Immunocytochemical analysis of HLA class II (DR) antigens in liver disease in man

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SUMMARY The in situ distribution of the major histocompatibility (HLA) class II (DR) antigens was studied in 113 liver biopsy specimens and five livers obtained at necropsy, using monoclonal antibody CR3/43. In 20 normal livers HLA-DR antigens were not detected in bile duct epithelium, hepatocytes, or portal vein endothelium. Normal arteriolar, sinusoidal and central venous endothelium often expressed HLA-DR. Kupffer cells always expressed these antigens. HLA-DR positive spindle cells were identified in the connective tissue of portal tracts, large hepatic veins, and liver capsule: most shared antigens common to all leucocytes and reacted with the histiocytic marker EBM11. Bile duct epithelium expresses HLA-DR in primary biliary cirrhosis, large duct obstruction, and drug induced cholestasis, indicating that HLA-DR positive spindle cells are phenotypically similar to histiocytes.

HLA-class II (DR) antigens function as histocompatibility antigens and regulate cell to cell interactions. They are necessary for antigen recognition and presentation to lymphocytes, and for T cell activation and differentiation.^{1–4}

HLA-DR antigens are normally expressed on B lymphocytes, cells of monocytic lineage,^{5,6} a small population of T helper cells, activated T cells,^{7,8} thymic epithelium,⁹ vascular endothelium,¹⁰ and lactating breast epithelium.¹¹ Epithelial cells such as keratinocytes,^{12–14} intestinal epithelium,¹⁵ and thyroid epithelial cells¹⁶ may express DR antigens, as well as carcinomas^{17,18} and malignant melanomas.¹⁹

HLA class I antigens have been extensively studied in normal and diseased liver.²⁰ Immunohistochemical studies on HLA-DR antigens have been limited to a small series of normal livers.^{21–23} The latter indicate that hepatocytes, arterial, and venous endothelium of normal liver do not express HLA-DR antigens. There are no data on the DR state of other liver stromal cells. Sinusoidal endothelium has been reported as DR positive, although the pattern of distribution and the intensity of staining vary among livers,²³ but Kupffer cells are always HLA-DR positive. In the latter study normal bile duct epithelium was reported as weakly DR positive. In cases of liver transplantation rejection,²⁴ however, and in primary biliary cirrhosis²⁵ focal HLA-DR expression was identified in bile duct epithelium.

This study was done to determine the HLA-DR state of cell populations in normal and diseased liver.

Material and methods

IMMUNOCYTOCHEMISTRY

One hundred and thirteen percutaneous needle liver biopsy specimens were collected fresh at the time of biopsy and immediately frozen in liquid nitrogen; tissue from five necropsy livers (<24 hours) was similarly handled. Cryostat sections (5–6 µm thick) were air dried at 20°C for two hours, fixed in acetone at –20°C for 15 minutes, and air dried at 20°C. The slides were either immediately used or stored at –20°C, individually wrapped in aluminium foil.

For localisation of HLA-DR antigens, the monoclonal antibody CR3/43 was used in a three stage immunoperoxidase technique and in an indirect immunoperoxidase method so that the sensitivity of the two methods could be compared. Table 1 lists the monoclonal antibodies and dilutions used.

THREE STAGE IMMUNOPEROXIDASE TECHNIQUE

Sections were incubated with the relevant antibodies for 30 minutes washed in Tris buffered saline (TBS), pH 7.6, for five minutes, and incubated with rabbit antimouse IgG diluted 1/50 in TBS, containing 30% normal human serum (v/v). Sections were washed in TBS and antirabbit IgG-peroxidase conjugate applied (diluted 1/100 in TBS containing 30% normal

Table 1 *Monoclonal antibodies used*

<i>Antibody</i>	<i>Specificity</i>	<i>Source</i>	<i>Method</i>	<i>Dilution</i>
CR3/43 [10]	HLA-DR	D Mason (Oxford)	Three stage	Nil
			Indirect	1/20
Dako leucocyte common	Common leucocyte antigens	Dako	Indirect	Mixture of the two antibodies (1/1)
Na/34 [26]	Langerhans' cells	A McMichael	Indirect	1/500 (purified ascitic fluid)
EBM/11 [27]	Mononuclear phagocyte system	NDP	Three stage	1/100
T4	T helper/inducer cells	Coulter clone	Indirect	1/40
Dako T8	T cytotoxic/suppressor cells	Dako	Indirect	Nil
Dako T1	Pan T lymphocytes	Dako	Indirect	Nil
Dako Pan B	Pan B lymphocytes	Dako	Three stage	Nil

All antibodies were in the form of culture fluid unless otherwise stated. Antibodies were diluted in TBS. NDP = Nuffield Department of Pathology.

human sera [v/v]). Peroxidase was shown by immersing washed sections for 10 minutes in 3,3-diaminobenzidine tetrachloride (0.8 mg/ml (BDH), containing H₂O₂ (0.08%, v/v) in TBS, washed in tap water, and counterstained with haematoxylin. As a control, sections were incubated with second antibody only.

