Heterogeneity of HLA-DR⁺ cells in normal human kidney. Immunohistological and cytochemical characterisation of discrete cell populations

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SUMMARY Biopsies of normal kidneys taken at time of transplantation were studied using a variety of immunofluorescent and cytochemical techniques. A heterogeneous population of HLA-DR⁺ cells was found, mainly confined to the intertubular interstitium. The majority of these cells (80%) were positive when stained with a rabbit anti-factor VIII antiserum suggesting that they were endothelial cells. A minority however (20%) were factor VIII⁻ but were positively stained with FMC17, a monoclonal antibody (McAb) directed against human monocyte/macrophage antigens. Positive staining of this subpopulation was also noted with RFD1, a McAb which reacts with an antigen on human interdigitating cells (ID cells). Cytochemical reactions revealed that these cells contain adenosine triphosphatase (ATPase) and acid phosphatase (ACP) and thus do not conform to the phenotype of tissue histiocytes. The phenotype of this latter population is identical with that of the ID cells found in tonsil, thymus and spleen and it is suggested that they play a major role in initiating the process of renal allograft rejection.

Acute graft rejection is responsible for over 75% of renal allograft failures.^{1,2} The graft rejection process is the result of host immunological mechanisms responding to alloantigens expressed by the cells of the donor kidney. Despite the fact that a majority of kidney grafts are now performed with a close HLA-ABC match, many grafts are still lost due to rejection. A close HLA-DR match between donor and recipient has been shown to improve significantly the one-year graft survival.^{3,4} This observation suggests therefore that the HLA-DR antigens may also play a significant part in initiating and driving the rejection process. In rats relatively large numbers of Ia⁺ (HLA-DR equivalent) cells have been identified in the interstitium of the normal rat kidney.⁵ These cells are stellate in appearance and may form part of a population of Ia⁺ cells widely distributed in all tissues in the rat, except the brain.⁶ In terms of their morphology, surface antigens and cytochemical activity these cells are indistinguishable from the "dendritic" cells identified in the mouse, which have been shown to have the functional capacity of antigen presentation.^{7,8} Batchelor

and his coworkers have tested the hypothesis that these cells play a role in the immunological response to rat renal allografts.⁹ They have shown that rejection of a long surviving immunologically enhanced kidney allograft could be provoked by infusion of an enriched suspension of donor strain dendritic cells into the recipient indicating that Ia⁺ dendritic cells (but not Ia⁺ B cells) have a major role in the activation of the graft rejection process.

Homogenates of normal human kidney have been shown to contain relatively large amounts of HLA-DR antigens.¹⁰ A population of cells in the intertubular interstitium express HLA-DR antigens on their surface membranes.^{11,12} However, HLA-DR antigens are expressed on a wide range of cell types including both lymphoid and non-lymphoid populations.¹²⁻¹⁴ A full understanding of the relevance of HLA-DR⁺ populations in renal allograft rejection demands an analysis of the cell types involved and their microenvironmental relations both in normal kidney and in rejecting allografts. Discrete characterisation of unique cell types within the tissue sections has recently been shown possible by the use of combinations of immunohistological and cytochemical techniques in frozen sec-

tions¹³ and this paper describes such an analysis of normal human kidney.

Material and methods

SPECIMEN PREPARATION

Needle biopsies were taken from normal human kidneys at the time of transplantation using the standard Trucut technique. The biopsies were mounted on cork, embedded in O.C.T. (Ames Corp) and frozen in isopentane cooled in a bath of liquid nitrogen. Six micron sections were cut in a cryostat maintained at -25°C , air dried for 2 h and fixed for 5 min in chloroform:acetone (1:1). They were freeze-dried for 2 h, individually wrapped and maintained at -20°C until required for staining. These techniques have been described in detail.¹³

ANTIBODIES AND CYTOCHEMISTRY

The types of antibodies used, their sources and staining characteristics are described in Table 1. Double immunofluorescent staining was performed using second layer antisera conjugated with fluorescein isothiocyanate (FITC) and tetraethyl rhodamine isothiocyanate (TRITC). Second layer antisera were made in goats (G), sheep (S) or swine (Sw) against the relevant Ig and were either eluted from immunoadsorbant columns or were IgG fractions of hyperimmune antisera. They were then conjugated to the appropriate fluorochrome (for example, FITC or TRITC) and were standardised on tissue sections (for example, tonsil) using first layer reagents of known activity and staining patterns. In the majority of experiments described below double labelling was performed using chicken anti Ia complexed to sheep antichickens FITC and the appropriate mouse monoclonal antibody complexed to goat antimouse Ig TRITC.

