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VARIABLE LOCALIZATION OF BLOOD GROUP ANTIGEN IN GROUP A KIDNEYS

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Summary.—The distribution of the blood group A antigen has been examined in 7 Group A kidneys using an indirect immunoperoxidase technique. Monoclonal antibody consistently demonstrated A antigen on the endothelium of all kidneys, particularly peritubular capillary endothelial cells and also the epithelial cells of distal tubules of all but 1 case. Nine hyperimmune anti-A alloantisera gave a variable pattern of staining on different endothelial cells, but no kidney was negative. Epithelial cell staining showed considerable variation both within an individual cell and between adjacent cells. Twenty-six out of 40 alloantisera from normal Group O blood donors failed to bind to endothelial cells of one kidney which was known to show strong expression of A antigen. Absorption was completely achieved using Group A red blood cells but not with a synthetic blood group substance. The variation in reaction intensity using different antisera emphasises that there is variation in antigen expression between cells and indicates the complexity of antibodies directed against the blood group A antigen.

ABO compatibility is a major pre-requisite for renal allograft transplantation as blood group antigens constitute important targets for rejection. Nevertheless, breaching of the ABO barrier has resulted in long term graft survival of a small number of reported cases (Murnaghan *et al.*, 1967; Wilbrandt *et al.*, 1969; Slapak, Naik and Lee, 1981; Brynger *et al.*, 1982). These exceptions suggest that either naturally occurring antibodies in these recipients are ineffective in binding to the graft and initiating vascular damage, or that there is variability in the surface expression of ABH antigens by the kidney endothelial cells.

Some variation in expression of blood group A antigen has been shown in previous reports, for example, both alloantisera and xenoantisera have demonstrated the presence of the A antigen on renal endothelium (Szulman, 1960; Holborow *et al.*, 1960; Bariety *et al.*, 1980) whilst staining of the epithelial cells of the distal tubules has varied (Holborow *et al.*, 1960; Bariety *et al.*, 1980). This apparent variation in expression of antigen may result from the recognition of distinct antigenic determinants of the A antigen by different antisera.

In this study we have re-investigated the distribution of the blood group A antigen in renal tissues by an indirect immunoperoxidase technique, using 1 mouse monoclonal antibody, 9 human immune and 41 human non-immune antisera. Antigen could be detected in all blood group A kidneys in cryostat sections and more detailed localization was possible in paraffin-embedded renal tissue.

MATERIALS AND METHODS

(1) *Kidneys.*—Renal tissue was obtained from 9 cadaveric donors; 7 were Group A (2 known A_1 and 1 known A_2) and 2 were Group O. Pre-transplant biopsies were collected and stored in liquid

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nitrogen until use. One whole kidney was also available for comparison of the distribution of the A antigen in frozen and formalin-fixed tissue. All kidneys exhibited normal renal morphology.

(2) *Antibodies.*—The monoclonal used was an IgM anti-A antibody (MH2/6D4) previously characterized (Voak *et al.*, 1980). Hyperimmune human anti-A serum was obtained from 9 patients investigated for suspected ABO haemolytic disease of the newborn. The IgG anti-A titres were determined by the indirect anti-human globulin test against Group A cells using standard serological techniques (Boorman, Dodd and Lincoln, 1977) and ranged from 1/200–1/1600. The sera showed no reaction with Group O erythrocytes, T and B lymphocytes and chronic lymphocytic leukaemia cells thus excluding the presence of antibodies to other blood group antigens and lymphocytotoxins. A selected Blood Group Reference Laboratory routine typing serum (BGRL 8082) whose IgG anti-A titre was 1/16 was also investigated. Forty non-immune blood group O alloantisera, were obtained from routine blood donors.

(3) *Immunoperoxidase technique.*—Renal tissue was examined by a modification of the indirect immunoperoxidase technique (Paul, van Es and Fleuren, 1979).

Cryostat sections (5 μm) were fixed in acetone, washed in phosphate buffered saline (PBS) and incubated with monoclonal anti-A antibody or undiluted human anti-A alloantisera for 60 min, followed by incubation with the appropriate second antiserum for 30 min. The second antiserum for the monoclonal anti-A antibody was an affinity purified rabbit anti-mouse serum (Sera Labs) whilst rabbit anti-human IgG, IgA or IgM (Behring) was used for the alloantisera. Subsequently, all sections were incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin (Miles-Yeda) for 30 min, followed by incubation for 10 min with a mixture of diaminobenzidine tetrahydrochloride and hydrogen peroxide (Graham and Karnovsky, 1966), and counterstained with Mayer's haemalum. The sections were washed between each incubation step with PBS (pH 7·3) for 30 min, with 3 changes of buffer. All incubations were carried out at 37°. Formalin fixed paraffin embedded renal tissue was examined using the same indirect immunoperoxidase technique after dewaxing in xylene, rehydration in alcohol and inhibition of endogenous peroxidase using 0·5% hydrogen peroxide in methanol for 10 min. Sections were examined independently by 2 observers and the intensity of staining was graded.

