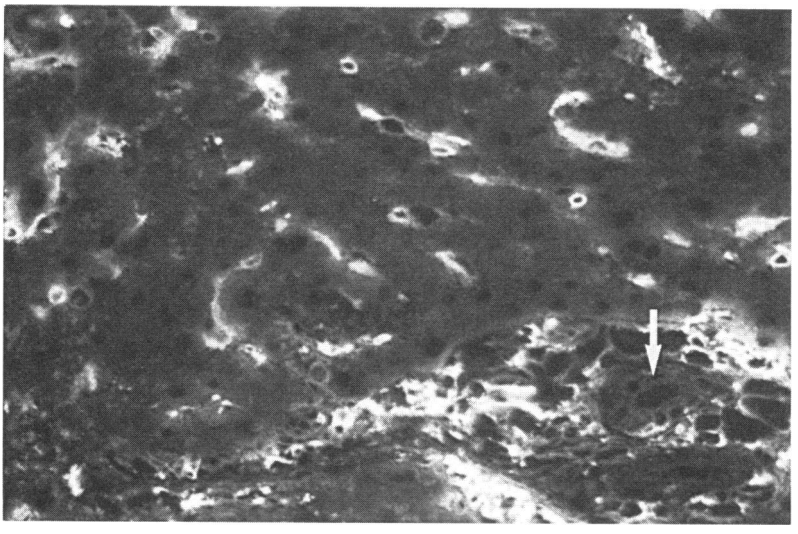

Fig. 3. IFL, HLA-D/DR. Unfixed normal liver. Positivity of sinusoidal cells, macrophage-like and spindle cells of portal tract. Negative bile duct (arrow) and hepatocytes. × 250.

observed. This was especially true for HLA-A,B,C expression (W6/32) which was weaker on sinusoidal cells and undetectable on hepatocytes, when tested by the conventional indirect IFL.

Pathological liver: HLA-A,B,C molecules expression (Table 2). A strong Class I cytoplasmic staining of hepatocytes, often associated with membrane-like reactivity, was observed in piecemeal necrosis areas in all groups (Fig. 2b, 4, 5a, 6a). In the same 'rosette' HLA-A,B,C positive hepatocytes adjacent to negative ones were found in occasional biopsies (Fig. 4). Sometimes other 'rosettes' with both cytoplasmic and membrane-like positivity or with membrane-like positivity alone were observed in the same field (Fig. 5a).

Large lobular areas of hepatocytes (more than 50%) with strong cytoplasmic staining (Fig. 2b, 6a), with or without a coexisting clear membrane-like positivity, were found preferentially in

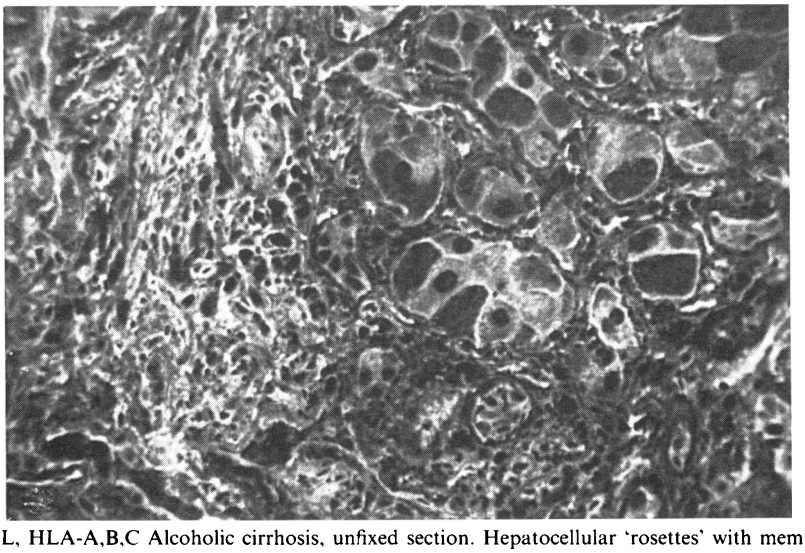

Fig. 4. IFL, HLA-A,B,C Alcoholic cirrhosis, unfixed section. Hepatocellular 'rosettes' with membrane-like staining. Positive and negative cytoplasmic staining. × 250.

