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Varying expression of major histocompatibility complex antigens on human renal endothelium and epithelium

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Summary. Pre-anastomosis wedge biopsies from 14 cadaveric donor kidneys were examined for the expression of class I (HLA-ABC) and class II (HLA-DR) antigens in renal tissue. Two monoclonal antibodies to class I antigens and four to class II antigens were used in an indirect immunoperoxidase technique. Consistent expression of both antigens was demonstrated on the surface of glomerular, peritubular capillary and venous endothelial cells. Renal arteries contained only class I antigens. Proximal tubules contained varying amounts of each antigen in their cytoplasm. Sixteen human lymphocytotoxic allo-antisera showed marked variation in their ability to detect HLA antigens on the kidney. The selection of donors for recipients of renal allografts involves the complement-dependent cytotoxicity test and the failure of some lymphocytotoxic antisera to bind to the kidney indicates that some suitable patients may be incorrectly excluded. The use of a binding assay using an immunoperoxidase technique should be included in cross-match techniques particularly for patients who have high levels of circulating cytotoxic antibodies.

Keywords: HLA antigens, renal transplantation, renal endothelium

Previous studies of the antigenic structure of the kidney have shown that class I antigens (HLA-ABC) have a wide distribution whereas class II antigens (HLA-DR) have restricted expression on renal vasculature (Baldwin *et al.* 1981; Hart *et al.* 1981; Natali *et al.* 1981; Hancock *et al.* 1982). Marked variation of class II antigens on proximal tubular epithelium has also been reported (Fuggle *et al.* 1983). The potential significance of class I and II antigens in the kidney depends on their site density and on variation in their arrangement on the endothelial cell surface. We have previously reported variation in endothelial expression of blood group A antigen and the inability of some preformed allo-antibodies to bind to the kidney (Trickett *et al.* 1983).

In view of the large number of highly presensitized patients requiring transplantation who already possess circulating antibodies to lymphocytes detected by complement-dependent cytotoxicity (CDC), it is important to distinguish those antibodies which bind to the kidney and initiate vascular damage, from those that have no deleterious effect. Auto-antibodies and most allo-antibodies that are cytotoxic to B lympho-

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cytes giving a positive B cell cross-match are probably irrelevant (Morris & Ting 1981; Ting & Morris 1983), whereas allo-antibodies cytotoxic to T lymphocytes are significant (Kissmeyer-Nielsen *et al.* 1966; Patel & Terasaki 1969).

In this report we have mapped the antigenic distribution of class I and II antigens on the endothelial cells of 14 kidneys using a range of mouse monoclonal antibodies. In addition, in order to assess the significance of tissue expression of these antigens we have investigated and compared the endothelial binding of human lymphocytotoxic allo-antisera.

Materials and methods

Kidneys. Pre-anastomosis wedge biopsies were taken from 14 cadaveric donor kidneys after nephrectomy and perfusion with hyperosmolar citrate (seven kidneys), Marshall's solution (four kidneys) or Eurocollins solution (three kidneys). The average cold ischaemia time before biopsy was 22.5 h.

Biopsies were collected and stored in liquid nitrogen until use. All kidneys exhibited normal renal morphology.

Monoclonal antisera. Two monoclonal antibodies to class I antigens were used. W6/32 (Barnstable *et al.* 1978) and 34/28 (Trucco *et al.* 1979) were obtained as supernatants from Sera Labs (MAS 1532 and MAS 017 respectively). Both these antibodies recognize determinants common to all class I (HLA-ABC) antigens.

Four monoclonal antibodies to class II antigens were also studied. FMC 4 (Beckman *et al.* 1980) was obtained as a supernatant from Sera Labs (MAS 061); CR3/43 (Sunderland *et al.* 1981) was obtained as ascites fluid and was a gift from Dr D.Y. Mason, John Radcliffe Hospital, Oxford. DA2 (Brodsky *et al.* 1980) and TAL-1B5 (Adams *et al.* 1983) were obtained as ascites fluid and supernatant respectively and were the gift of Dr W. Bodmer, Imperial Cancer Research Fund, London. FMC 4, CR3/43 and DA2 are monoclonal antibodies recognizing monomorphic determinants on class II (HLA-DR) antigens. TAL-1B5 recognizes an epitope common to human class II α chains (Adams *et al.* 1983). All the monoclonals used were of mouse immunoglobulin subclass IgG_1, with the exception of W6/32 which was IgG_{2a}.