Acid phosphatase activity (ACP) and adenosine triphosphatase activity (ATPase) were demonstrated using established techniques.¹¹ In some cases cytochemical staining was performed on tissue sections previously stained by immunofluorescent techniques (IF). After IF staining the slides were washed in phosphate-buffered saline (PBS) for 10 min, then in distilled water for 10 min. They were then fixed in ice-cold calcium formalin for 3–5 min, again washed in distilled water and then incubated in the appropriate cytochemical incubation medium. The sections were examined on a standard Zeiss microscope with an epifluorescence condenser containing selective filters for FITC and TRITC.

EXPERIMENTAL CONTROLS

Frozen sections of tonsil, thymus, lymph node and synovial membrane were used as control tissues to

validate the staining characteristics of the various antibodies used. Sections of kidney were incubated with irrelevant antibody and with 2nd and 3rd layer antisera alone to control for non-specific staining.

Results

LOCALISATION OF HLA-DR⁺ CELLS

Indirect immunofluorescence techniques using either heterologous anti-HLA-DR antisera or monoclonal anti-HLA-DR antibody revealed a consistent pattern of positive cells in all sections studied. The HLA-DR⁺ cell population were found almost exclusively in the intertubular interstitium (Fig. 1). This localisation occurred in sections of both cortex and medulla. Glomerular capillary loops and mesangial cells were also HLA-DR⁺ (Fig. 2d). There was no membrane staining of tubular epithelial cells, although there was a very faint background cytoplasmic fluorescence.

MORPHOLOGY OF HLA-DR⁺ CELLS

The morphology of the HLA-DR⁺ cells appeared extremely heterogeneous, even allowing for the fact that tissue sectioning may cut cells at different angles. Three fundamentally different cell shapes could be identified (Table 2). The majority of cells were either round or crescent-shaped, being elongated and curved in close apposition to kidney tubules and were relatively small in size. A minority were larger and stellate in appearance and some of the latter cells had processes which extended between kidney tubules. No obvious relation was found between anatomical localisation and morphology. Cells of all morphological types were found in all areas of the kidney, although there was a preponderance of cells of an interdigitating morphology in the cortex. Examples can be seen in Figs. 1, 2b and 2d, 4a and 4c, and 5a.

IMMUNOLOGICAL CHARACTERISATION

To characterise the HLA-DR⁺ cells, combinations of immunohistological and cytochemical reactions were performed on the same or serial sections.

Reagents detecting lymphocyte surface markers (eg BA1 and UCHL1) failed to react with the HLA-DR⁺ cells. Conversely, W6/32 a mouse monoclonal antibody directed against HLA-ABC antigens "stained" virtually all the HLA-DR⁺ cells. When both reagents (HLA-DR and HLA-ABC) were tested in combination on the same sections, an almost complete concordance of reaction pattern was seen in all morphological areas of the kidney (Fig. 2a and 2d). Although all HLA-ABC⁺ cells were HLA-DR⁺, occasional individual cells were HLA-DR⁺, HLA-ABC⁻.

Fig. 1 Kidney cortex stained with an anti-HLA-DR McAb and goat anti-mouse conjugated to peroxidase as second layer. A light haematoxylin counterstain has also been applied. The glomerular capillary loops are HLA-DR⁺ as are the linear peritubular capillaries. Interspersed in the interstitium are occasional HLA-DR⁺ cells of more complex morphology (examples arrowed).

Fig. 2 Normal kidney stained with a combination of W6/32 a McAb against human HLA ABC antigens, (Fig 2a and c) and chicken anti HLA-DR (Fig 2b and d). Second layer reagents were goat-anti mouse TRITC and sheep anti chicken FITC and they were photographed using filters selective for TRITC and FITC. Fig. 2a shows an area of medulla with HLA-ABC⁺ cells arranged longitudinally between tubules and Fig. 2b shows the same field labelled with anti HLA-DR. Fig. 2c shows a glomerulus stained with chicken anti HLA-DR. There is almost 100% concordance between HLA-ABC⁺ and HLA-DR⁺ cells.