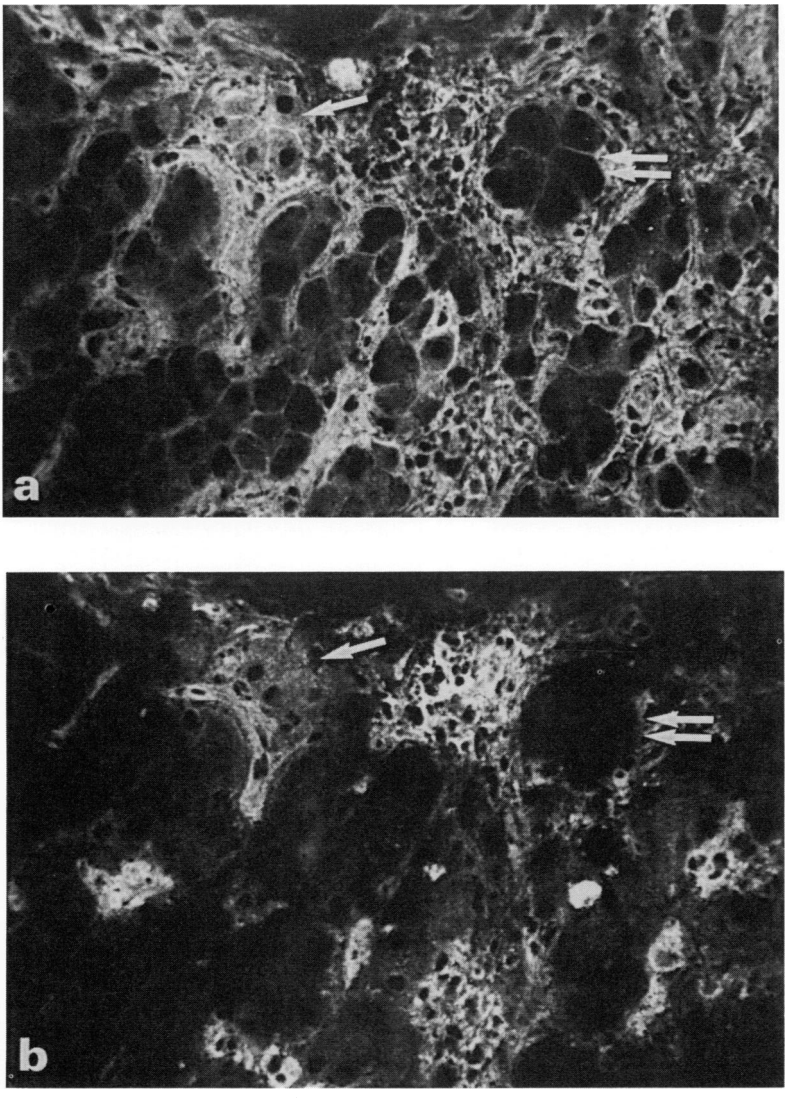

Fig. 5 (a) IFL, HLA-A,B,C. PBC stage 2, unfixed section. A piecemeal necrosis area showing two hepatocellular 'rosettes', one with cytoplasmic and membrane-like staining (arrow), and one with membrane-like but not cytoplasmic positivity (double arrows). × 250. (b) IFL, HLA-D/DR. PBC stage 2, unfixed section. Consecutive to Fig 5a. There is faint cytoplasmic staining of the 'rosette' (arrow) with HLA-A,B,C cytoplasmic positivity in Fig. 5(a) and negativity of the other 'rosette' (double arrows). × 250.

autoimmune, cryptogenic and HBV R^+ cases and less commonly in HBV R^-, alcoholic and PBC biopsies. A clear membrane-like positivity (more than 50% of hepatocytes) was observed in HBV R^-, PBC and alcoholic cases, while it was less common in autoimmune and HBV R^+ cases. The presence of cytoplasmic and membrane-like positivity in different areas of the same section as well as in the same hepatocyte was detectable.