Monoclonal antibodies were tested at a range of dilutions on kidney sections and subsequently used at the concentration which produced clear distinct positive staining.

Affinity-purified rabbit anti-mouse immunoglobulin was obtained from Sera Labs, rabbit anti-human immunoglobulin IgG from Behring and horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin from Miles–Yeda.

Human allo-antisera. Six maternal allo-antisera defining the class I antigens HLA-A2, A3, B8, and B12 together with 10 maternal sera defining class II antigens HLA-DR1, DR3, DR4 and DR7 were obtained locally, and were characterized on panels of lymphocytes from normal individuals by complement-dependent cytotoxicity (CDC).

Immunoperoxidase technique

Monoclonal antibodies. Renal tissue was examined by an indirect immunoperoxidase technique (Trickett *et al.* 1983).

Cryostat sections (5 μm) were air dried, fixed in acetone for 10 min and washed in phosphate-buffered saline (PBS) pH 7.2 for 30 min with three changes of buffer. The sections were then incubated with the appropriate monoclonal antibody for 60 min, followed by incubation with rabbit anti-mouse immunoglobulin, diluted 1:150 in PBS containing 0.75% human AB serum, for 30 min. Subsequently all sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin, diluted 1:800 in PBS for 30 min. The sections were washed between each incubation step with PBS pH 7.2 for 30 min, with three

changes of buffer. Incubations were carried out at both 20°C and 37°C in a humidified atmosphere. Slides were then incubated with diaminobenzidine tetrahydrochloride (Sigma) 0.5 mg/ml in 0.2 M Tris-HCL buffer pH 7.6 containing 0.01% hydrogen peroxide (Graham & Karnovsky 1966) for 10 min. The peroxidase reaction was stopped by washing in PBS, followed by tap water for 5 min and the slides were then counterstained with Mayer's haemalum, dehydrated in ethanol, cleared in xylene and mounted in DPX. Sections were examined independently by two observers and the intensity of staining was graded.

Specificity controls included absorption of specific antibodies with two human B lymphoblastoid cell lines, Bristol 8 which expresses HLA-ABC and HLA-DR antigens, Daudi which expresses HLA-DR but lacks HLA-ABC (Brodsky *et al.* 1979), and cells expressing only HLA-ABC from a T cell chronic lymphocytic leukaemia patient. Absorption of monoclonal antibodies was performed by incubation with an equal volume of the appropriate washed packed cells for 60 min at 4°C, 20°C and 37°C. A further control included incubation of sections with second and third antibodies alone.

Human allo-antisera. The indirect immunoperoxidase technique was performed as described above by substituting human lymphocytotoxic sera for the monoclonal antisera together with rabbit anti-human IgG, diluted 1:4000 in PBS, as the second antiserum. Positive controls included a kidney with defined Ig deposits, whilst negative controls included pooled human AB sera, previously screened for the absence of lymphocytotoxic antibodies and blocking activity in mixed lymphocyte culture. ABO incompatibility restricted the use of allo-antisera for some kidneys.

Results

Class I antigens

The monoclonal antibody W6/32 consistently showed intense staining for class I antigen on the glomerular and peritubular capillary endothelium of all 14 kidneys. Biopsies of seven kidneys contained veins whose endothelium also showed uniform

Table 1. Distribution of HLA antigens in 14 kidneys using monoclonal antibodies

	Class I (HLA ABC)	Class II (HLA-DR)
Monoclonal antibody	W6/32	FMC4 CR3/43 TAL-1B5
Arterial endothelium	++	−*
Glomerular capillary endothelium	+++	+++
Peritubular capillary endothelium	+++	+++
Venous endothelium	++	++
Proximal tubule epithelium‡	−→+	−→+++
Distal tubule epithelium‡	−→+	−→++†

Staining intensity: +++ strong, ++ moderate, + weak, − negative.