Fig. 3 Normal kidney double stained with anti HLA-DR McAb and rabbit anti factor VIII RAg. Picture 3a (taken through a fluorescein sensitive filter) shows part of a glomerulus and some proximal tubules. The glomerular capillary loops and the intertubular cells are HLA-DR⁺. Picture 3b (taken through a rhodamine sensitive filter) shows the same field in which there is confluent positive staining of the glomerular endothelium and some, though not all of the intertubular HLA-DR⁺ structures are stained.

Table 1 Antibodies used in this study

Designation	Source	Specificity	Reference
Chicken heterologous antiserum	Chicken heterologous antiserum to purified human p28,33 antigens	B cells Some activated T cells Interdigitating cells Some macrophages	Janossy G <i>et al</i> ¹⁶
OKla	Mouse monoclonal antibody against human p28,33 antigens	As above	Reinherz EL <i>et al</i> ¹⁵
YE2/36	Mouse monoclonal antibody against human p28,44 antigens	As above	Bricknell PM <i>et al</i> ¹⁷
FMC-17	Mouse monoclonal antibody against human macrophage and monocyte antigen	Circulating monocytes Cells of macrophage/monocyte lineage	Seymour GJ <i>et al</i> ¹⁸
Rabbit anti-factor VIII	Rabbit heterologous antiserum against human factor VIII RAg	Endothelial cells	Dako Lab
W6/32	Mouse monoclonal antibody against human HLA-ABC antigens	All leucocytes Most epithelial cells	Barnstable CJ <i>et al</i> ¹⁹
RFD1	Mouse monoclonal against human ID cells	ID cells in lymph node, tonsil and thymus. 20% of B cells, 3-4% of immature myeloblasts	Rawlings Bofill <i>et al</i> (in preparation)
BA1	Mouse monoclonal against human B cells	B cells in peripheral blood lymph nodes and tonsil B cell precursors in bone marrow	Abrahamson CS <i>et al</i> ²⁰
UCHT1	Mouse monoclonal against human T cells (pan T)	T cells in peripheral blood lymph node and thymus	Callard RE <i>et al</i> ²¹

Table 2 HLA-DR⁺ cells in normal kidney

Morphology	HLA-ABC	Factor VIII	RFD1	FMC17	ACP	ATPase
Crescent-shaped, } round, or oval Stellate	+	+	-	-	-	+
	+	-	+	+	-	+

Combined reactions using anti-HLA-DR and anti-factor VIII antisera revealed that the HLA-DR⁺ cells were heterogeneous not only in terms of morphology (see above) but also in terms of surface markers. Anti-factor VIII antiserum demonstrated a network of positive cells interspersed between the tubules corresponding in location to the capillary endothelium (Fig. 3a and 3b). A proportion of HLA-DR⁺ cells (approx 20%) were factor VIII⁻ and these were predominantly the large cells in the intertubular interstitium. The close anatomical

association of all cells in the intertubular interstitium and the fact that the factor VIII staining was cytoplasmic made it difficult to measure the exact proportions of HLA-DR⁺ cells that were either factor VIII⁺ or factor VIII⁻.

Clarification of the picture emerged when HLA-DR⁺ cells were stained with FMC17, a McAb detecting cells of the monocyte/macrophage series. While the majority of DR⁺ cells were FMC17⁻, a proportion (approx 20%) were FMC17⁺ (Fig. 4b and d). Combined staining using anti-HLA-DR

Fig. 4 Sections of normal kidney stained with anti-HLA-DR antiserum, FMC-17 McAb and Mg⁺⁺ dependent ATPase. Picture 4a (taken through a fluorescein sensitive filter) shows a selection of HLA-DR⁺ cells. Picture 4b (taken through a rhodamine sensitive filter) shows the same field in which two of the HLA-DR⁺ cells (arrowed) are unequivocally FMC-17⁺. Picture 4c shows several HLA-DR⁺ cells and a large blood vessel (labelled*). Picture 4d shows the same field which has been subsequently labelled with ATPase. The vessel wall is seen to be strongly ATPase⁺ and practically all of the HLA-DR⁺ cells are also ATPase⁺.