By using the double IFL techniques a close association was found between hepatocytes with high HLA-A,B,C cytoplasmic reactivity (with or without membrane-like staining) and a strong infiltration of lymphocytes with suppressor-cytotoxic phenotype in periportal and parenchymal areas. The same correlation was not detected in areas with normal or membrane-like expression of HLA-A,B,C.

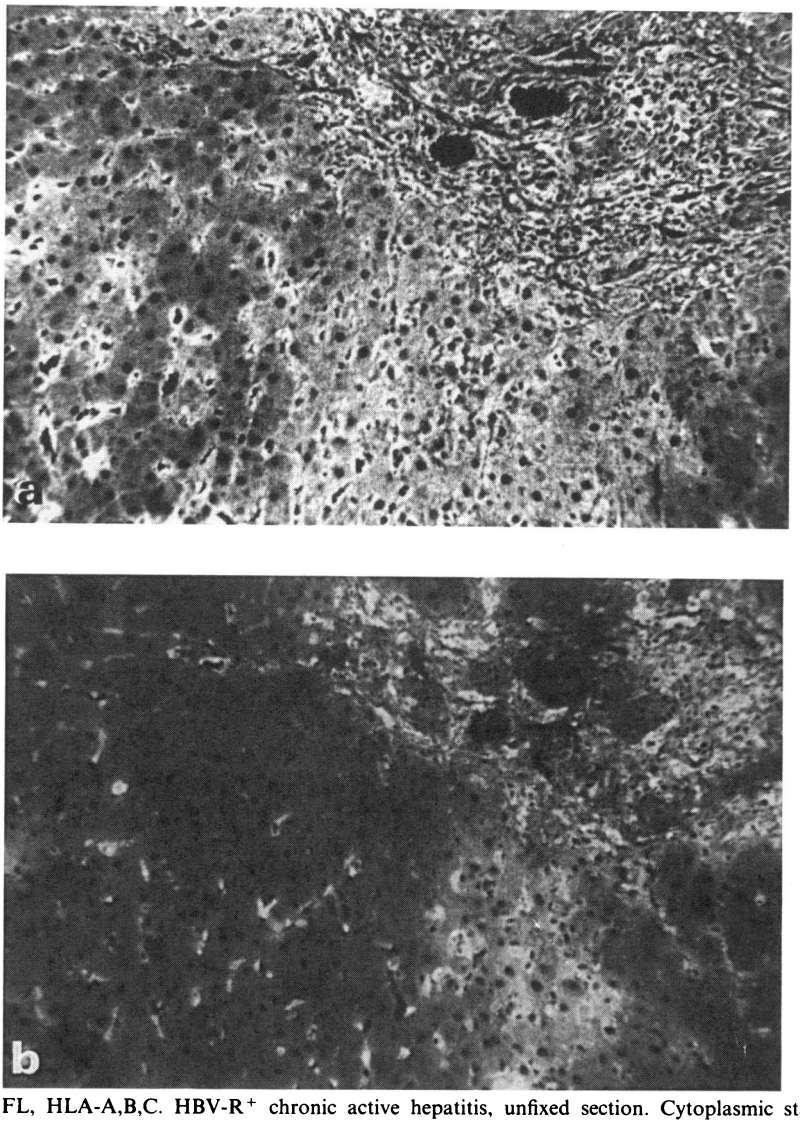

Fig. 6(a) IFL, HLA-A,B,C. HBV-R^+ chronic active hepatitis, unfixed section. Cytoplasmic staining of hepatocytes. × 100. (b) IFL, HLA-D/DR. HBV – R^+ chronic active hepatitis, unfixed section. Consecutive to Fig. 6a. Large lobular area with positive hepatocytes, corresponding to strong HLA-A,B,C positivity in Fig. 6(a). × 100.