* Weak arterial staining seen in one kidney with FMC4, see text.

† Distal tubule epithelium showed variable staining with CR3/43 only.

‡ Variable staining.

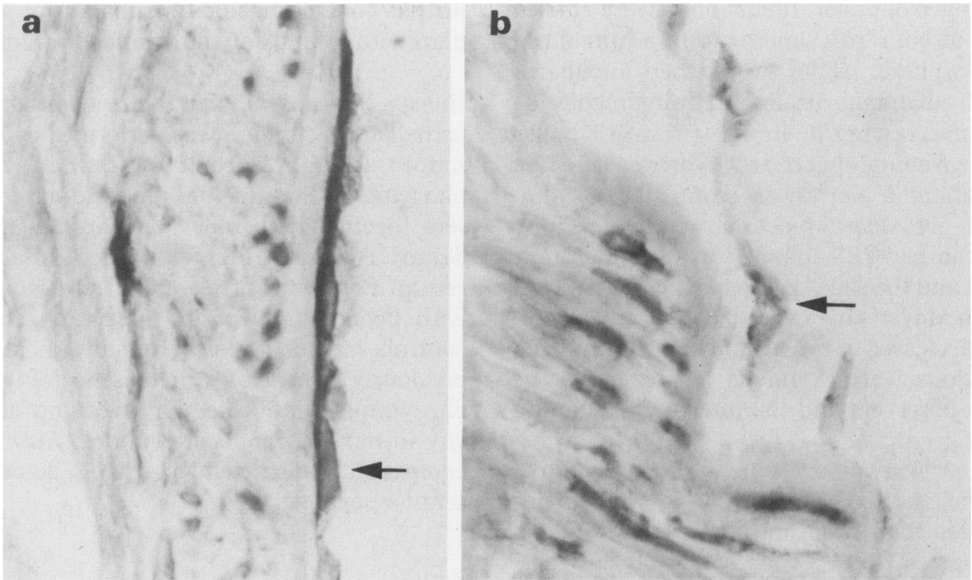

Fig. 1. Arterial endothelium (arrow) containing class 1, antigens (*a*). In comparison, there are no class II, antigens (*b*). Indirect immunoperoxidase, DAB-H_2O_2, with haematoxylin nuclear counterstain. × 1100.

distribution of antigen (Table 1). Ten kidney biopsies contained interlobular arteries which showed similar staining (Fig. 1) but a further kidney had arterial endothelium without demonstrable antigen. The other monoclonal antibody 34/28, showed less intense staining throughout and arteries were often negative.

The cytoplasm of proximal tubular epithelial cells was negative in 11 kidneys and positive in three kidneys with the W6/32 antibody; 34/28 antibody however was always negative. Distal tubule epithelium showed only trace amounts in two kidneys with W6/32, whilst no antigen was detectable in any distal tubules with 34/28 antibody.

Specificity for class I antigens was confirmed by abolition of staining following absorption of W6/32 with Bristol 8 cells whilst absorption with Daudi failed to remove antibody activity.

Each human allo-antiserum to class I antigens showed greater variation in staining pattern and intensity than the monoclonal antibodies. Individual kidneys showed inconsistent staining patterns, with the panel of allo-antisera (Fig. 2 and Table 2). In three kidneys (Patients 7, 11, 13) the presence of HLA-A2 could not be demonstrated by one allo-antiserum although it was detected by the other. Antisera to HLA-A3, B8 and B12 did not detect antigen on any of the kidneys expected to express them. In addition antisera to HLA-A3 and B8 demonstrated unexpected 'additional' activity which could not be explained from previous characterization by lymphocytotoxicity.

Class II antigens

Class II antigens were detected on glomerular, peritubular capillary and venous endothelium whilst the arterial endothelium was negative (Table 1 and Figs 1 and 3)

FMC 4, CR3/43 and TAL-1B5 monoclonal antibodies showed only minor differences in the detection of these antigens.