Control sections were incubated with each second antiserum and with peroxidase-conjugated goat anti-rabbit immunoglobulin to exclude the presence of pre-existing immunoglobulin (Ig) in the kidney. Pooled human AB sera, previously screened for the absence of lymphocytotoxic antibodies and blocking activity in the mixed lymphocyte culture were used as controls whilst a kidney with defined Ig deposits was included as a positive control for all cases.

(4) *Specificity controls.*—The specificity of the monoclonal anti-A and the human alloantisera was confirmed using the 2 Group O kidneys. Absorption of sera with an equal volume of washed packed Group A or B erythrocytes for 60 min at 4°, 20° and 37° confirmed the specificity of all antisera. Four alloantisera were similarly absorbed with Synsorb A or B (Chembiomed, University of Alberta) at 200 mg/ml as above.

RESULTS

(1) *Monoclonal anti-A antibody (MH2/6D4)*

Blood group A antigen was consistently present in the endothelial cells of the peritubular capillaries (PTC) in frozen sections of all 5 Group A kidneys (Table I). Biopsies of 4 kidneys contained interlobular arteries which also showed positive endothelial staining. Distribution of antigen on PTC endothelium was uniform between kidneys, whereas arterial and glomerular capillary endothelial cells had variable intensity. Mesangial cells and epithelial cells in glomeruli and proximal tubules were unstained. Four kidneys showed staining of the cytoplasm of the distal tubular epithelial cells which often varied both in intensity and cytoplasmic distribution. Collecting ducts could not be readily distinguished from distal tubules in cryostat sections, but showed definite localization in 1 kidney.

Comparison of formalin fixed, paraffin-embedded sections and frozen sections from 1 kidney showed that the distribution in endothelial cells was similar. Distal tubular epithelial cells and collecting ducts showed a similar distribution of the A antigen in the cell cytoplasm of frozen and fixed tissue with a similar variation between adjacent cells. Improved localization in fixed tissue showed that antigen was present on the surface of both endothelial and epithelial cells.

Absorption of the monoclonal antibody with human Group A erythrocytes abol-

TABLE I.—*Immunoperoxidase staining of kidneys with monoclonal anti-A (MH2/6D4)*

Kidney	Sections	Blood group	Arterial endothelium	Glomerular capillary endothelium	Peritubular capillary endothelium	Proximal tubule epithelium	Distal† tubule epithelium
R.G.	Frozen	A_1	+ +	+ +	+ + +	−	− → + + +
	Paraffin	A_1	+ + +	+ + +	+ + +	−	− → + + +
A.M.	Frozen	A_1	+	+ +	+ + +	−	− → + +
K.M.	Frozen	A	+ + +	+ + +	+ + +	−	− → + +
H.D.	Frozen	A	+	+	+ + +	−	−
R.L.	Frozen	A	*	+ + +	+ + +	−	− → + +
J.W.	Frozen	O	−	−	−	−	−
J.B.	Frozen	O	−	−	−	−	−

Staining intensity: + + + strong, + + moderate, + weak, − negative.
* No large vessel present in biopsy.
† Variable staining.

ished all staining whilst absorption with human Group B erythrocytes failed to reduce staining intensity. Monoclonal anti-A antibody showed no staining of the 2 Group O kidneys and no staining was observed in other negative control sections.

(2) *Human alloantisera*

Blood group A antigen was clearly demonstrated in glomerular and arterial endothelial cells by all human alloantisera (Fig. 1, a, b).

Three different patterns of staining could be defined by the reaction with peritubular capillary endothelial cells of 7 Group A kidneys (Table II). Six immune alloantisera gave consistently strong staining of the peritubular capillary endothelium whilst 3 immune alloantisera gave reactions of varying intensity. The non-immune anti-A typing serum (BGRL 8082) gave weak reactions or none at all.

All human alloantisera stained the epithelial cells of the distal tubules and collecting ducts of the Group A kidneys but the intensity of the reaction varied considerably between adjacent epithelial cells (Fig. 2). Some distal tubules remained negative.

Examination of frozen sections of one kidney (RG) showed similar patterns of staining for both IgG and IgM anti-A antibodies, although the latter were weaker in intensity. No IgA anti-A antibodies were demonstrated.

Absorption of the alloantisera with human Group A erythrocytes removed all observed staining in frozen sections; absorption with human Group B erythrocytes failed to reduce the staining. Absorption of 4 alloantisera with Synsorb A reduced but did not remove the observed staining in frozen sections, absorption with Synsorb B failed to reduce the staining. Two Group O kidneys and other control sections were all negative.