By the same technique, a cytoplasmic reactivity for HLA-A,B,C molecules was in general detected in areas with nuclear HBcAg and/or Delta antigen positivity, while membrane-like staining of HLA-A,B,C products was more often seen in areas with hepatocytes containing cytoplasmic HBsAg.

Pathological liver: HLA-D/DR molecules expression (Table 2). In piecemeal necrosis areas a weak HLA-D/DR cytoplasmic staining was detected in hepatocytes of 6/10 HBV R^+ cases (Fig. 5b) and, less commonly, of autoimmune (3/9), alcoholic (2/8) and HBV R^- biopsies (1/12). Positive and negative hepatocellular 'rosettes' were often observed in the same field (Fig. 5b).

In the parenchyma, large areas of hepatocytes with a weak HLA-D/DR^+ cytoplasmic staining were observed in HBV R^+ cases (Fig. 6b) and to a lesser extent in the other groups.

Table 2. Morphological parameters studied by IFL

		Sinusoidal cells			PMN			Parenchyma		
					det	DR+	ABC+	DR+	ABC+	
Condition	No	N	I	D	n	C	C/M	C	C>50%	M>50%
PBC	15	4	11	0	9	3	7	1	6	6
Autoimmune	13	3	7	3	9	3	9	3	9	3
Cryptogenic	5	0	4	1	5	2	5	1	5	0
HBV R^+	13	7	6	0	10	6	9	6	10	2
HBV R^-	18	9	5	4	13	1	12	1	11	8
Alcoholic	12	6	2	4	8	2	7	0	7	4

Sinusoidal cells: N, normal number and positivity; I, increased; D, decreased.

PMN, piecemeal necrosis: det, detected on the cryostat sections; C, cytoplasmic positivity; M, membrane-like positivity; >50%, detected in more than 50% of hepatocytes.

In PBC and autoimmune cases, groups of hepatocytes expressing HLA-D/DR molecules were often in close contact with clusters of strongly DR^+ sinusoidal cells. The phenomenon was occasionally observed in the other groups and was correlated with the number of such clusters.

Both in piecemeal necrosis and parenchymal areas, HLA-D/DR^+ hepatocytes turned out to be strongly positive for cytoplasmic HLA-A,B,C on consecutive sections (Fig. 5a–5b, 6a–6b).

The presence of cytoplasmic HBsAg was generally found in HLA-D/DR negative lobular areas with a weak positivity of neighbouring sinusoidal cells, while the presence of HBcAg or Delta antigen was more often associated with HLA-D/DR staining of hepatocytes in areas where sinusoidal cells were strongly positive.

An increased number of HLA-D/DR positive sinusoidal cells (probably Kupffer cells) was observed in PBC, autoimmune and cryptogenic cases, while their number and IFL staining were less prominent in alcoholic, HBV R^+ and HBV R^- cases.

Pathological liver: HLA-D/DQ expression. No staining of hepatocytes was detected in our series. Sinusoidal cell pattern of positivity was similar to HLA-D/DR staining, but the number of positive cells was generally lower.

Bile ducts: HLA-A,B,C, D/DR and D/DQ molecules expression. The expression of HLA Class I and II molecules on bile ducts have been previously reported (Ballardini *et al.*, 1984). In this study, bile ducts turned out to be positive for both HLA-D/DR and HLA-D/DQ in the five PBC cases tested for both molecules.