The glomerular and peritubular capillary endothelium showed strong and consistent expression of class II antigens in 12/14 kidneys with FMC4, and in 13/14 kidneys

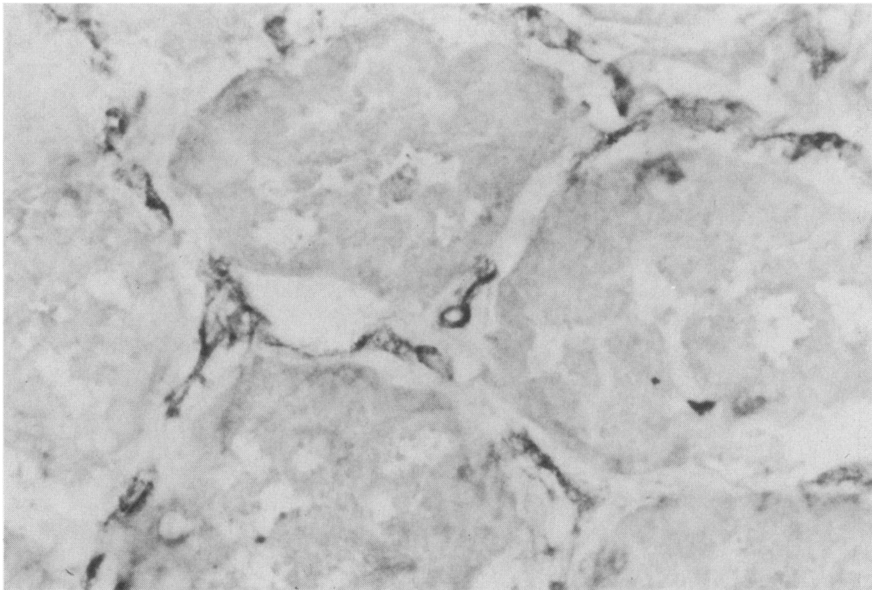

Fig. 2. Peritubular capillary endothelium containing antigens demonstrated by human HLA-A2 allo-antiserum and indirect immunoperoxidase with haematoxylin counterstain. × 750.

Table 2. Reactivity of six class I (HLA-ABC) allo-antisera in 13 kidneys

	Donor tissue type				Antisera to HLA-A, B antigens					
					A2		A3	B8		B12
Donor kidney	HLA-A		HLA-B		1	2	3	4	5	6
1	2	10	12	40	+	++	−	−	−	⊟
2	1	2	7	8	+++	++	−	⊟	⊟	NT
3	2	3	7	w44	++	++	NT	(+)	(+)	NT
4	1	2	7	w44	+	+	NT	−	(+)	NT
5	2	w24	7	w35	+	+++	−	−	−	−
6	2	—	7	14	+	++	−	−	−	−
7	2	26	5	40	+	⊟	−	−	−	NT
8	1	—	8	27	−	−	(+)	⊟	⊟	−
9	2	3	18	w50	++	+	⊟	−	−	−
10	2	—	7	17	++	++	NT	−	(+)	NT
11	2	28	5	w35	⊟	++	NT	−	(+)	NT
12	2	w31	5	w39	++	++	−	−	−	NT
13	2	—	17	w44	⊟	+	−	−	−	NT

Staining intensity: +++ strong, ++ moderate, + weak, − negative.

NT Not tested.

□ Donor lymphocytes typed + for antigen but renal endothelium and epithelium −.

( ) Donor lymphocytes − for antigen but renal endothelium +.