INDIRECT IMMUNOPEROXIDASE TECHNIQUE

Sections were incubated in series with monoclonal antibodies (table 1) for one hour, in TBS for five minutes, and rabbit antimouse IgG conjugated with peroxidase (Dakopatts) for 30 minutes, as described above. After a five minute wash in TBS peroxidase was shown as in the three stage immunoperoxidase technique.

HISTOCHEMISTRY

Three normal necropsy livers (< 18 hours after death) were used for showing ATP-ase,²⁸ non-specific esterase,²⁹ and acid phosphatase activities³⁰ in an attempt to characterise the DR positive spindle cells in portal tracts.

Results

HLA-DR IN NORMAL LIVER

Table 2 shows the distribution of HLA-DR antigens in 20 normal livers. Bile duct epithelium and portal vein endothelium did not contain detectable amounts of HLA-DR (fig 1). In the stroma of all portal tracts (fig 7), perivascular connective tissue of central large hepatic veins (fig 2), and in liver capsule, spindle cells were strongly positive for HLA-DR, as were similar

Table 2 *Distribution of HLA-DR antigens in normal liver (n = 20)*

<i>Site: cell type</i>	<i>Result</i>
<i>Portal tracts:</i>	
Bile duct epithelium	Negative
Portal vein endothelium	Negative
Arteriolar endothelium	+ (12/20)
Spindle cells	+ (20/20)
<i>Parenchyma:</i>	
Hepatocytes	Negative
Sinusoidal endothelium	+ (10/20)
Central venous endothelium	+ (9/20)

cells in close contact with bile duct epithelium. Arteriolar endothelium was positive in 12 of 20 cases.

All hepatocytes were HLA-DR negative, while sinusoidal endothelium expressed HLA-DR either focally (four of 20), or diffusely throughout lobules (six of 20). Central vein endothelium was positive in nine of 20 cases. Kupffer cells were always strongly HLA-DR positive (fig 3).

IMMUNOCYTOCHEMICAL CHARACTERISTICS OF HLA-DR POSITIVE SPINDLE CELLS

Spindle cells in portal tracts did not express antigens characteristic of Langerhans' cells, T or B lymphocytes, nor did they exhibit ATPase or acid phosphatase activities. A minority contained non-specific esterase and also expressed leucocyte common antigen. EBM/11, a monoclonal antibody with high cellular specificity for macrophages,^{27 31-34} stained spindle cells in portal tracts and sinusoidal cells (fig 4).

Fig 1

Fig 2

Fig 3 (a)

Fig 3 (b)

Fig 4

Fig 5

Fig 1 Normal portal tract stained for HLA-DR. Spindle cells in stroma, adjacent to bile duct (arrow heads), are HLA-DR positive while bile duct epithelium (large arrows) and portal vein endothelium (small arrows) are HLA-DR negative.

Fig 2 Normal centrilobular area. Central vein endothelium and sinusoidal lining cells are HLA-DR positive but HLA-DR is not detectable in hepatocytes.

Fig 3 Centrilobular area with large hepatic vein stained for HLA-DR (a) and leucocyte common antigen (b). HLA-DR positive spindle cells are present in wall of vein and Kupffer cells (arrows) (a). Some stromal cells around central vein and occasional sinusoidal cells (arrows) also contain leucocyte common antigen (b).

Fig 4 Normal portal tract stained with EBM/11. Spindle cells similar to those in fig 1 are EBM/11 positive. Cluster of EBM/11 positive macrophages is also present (arrow).

Fig 5 Portal tract from a case of primary biliary cirrhosis. Bile duct epithelium (arrow) and spindle cells as well as inflammatory cells are HLA-DR positive.