DISCUSSION

In the present study, both cellular distribution and expression of Class I MHC molecules were shown to be more widely displayed in normal and pathological liver specimens than previously reported (Barbatis *et al.*, 1981; Fleming *et al.*, 1981; Montano *et al.*, 1982; Daar *et al.*, 1984; Lautenschlager *et al.*, 1984; Nagafuchi *et al.*, 1985; Nagafuchi & Scheuer 1986a,b; Fukusato *et al.*, 1986). The use of unfixed or differently fixed material and the different sensitivity of the technique used in previous studies may well account for these discrepancies. Of note is that it has been recently demonstrated that beta-2-microglobulin (closely related to Class I MHC molecules) is detectable in plasma membrane of normal, fixed hepatocytes (Nagafuchi *et al.*, 1985). Theoretically, fixation should improve cellular morphology and cell membrane preservation but, in our experience, it

greatly lowers cytoplasmic staining of Class I and II molecules. The cytoplasmic pattern of positivity is of particular interest, being possibly related to active cellular synthesis, although the possibility of endocytosis cannot be completely ruled out. Since established criteria to adopt fixed or unfixed sections in IFL studies are lacking, we decided to use unfixed material, which allowed us to examine a broader spectrum of positive liver cell structures. When comparing the results of studies on liver cell expression of HLA molecules one should therefore take into account different prestaining conditions.

In pathological liver a common pattern was observed: Class I molecules were commonly seen in hepatocytes undergoing active destruction in piecemeal necrosis areas. This observation correlates well with the increased number of T cells of suppresor-cytotoxic phenotype, preferentially found around and within the affected areas (Alexander & Williams, 1984).

It was previously reported that surface expression of Class I molecules on hepatocytes is primarily detectable in HBV-related cases without viral replication (Montano *et al.*, 1982). The higher percentage (44%) of membrane-like reactivity observed in biopsies of HBV R^- cases, compared with that found in HBV R^+ biopsies (15%), partially supports previous conclusions. Adequate membrane expression of HLA-A,B,C molecules in HBV R^- cases has been proposed as a prerequisite for reaching a complete clearance of virally infected cells, while the incapacity to produce or display these molecules could maintain viral replication. Following this interpretation, treatment with α-interferon (α-IFN) which is known to enhance Class I expression on a variety of cells, has been proposed for HBV R^+ cases (Thomas & Pignatelli, 1985) and recently implemented (Pignatelli *et al.*, 1986a). It remains to be established whether hepatocytes, which in our experience largely synthesize Class I molecules and have a concomitant active viral replication, do not have the full capacity to express these products at the cell surface. α-IFN may have this potentiating role, and since it has been recently reported that α-IFN can also modulate HBcAg and HBeAg expression on hepatocyte surface (Pignatelli *et al.*, 1986b), both facts could contribute to explain the effect of treatment.

The enhanced hepatocellular Class I expression in a high proportion of autoimmune and cryptogenic cases (70% and 100% respectively), where the membrane-like reactivity is hardly distinguishable from cytoplasmic staining on unfixed sections, parallels similar observations in other autoimmune conditions, including bile ducts in PBC (Hanafusa *et al.*, 1983; Bottazzo *et al.*, 1985; Ballardini *et al.*, 1984). In contrast with HBV-infected hepatocytes, in the autoimmune patients the relevant target autoantigen should be normally available on the cell surface and the enhanced Class I expression should ultimately facilitate its recognition.