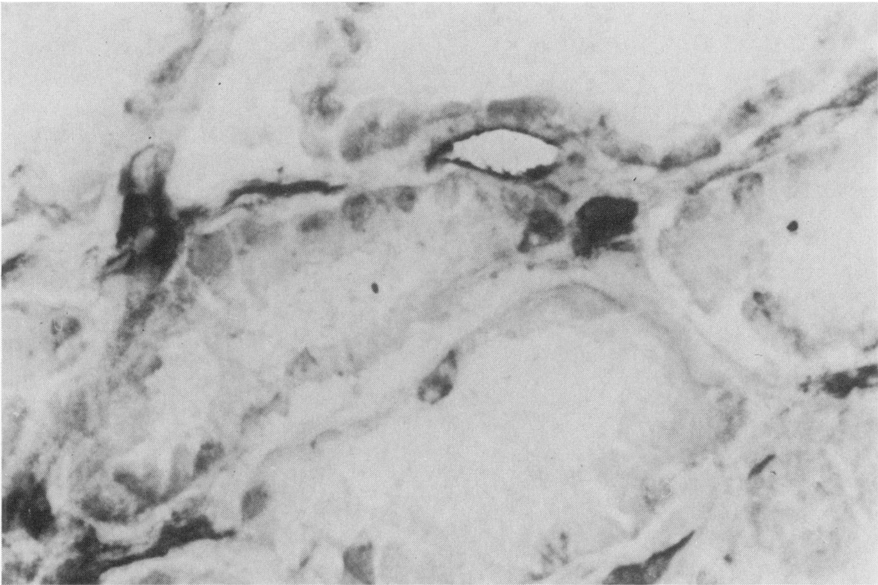

Fig. 3. Peritubular capillary endothelium containing class II antigens. Monoclonal antibody (CR3/43), indirect immunoperoxidase with haematoxylin counterstain. × 750.

with TAL-1B5. Less intense staining was seen in two kidneys using FMC 4 and in one kidney with TAL-1B5. Veins in seven kidneys showed moderate endothelial staining with little variation. The glomerular arterioles showed variable staining in 2/14 kidneys for FMC 4 and 1/14 kidneys with TAL-1B5. Interlobular arteries were present in 11 kidneys but showed no reaction with CR3/43 and TAL-1B5 (Fig. 1). FMC 4 was negative in 10 kidneys but showed weak endothelial staining in one renal artery.

The monoclonal antibody DA2 gave either weak staining or none by the indirect immunoperoxidase technique used in this study. Tubular epithelium showed a marked variation in the expression of class II antigens. Proximal tubular epithelium showed diffuse staining with CR3/43 in nine kidneys, but none in five kidneys. FMC 4 and TAL-1B5 were positive in five kidneys only. Distal tubular epithelium was positive in four kidneys with CR3/43, but negative in all 14 kidneys with FMC 4 and TAL-IB5.

Absorption of FMC 4, CR3/43 and TAL-1B5 with Bristol 8 cell line abolished all staining whilst absorption with T-CLL cells failed to remove antibody activity, confirming specific class II activity.

Human allo-antisera to class II antigens gave the same pattern of antigen distribution as the monoclonal antisera but reactions were less intense (Table 3). However the HLA-DR1 positive kidney (Patient 8) gave a negative result with two DR1 allo-antisera. Five allo-antisera showed additional reactions incompatible with results obtained from lymphocytotoxicity (Table 3).

Discussion

Monoclonal antibodies demonstrated strong expression of class I antigens on renal veins, glomerular and peritubular capillary endothelium and on the majority of arteries. The consistent expression of these antigens reflects the known deleterious effect of preformed, cytotoxic allo-antibodies which give a positive T-cell cross-match (Kissmeyer-Nielsen *et al.* 1966; Patel & Terasaki 1969). Our findings are in agreement with previous reports of the widespread distribution of class

Table 3 Reactivity of 10 class II (HLA-DR) allo-antisera in eight kidneys

			Antisera to HLA-DR antigens									
			DR1			DR3	DR4			DR7		
Donor kidney	Donor HLA-DR type		1*	2	3	4	5	6	7	8	9	10
1	4	w6	++	(++)	(+)	−	+	+	+	−	−	−
2	4	3	−	−	−	+	+++	+	++	−	−	−
3	2	w6	++	(++)	(++)	−	−	−	−	−	−	−
4	2	7	(+)	−	−	−	−	−	−	++	+	+
5	—	w6	−	NT	−	(+)	(+)	−	−	−	NT	NT
6	5	w6	+	−	−	(++)	−	−	−	−	−	−
7	4	—	+	NT	−	−	+++	+++	+	−	NT	NT
8	1	7	⊟	NT	⊟	−	(+++)	−	−	++	NT	NT

Staining intensity: + + + strong, + + moderate, + weak, − negative.
NT, Not tested.
□ Donor lymphocytes typed + for antigen but renal endothelium and epithelium −.
( ) Donor lymphocytes − for antigen, renal endothelium +
* Serum had additional reactivity to some DR4, 5 and w6 positive cells by lymphocytotoxicity.