Alloantisera from 40 normal group O blood donors were investigated for their ability to bind a group A_1 kidney (RG), previously demonstrated to show strong expression of the A antigen. Twenty-six of these alloantisera failed to bind to any structures. The remaining 14 sera bound to the endothelium of the PTC and the epithelial cells of distal tubules.

DISCUSSION

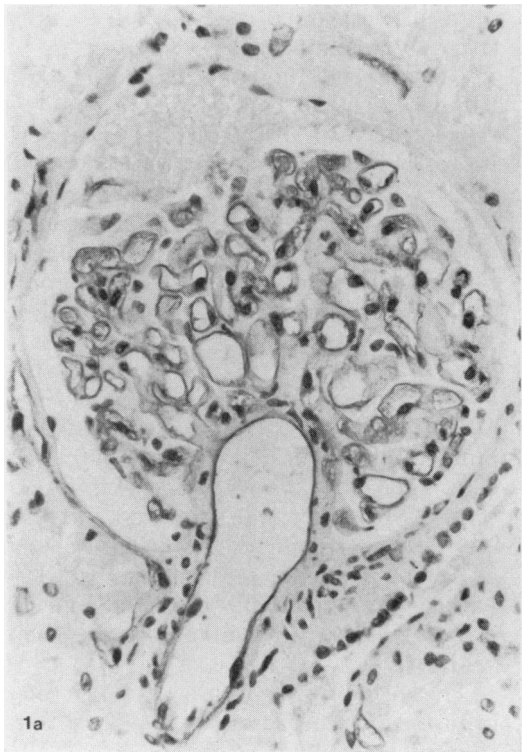
Vascular endothelial cells provide the initial contact between a renal allograft and the immune system of the recipient. Irreversible vascular rejection is seen in the majority of ABO-incompatible renal grafts (Wilbrandt *et al.*, 1969), which is presumed to be due to early endothelial damage (Porter, 1974). There are however 4 reports of 6 cases of long term graft survival (more than 12 months) in blood group O recipients of Group A_1 and A_2 grafts (Murnaghan *et al.*, 1967; Wilbrandt *et al.*, 1969; Slapak *et al.*, 1981; Brynger *et al.*, 1982).

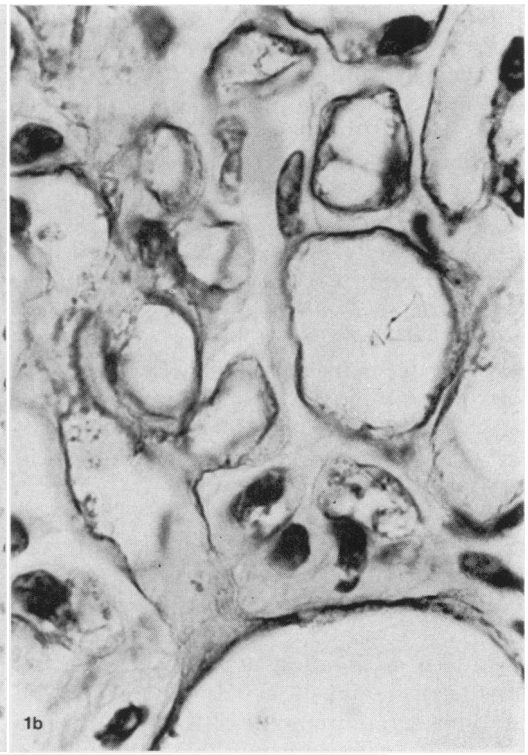
Survival of grafts breaching the ABO barrier may arise from variable expression of A antigen on graft endothelium. Our

TABLE II.—*Variation in staining of peritubular capillary endothelium in frozen sections of 9 kidneys using human alloantisera*

Pattern	Serum		IgG anti-A titres	Kidney (blood group subtype) R.G. (A_1)	A.M. (A_1)	K.M. (A)	H.D. (A)	R.L. (A)	P.O. (A)	L.P. (A_2)	J.W. (O)	J.B. (O)
1	Immune	(6)	1/200–1/1600	+ + +	+ + +	+ +	+ + +	+ +	+ +	+ + +	–	–
2	Immune	(3)	1/100–1/800	+ +	+	+ +	+ + +	–	+	N.D.	–	–
3	Blood group reference typing serum	(1)	1/16	–	+	–	–	–	–	–	–	–

Staining intensity: + + + strong, + + moderate, + weak, – negative.
ND = not done.