In all subgroups studied, including autoimmune cases, hepatocytes did not show the striking feature of 'aberrant' HLA-D/DR expression described in classical organ-specific autoimmune diseases and in the bile duct of PBC. The inappropriate expression of Class II molecules in the latter disorders has led to the hypothesis that epithelial cells may act as antigen-presenting cells, presenting their own surface autoantigens, thereby activating autoreactive helper T cells and bypassing the necessity for classical antigen-presenting cells such as macrophages (Bottazzo *et al.*, 1983). The same hypothesis does not seem to apply to chronic liver disorders. HLA-D/DR expression in fact was weak and bore a poor relation to any of the aetiological subgroups examined, even though it was more often detected in areas of piecemeal necrosis and HBcAg positivity. Moreover, HLA-D/DQ positive hepatocytes were never detected even in biopsies showing HLA-D/DR positive hepatocytes. It is known that γ-IFN is a potent HLA-D/DR inducer in a variety of cells, including epithelial cells (e.g. thyrocytes) which normally do not synthesize Class II products (Todd *et al.*, 1985). One can postulate that the heavy infiltration of activated T lymphocytes should lead to increased local production of γ-IFN. Despite the fact that this phenomenon is likely to occur in the diseased liver, hepatocytes seem to respond poorly to the action of the putative inducer. The possibility remains that hepatocytes do not actively participate in the initiation process by presenting their surface antigens in the expression of Class II molecules, but that classical antigen-presenting cells may play the critical role. This is supported by the increased number and IFL positivity for both HLA-D/DR and DQ molecules of sinusoidal (possibly Kupffer cells) scattered in the affected tissue, mainly in autoimmune and PBC biopsies. For at least some antigens, only cells co-expressing HLA-D/DR and HLA-D/DQ can function as antigen presenting cells (Gonwa *et al.*,

1983). From out data, sinusoidal and bile duct cells, not hepatocytes, can be proposed as antigen presenting cells in pathological liver conditions.

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REFERENCES

ALEXANDER, G. & WILLIAMS, R. (1984) Characterization of the mononuclear cell infiltrate in piecemeal necrosis. *Lab. invest.* **50,** 247.

BALLARDINI, G., MIRAKIAN, R., BIANCHI, F.B., PISI, E., DONIACH, D. & BOTTAZZO, G.F. (1984) Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. *Lancet* **ii,** 1009.

BARBATIS, C., WOODS, J., MORTON, J.A., FLEMING, K.A., MCMICHAEL, A. & MCGEE, J.O.D. (1981) Immunohistochemical analysis of HLA (A,B,C) antigens in liver disease. *Gut* **22,** 985.

BOTTAZZO, G.F., DEAN, B.M., MCNALLY, J.M., MACKAY, E.H., SWIFT, P.G.F. & GAMBLE, D.R. (1985) *In situ* characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. *New Engl. J. Med.* **313,** 353.

BOTTAZZO, G.F., PUJOL-BORREL, R., HANAFUSA, T. & FELDMANN, R. (1983) Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. *Lancet* **ii,** 1115.

DAAR, S., FUGGLE, S.V., FABRE, J.W., TING, A. & MORRIS, P.J. (1984) The detailed distribution of HLA-A,B,C antigens in normal human organs. *Transplantation* **38,** 287.

FLEMING, K.A., MCMICHAEL, A., MORTON, J.A., WOOD, J. & MCGEE, J.O.D. (1981) Distribution of HLA class I antigens in normal human tissue and in mammary cancer. *J. clin. Pathol.* **34,** 779.

FUKUSATO, T., GERBER, M.A., THUNG, S.N., FERRONE, S. & SCHAFFNER, F (1986) Expression of HLA Class I antigens on hepatocytes in liver diseases. *Am. J. Path.* **123,** 264.

HANAFUSA, T., PUJOL-BORREL, R., CHIOVATO, R., RUSSEL, R.C.G., DONIACH, D. & BOTTAZZO, G.F. (1983) Aberrant expression of HLA antigens on thyrocytes in Graves' disease: relevance for autoimmunity. *Lancet* **ii,** 1111.

GONWA, T.A., PICKER, L.J., RAFF, H.V., GOYERT, S.M., SILVER, J. & STOBO, J.D. (1983) Antigen-presenting capabilities of human monocytes correlates with their expression of HLA-DS, an Ia determinant distinct from HLA-DR. *J. Immunol.* **130,** 706.

KO, H., FU, S.M., WINCHESTER, R.J., YU, D.T.Y. & KUNKEL, H.G. (1979) Ia determination on human T lymphocytes. Occurence on mitogen and antigen activated T cells. *J. exp. Med.* **150,** 246.