I antigens in the kidney (Baldwin *et al.* 1981; Hart *et al.* 1981; Hancock *et al.* 1982), and their detection on tubular epithelial cells also confirms previous work (Hancock *et al.* 1982; Fuggle *et al.* 1983). As class I antigens are seen within epithelial cytoplasm and not solely present on the cell surface they are likely to be the result of passive re-absorption of HLA antigens from the glomerular ultrafiltrate and derived from the serum (Pellegrino *et al.* 1974).

Class II antigens were more restricted in their distribution on renal tissue being confined to the glomerular, peritubular capillary and venous endothelium. Several juxtaglomerular arterioles expressed class II antigens suggesting that they were efferent (postglomerular) in origin. Interlobular arteries were negative with the exception of one kidney. These observations extend previous findings (Baldwin *et al.* 1981; Natali *et al.* 1981; Hancock *et al.* 1982; Fuggle *et al.* 1983). The lack of class II antigens on renal arteries may influence the nature of the rejection process.

It is surprising that the majority of renal transplants performed in the presence of a positive B cell cross-match are successful (Morris & Ting 1981) as there is very limited variation in the expression of class II antigens. However many B cell positive cross-matches are not the result of antibodies to class II antigens (d'Apice & Tait 1980) and some are due to non-HLA autologous antibodies (Ting 1983; Ting & Morris 1983). Infrequently hyperacute and accelerated rejection can be associated with antibodies directed against class II antigens (Ahern *et al.* 1982; Mohanakumar *et al.* 1981) which confirms that their presence on the renal vasculature may be responsible. Rapid allograft rejection due to those antibodies will depend not only on class II (HLA-DR) antigen expression but also on their ability to bind to the kidney.

The detection of class II antigens within tubular cytoplasm varies as in a previous report (Fuggle *et al.* 1983). There was no correlation between expression of epithelial class II antigens and HLA type, perfusion time or perfusate. The variability of proximal tubular class II antigens may result from re-absorption of HLA-DR antigens from the glomerular ultrafiltrate as for HLA-ABC antigens (Wilson *et al.* 1979).

Considerable variation in detection of class I and II antigens was seen with human lymphocytotoxic allo-antisera in contrast to results obtained with the monoclonal antibodies. The pattern of reactivity was similar but detection on individual structures varied considerably. Endothelium was often 'patchy' in staining pattern, particularly with class I allo-antisera. Several lymphocytotoxic allo-antisera (Tables 2 and 3) did not bind to any renal, endothelial or epithelial structures, despite the fact that they were well characterized and gave reproducible results by complement-dependent cytotoxicity on testing lymphocytes. There are several possible explanations for these findings. The detection of antigenic determinants is dependent on concentration, subclass and avidity of the antibody. Antigen may be present at levels below the threshold sensitivity of the test system whilst the antigenic determinants may in some instances be subject to conformational changes in their topographical expression on the cell surface. This 'antibody-dependent' expression may account for the previous unsuccessful attempts to reproducibly demonstrate individual class I antigens (Baldwin *et al.* 1981).

Currently, testing of the recipients serum with the potential donor's lymphocytes is very important in selecting donors and recipients for renal transplantation (Ting 1983). However these cross-match tests use non-renal targets and may in some instances lead to the exclusion of suitable recipients. The nature and specificity of antibodies producing a positive T and/or B cell cross-match needs further evaluation as complement-dependent cytotoxic allo-antibodies vary in their ability to bind to renal endothelium. The finding that some class I lymphocytotoxic antibodies fail to bind has important clinical implications. A kidney-binding assay such as the immunoperoxidase technique described, should prove useful in the successful selection of kidney donors for highly presensitized patients if the recipient's circulating cytotoxic antibodies are ineffective in binding to the donor kidney. Studies are currently in progress to determine the suitability of such a procedure.

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