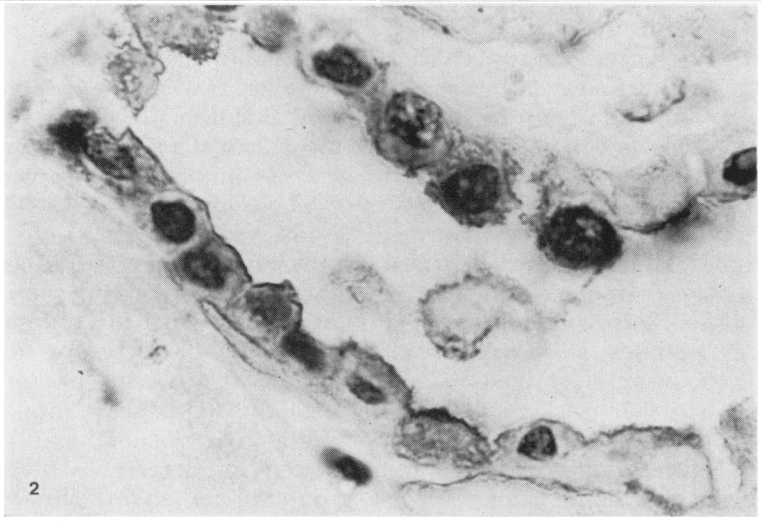


FIG. 1a.—Glomerulus and arteriole showing localization of blood group A antigen on the endothelial surfaces, using human alloantiserum. Indirect immunoperoxidase/haematoxylin × 297.

FIG. 1b.—Glomerular detail showing distinct endothelial distribution of blood group A antigen, using human alloantiserum. Indirect immunoperoxidase/haematoxylin × 1088.

FIG. 2.—Collecting duct showing localization of blood group A antigen to surface of epithelial cells and sometimes to cytoplasm, using human alloantiserum. Indirect immunoperoxidase/haematoxylin × 995.

studies revealed a consistent and strong demonstration of blood group A antigen on the peritubular capillary (PTC) endothelium when the monoclonal antibody was used. However, some evidence for variable expression of antigen was suggested by the variation in intensity of staining in arterial and glomerular capillary endothelial cells, which occurred in 2 of the 5 Group A kidneys. Furthermore, the demonstration that alloantisera produced 3 different categories of staining, showing no correlation with titres of IgG anti-A activity in the 9 immune sera; emphasizes the variable expression of antigen by endothelium and differences in antibody specificity.

The ABH antigens have been shown to be complex glycolipid and glycoprotein macromolecules (Wilczynska, Miller-Podraza and Koscielak, 1980; Finne, 1980). As Group O sera contain a spectrum of anti-A antibodies, it is not unexpected that they should show variable recognition of different components of the blood group A antigen. Their complexity is further demonstrated by the inability to abolish staining with a synthetic trisaccharide blood group A substance (Synsorb A) whereas absorption with Group A erythrocytes removed all activity.

Variation in staining of the distal tubular epithelial cells, most clearly identifiable in paraffin sections, demonstrates that antigen may be either on the surface or in the cytoplasm of some distal tubular cells, whilst others have none. Collecting ducts were sometimes positive but all other renal cells were unstained as has been reported by other workers (Hinglais *et al.*, 1981).

The marked variation in distal tubule and collecting duct staining suggests that A antigen distribution varies within a kidney, independent of the antibody used to define it. Secretor status has been reported to influence the distribution of blood group antigen in collecting ducts but not in distal tubules (Szulman, 1960; Bariety *et al.*, 1980). Positive staining of collecting ducts was only seen in the paraffin embedded sections of a kidney from an individual who was Lewis (a^-b^+), *i.e.* a secretor (Hinglais *et al.*, 1981). Distal tubule epithelial cell staining is likely to reflect variation in the physiological activity of different segments of the tubule. ABH glycosyltransferase enzymes have been located in homogenised preparations of normal kidney tissue which suggests that synthesis of some ABH macromolecules may occur in the kidney (Oriol *et al.*, 1980).

Frozen sections of 1 kidney showed no epithelial staining with the monoclonal anti-A antibody despite 8 out of 9 human antisera being positive. This finding and the variability between the staining observed with the alloantisera indicates that any study of the distribution of blood group A antigen requires investigation with several antibodies possessing specificity for different components of the A antigen.

The consistent expression of blood group A by peritubular capillary endothelium suggests that antigen variation is not sufficient to allow regular successful transplantation across the ABO barrier. However, the variable expression by some renal endothelial cells suggests that the rare survival of ABO incompatible allografts is, in part, due to a very low density of blood group A antigens on the cells of Group A kidneys, either of Group A_1 or A_2 subtype. Furthermore, if there is also an inability of the immunosuppressed recipient's anti-A antibody to bind to the graft, there will be little or no endothelial damage. The occurrence of variation in antibody avidity is supported by the finding that 26 out of 40 Group O sera showed no detectable binding to blood group A renal endothelium of a Group A_1 kidney (RG), despite the fact that A antigen was strongly expressed by this kidney.

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