LAUTENSCHLAGER, I., TASKINEN, E., INKINEN, K., LEHTO, V.P., VIRTANEN, I. & HAYRY, P. (1984) Distribution of the major histocompatibility complex antigens on different cellular components of human liver. *Cell. Immunol.* **85,** 191.

LEEVY, C.M., POPPER, H. & SHERLOCK, S. (1976) Disease of the liver and biliary tract. Standardization of nomenclature, diagnostic criteria and diagnostic methodology. In: *Fogarty International Centre Proceedings* N. 22, DHEW Publication (NIH) 76–725. US Government Printing Office, Washington DC, 9–11.

MONTANO, L., MIESCHER, G.C., GOODALL, A.H., WIEDEMANN, K.H., JANOSSY, G. & THOMAS, H.C. (1982) Hepatitis B virus and HLA antigen display in the liver during chronic hepatitis B virus infection. *Hepatology* **2,** 557.

MOST, J., KNAPP, W. & WICK, G. (1986) Class II antigens in Hashimoto thyroiditis. I. Synthesis and expression of HLA-DR and HLA-DQ by thyroid epithelial cells. *Clin. Immunol. Immunopathol.* **41,** 165.

NAGAFUCHI, Y., HOBBS, K.E.F., THOMAS, H.C. & SCHEUER, P.G. (1985) Expression of beta-2-microglobulin on hepatocytes after liver transplantation. *Lancet* **i,** 551.

NAGAFUCHI, Y. & SCHEUER, P.G. (1986a) Expression of beta-2-microglobulin on hepatocytes in acute and chronic type B hepatitis. *Hepatology* **6,** 20.

NAGAFUCHI, Y. & SCHEUER, P.G. (1986b) Hepatic β_2-microglobulin distribution in primary biliary cirrhosis. *J. Hepatol.* **2,** 73.

NATALI, P.G., DE MARTINO, C., QUARANTA, V., NICOTRA, N.R., FREZZA, F., PELLEGRINO, N.A. & FERRONE, S. (1981) Expression of Ia-like antigens in normal human non lymphoid tissues. *Transplantation* **31,** 75.

NATALI, P.G., SEGATTO, O., FERRONE, S., TOSI, R. & CORTI, G. (1984) Differential tissue distribution and ontogeny of DC-1 and HLA-DR antigens. *Immunogenetics* **19,** 109.

PIGNATELLI, M., WATERS, J., BROWN, D., LEVER, A.M.L., IWARSON, S., SCHAFF, Z., GERETY, R. & THOMAS, H.C. (1986a) HLA Class I antigens on hepatocyte membrane during recovery from acute hepatitis B infection and during interferon therapy in chronic hepatitis B virus infection. *Hepatology* **6,** 349.

PIGNATELLI, M., LEVER, A.M.L. & THOMAS, H.C. (1986b) Modulation of hepatitis B virus protein expression by interferon (IFN): homology between the precore region of HBV-DNA and a sequence regulating the cellular interferon induced antiviral system. *J. Hepatol.* **3** (suppl 1), S76.

THOMAS, H.C. & PIGNATELLI, M. (1985) Is modulation of HLA display by interferon important in preventing the development of the chronic carrier state of hepatitis B virus in adults? *Gastroenterol. clin. Biol.* **4,** 287.

TODD, I., PUJOL-BORREL, R., HAMMOND, L., BOTTAZZO, G.F. & FELDMANN, M. (1985) Interferon-gamma induces HLA-DR expression by thyroid epithelium. *Clin. exp. Immunol.* **61,** 265.

WOOD, G.S. & WARKE, R. (1982) Suppression of endogenous avidin-binding activity in tissues and its relevance to biotin-avidin detection systems. *J. Histochem. Cytochem.* **29,** 1196.

ZINKERNAGEL, R.M. & DOHERTY, P.C. (1979) MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction-specificity, function and responsiveness. *Adv. Immunol.* **27,** 51.