Table 3 Distribution of HLA-DR antigens in biliary and hepatocellular liver disease (n = 51)

Condition	Portal tracts*						
	No of cases	Bile duct epithelium		Endothelium		Spindle cells	Lymphocytes/ Plasma cells
		Focal	Diffuse	Vein	Artery		
Primary biliary cirrhosis	7	5	1	4	7	++	+++
Large bile duct obstruction	4	2	1	0	2	++	+++
Cholestasis (drug induced)	3	1	1	0	2	++	+++
Sclerosing cholangitis	3	1	0	1	3	++	+++
Acute non-B hepatitis	1	0	0	0	1	++	+++
Non-resolving hepatitis	2	1	0	0	2	++	+++
Chronic active hepatitis	5	1	0	0	3	++	+++
Granulomatous hepatitis	4	1	0	1	2	++	+++
Non-specific active hepatitis	20	2	0	0	6	++	+++
Total	49	14	3	6	28	51	51

++, +++ = Subjective assessment of staining intensity.

HLA-DR IN LIVER DISEASES

Tables 3 and 4 show the distribution of HLA-DR antigens in liver diseases. HLA-DR antigens were expressed in the bile duct epithelium (fig 5) in most cases of primary biliary cirrhosis (six of seven). In five cases the expression was focal, varying from bile duct to bile duct in the same or adjacent portal tracts. In one case of primary biliary cirrhosis (stage 3) all bile ducts were HLA-DR positive. This phenomenon did not occur exclusively in primary biliary cirrhosis. It was often observed in chronic large bile duct obstruction (three of four), drug induced cholestasis (two of three), and in bile ducts adjacent to metastatic carcinoma (three of six). In sclerosing cholangitis (one of three), alcoholic (two of 30), and in a spectrum of other liver diseases (nine of 41) HLA-DR positive bile duct epithelium was observed.

Sinusoidal and central vein endothelium usually contained HLA-DR antigens in most types of liver disease studied (table 3). The intensity of staining was greatest in primary biliary cirrhosis and hepatocellular liver disease. An interesting finding was the expression of HLA-DR in portal vein endothelium (four of seven) of cases of primary biliary cirrhosis, regardless of the stage of the disease (table 3). This phenomenon rarely occurred in other types of liver disease (tables 3 and 4).

For HLA-DR localisation the three stage immunoperoxidase technique was more sensitive than the indirect method.

Discussion

The major histocompatibility class II (DR) antigens are important immune response regulators. Their presence on the surface of a cell enables it to present antigens to immunocompetent T lymphocytes. These lymphocytes undergo activation and differentiation, acquiring destructive or immune response regulatory

capacity. During liver transplant rejection³⁵ and in graft versus host disease following bone marrow transplantation^{36,37} the epithelium of intrahepatic bile ducts and the hepatic vein and sinusoidal endothelium are primarily destroyed. Portal tracts and sinusoids are the main sites of the inflammatory response in biliary and hepatocellular liver diseases.

This study shows that a previously unreported population of HLA-DR positive spindle cells exists in the stroma of normal portal tracts, subcapsular and perivascular connective tissue, and around normal bile duct epithelium. These cells are distinct from HLA-DR positive Kupffer cells and central venous endothelium. HLA-DR positive spindle cells are morphologically indistinguishable from fibroblasts but the latter are usually HLA-DR negative. Further characterisation of the HLA-DR positive spindle cells indicated that they did not have antigens in common with Langerhans', T or B cells and that they do not exhibit ATP-ase or acid phosphatase activities; most react with leucocyte common antibody. A few contain non-specific esterase, but most were EBM/11 positive. The latter antibody stains most cells of the human mononuclear phagocyte system with high cellular specificity.^{27,31-34}

Portal tracts of rat liver³⁸ contain HLA-DR positive dendritic cells. These cells are histochemically similar to the Steinman cell, which is a potent stimulator of immune responses.³⁹⁻⁴¹ Interdigitating and dendritic reticulum cells have also been shown in human liver by electron microscopy.⁴² It is unlikely that these HLA-DR positive spindle cells, however, are identical with interdigitating reticulum cells of lymphoid organs because these cells are ATP positive, while the HLA-DR positive spindle cells of liver do not contain this enzyme.⁴³ On balance, therefore, the histochemical and immunohistochemical evidence suggests that these HLA-DR positive spindle cells form part of the mononuclear phagocyte system.