Fig. 5 Normal kidney stained with anti HLA-DR antiserum and RFD1. Picture 5a taken through an FITC sensitive filter shows numerous DR⁺ cells some of which (labelled*) are clearly endothelial. Picture 5b (taken through a TRITC sensitive filter) shows the same field labelled with RFD1 in which the endothelial cells (*) are clearly negative and in which seven cells with an interdigitating morphology (arrowed) are clearly RFD1⁺.

antiserum and RFD1 (a McAb detecting human ID cells) confirmed that approximately 20% of the DR⁺ cells were RFD1⁺ and these were clearly of a stellate or interdigitating morphology (Fig. 5a and 5b). It was also clear that endothelial cells were RFD1⁻.

COMBINED CYTOCHEMICAL REACTIONS

Sets of sections from different biopsies of normal kidney were "stained" with HLA-DR antiserum and then placed in incubation medium to demonstrate enzyme activity. Using this technique it was

found that the HLA-DR⁺ cells in the interstitium failed to express any ACP activity irrespective of their localisation, morphology or other immunohistological characteristics. Occasional isolated kidney tubules were ACP⁺. In contrast, combined reactions to detect ATPase activity demonstrated that all the HLA-DR⁺ cells expressed discrete membrane activity for this enzyme (Fig. 4c-d).

Discussion

The results described here reveal that normal kidney

contains a significant population of cells expressing HLA-DR antigens and furthermore that this population is heterogeneous. The presence of HLA-DR antigens in homogenates of kidney has been previously reported although this study failed to localise the HLA-DR expressing cells.¹⁰ Koyama *et al* demonstrated HLA-DR⁺ cells in the intertubular interstitium and in the glomeruli which were thought to be endothelial cells,¹¹ while a further study demonstrated HLA-DR⁺, HLA-ABC⁺ cells in the interstitium, a proportion of which were thought to be dendritic cells analogous to the dendritic cells described by Steinman and his co-workers in the mouse.^{7,12} However, this subpopulation was not analysed for additional immunohistological and cytochemical markers which are characteristic of dendritic cells in other locations.

The morphology and phenotype of the HLA-DR⁺ cells which are factor VIII⁺, RFDI⁺, FMC17⁺, ATPase⁺ described above strongly supports the suggestion of Koyama *et al* that the majority of HLA-DR⁺ cells are indeed endothelial. However, approximately 20% of the HLA-DR⁺ population are factor VIII⁻ but FMC17⁺, RFDI⁺. These immunocytochemical characteristics, together with the stellate morphological appearance of these cells, offers strong evidence that they are indeed dendritic or interdigitating cells. Their cytochemical reactions (that is, ATPase⁺, ACP⁻) are at variance with those of HLA-DR⁺ activated macrophages and provides further evidence that this is a unique cell population. This cell type is found in the paracortical area of the lymph node and the corticomedullary junction of the thymus.^{16,20,22} These cells also accumulate in the synovia of patients suffering from active rheumatoid arthritis and in the skin during delayed type hypersensitivity reactions.^{23,24} It has been suggested that such cells are analogous to the dendritic cells of mice that are known to possess antigen presenting capacity.^{8,22,25}

While there is good evidence for the implication of dendritic cells of donor origin in allograft rejection in rodents, this has not been established in the human.⁹ There is indirect evidence from retrospective reviews of allograft survival, that DR antigens have a crucial role in the ultimate acceptance or rejection of the allograft.^{3,4,26} However, this begs the question as to which population of DR⁺ cells are implicated and whether all of the DR⁺ cells have similar capacities for antigen presentation. Although the HLA-DR antigens on the capillary endothelium may act as target for host derived rejection processes, it is tempting to speculate that the possible antigen presenting capacity of the above described HLA-DR⁺, FMC17⁺, RFDI⁺ ATPase interdigitating cells of the donor kidney may trigger the early

episodes of acute graft rejection. Weight is added to this hypothesis by the focal nature of the interstitial cellular infiltrates that accompany the acute rejection process.

Confirmation of the involvement of HLA-DR⁺ ID cells in human renal allograft rejection is still awaited but it would seem that the above description of their phenotype and localisation, should provide a firm basis for further elucidation of their role in the in situ rejection process.

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