Condition	Parenchyma				
	Hepatocytes	Sinusoidal endothelium		Central vein endothelium Diffuse	Kupffer cells
		Focal	Diffuse		
Primary biliary cirrhosis	0	0	7	7	++++
Large bile duct obstruction	0	0	4	4	+++
Cholestasis (drug induced)	0	0	2	2	+++
Sclerosing cholangitis	0	1	3	3	+++
Acute non-B hepatitis	0	0	1	1	+++
Non-resolving hepatitis	0	0	2	2	+++
Chronic active hepatitis	0	0	5	5	+++
Granulomatous hepatitis	0	0	4	4	+++
Non-specific active hepatitis	0	6	7	14	+++
Total	0	7	35	42	51

Table 4 Distribution of HLA-DR in alcoholic and other liver diseases

Condition	No of cases	Bile duct		Endothelium	
		Focal	Diffuse	Portal vein	Hepatic artery
Alcoholic fibrosis and fatty infiltration	11	0	0	0	3
Alcoholic hepatitis	10	1	0	0	6
Alcoholic hepatitis with cirrhosis	9	1	0	0	3
Idiopathic cirrhosis	2	1	0	0	2
Metastatic carcinoma	6	2	1	2 (Focal)	1
Methotrexate induced fibrosis	2	1	0	0	2
Miscellaneous	10	0	0	0	4
Total	49	6	1	2	21

These results agree with the recent observation²⁵ that HLA-DR antigens are expressed in bile duct epithelium in most cases of primary biliary cirrhosis. In the latter study HLA-DR expression on bile duct epithelium was regarded as a primary phenomenon, characteristic of primary biliary cirrhosis. The latter study, however, contained only a few cases of other biliary tract disorders. It is clear from the present data that HLA-DR positive bile duct epithelium is often observed in chronic large bile duct obstruction, drug induced cholestasis, and bile duct epithelium adjacent to metastatic carcinoma. In chronic active hepatitis, alcoholic liver disease, and sclerosing cholangitis bile duct epithelium is occasionally HLA-DR positive. It is unlikely, therefore, that HLA-DR expression in bile duct epithelium is a primary aetiological factor in primary biliary cirrhosis. The presence of HLA-DR in biliary epithelium in a spectrum of liver disorders may be a secondary phenomenon associated with inflammation.

Normal sinusoidal and central vein endothelium usually contain HLA-DR, and the intensity of staining for the antigens was increased in all liver diseases studied. Portal vein endothelium, which is HLA-DR negative in normal liver, was positive in four of seven

cases of primary biliary cirrhosis, regardless of the stage of the disease, and occasionally in veins adjacent to metastatic carcinoma. The specific factors that induce or increase HLA-DR antigen content of these hepatic endothelia are unknown. It has been shown, however, that HLA-DR negative human vascular endothelium can be induced to express HLA-DR antigens by activated T lymphocytes and γ -interferon.⁴⁴ Other studies²⁵ indicate that most T lymphocytes in the portal tracts and sinusoids, regardless of subtype, are HLA-DR positive. The presence of these activated T lymphocytes could induce HLA-DR expression in adjacent bile duct epithelium or blood vessel endothelium, but it is difficult to explain why this occurs more commonly in certain types of liver disease such as primary biliary cirrhosis.

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The Design and Analysis of Long-Term Animal Experiments. Statistical Methods in Cancer Research. Vol. III. IARC Scientific Publications No. 79. JJ Gart, D Krewski, PN Lee, RE Tarone, J Wahrendorf. (Pp. 219; £28.) Oxford University Press. 1987. ISBN 92 832 1179 8

Most attempts to understand and control industrial or domestic exposure to chemicals and radiation as the causes of cancer, and equally, much research into the stages of carcinogenesis are based on long term experiments in animals. The design and analysis of such studies is far more complex than is often realised, but if the work is to be worthwhile, extraneous factors must be rigorously controlled and the results must be evaluated by appropriate statistical procedures.

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AD DAYAN

Joint Royal College of Surgeons/Imperial Cancer Research Fund Histopathology Unit

As part of this unit, which has been established for the purpose of advancing the science of diagnostic histopathology, a panel of experienced histopathologists provides advice on diagnostic problems to any pathologist seeking a second opinion. Material can be sent as wet tissue, fixed tissue, paraffin blocks, or sections. If stained sections are submitted additional unstained sections should be sent whenever possible. Where blocks are sent, they will be returned in due course when the sender indicates that this is necessary. Special arrangements can be made, where possible, for the examination of frozen sections or material requiring electron microscopy. There is no charge for these services.

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Corrections

An error occurred in the summary of *J Clin Pathol* 1987;40:879-84. (Barbatis *et al.*) The last paragraph should read: Bile duct epithelium expresses HLA-DR in primary biliary cirrhosis, large duct obstruction and drug induced cholestasis indicating that HLA-DR expression in bile duct epithelium is not exclusive to primary biliary cirrhosis.

(See also correspondence from Professor Wright)

In the paper by Kvale D *et al.* (*J Clin Pathol* 1987;40:621-5), the first line of the summary should read: The serum concentrations of IgA and IgM associated secretory component... and not serum concentrations of IgAp and IgMr....

Notices

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