

Ia ANTIGENS IN RAT KIDNEY, WITH SPECIAL REFERENCE TO THEIR EXPRESSION IN TUBULAR EPITHELIUM*

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Antigens of the Ia type are classically found on B lymphocytes (1), some activated T lymphocytes (2), and accessory cells of the macrophage/dendritic cell lineages (3, 4) in mice, and have a similar limited tissue distribution in the rat (5, 6, 7). They are antigenic determinants on surface glycoproteins that have important roles in antigen presentation to T lymphocytes, in cell interactions leading to B lymphocyte activation and in activation of T lymphocytes by alloantigens in graft-versus-host disease and in the mixed leukocyte reaction (reviewed in reference 8). Thymic epithelium, while a nonlymphoid cell-type, expresses Ia antigens in a lymphoid environment (9, 10). Ia molecules may be Ir gene products (11) and it has been suggested that they could represent primary recognition receptors for antigen (12, 13, 14).

Ia antigens have recently been described in association with a number of nonlymphoid cells, including vascular endothelium (15) and a number of epithelia in guinea pigs (16, 17), man (18–21), mice (22, 23), and rats (24–26). The work of Hart and Fabre (24) and Mayrhofer et al. (26) strongly suggests that the Ia antigens are synthesized by the epithelial cells and not passively acquired. These findings pose questions as to whether Ia molecules function in nonlymphoid cells in an immunological context or whether they have different functions, perhaps related to tissue organization (6) or to lymphoid cell migration (16). Antigen in association with human vascular endothelial cells has been shown to stimulate T lymphocyte blastogenesis in vitro (15). Ia molecules associated with epithelial cells could therefore be important in the handling of antigens at mucosal surfaces and clues to their function might follow definition of their subcellular localization.

We have recently described the expression and distribution of Ia antigens in rat intestinal epithelium (26). In these cells, the distribution of immunoperoxidase reaction product in cryostat sections of fresh-frozen tissue suggested that much of the antigen was intracellular, while Hart and Fabre (24) have described Ia antigens within the cytoplasm of rat renal tubular cells. We have further defined the subcellular localization of Ia molecules in the epithelium of rat kidney by the use of tissue fixation, immunoperoxidase detection of antigen, and histochemical identification of lysosomes.

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We have also examined the genetic control of Ia antigen expression in renal epithelium and noted its distribution on other cell types in the kidney.

Materials and Methods

Animals. Male DA (RTI^a), PVG/c (RTI^b), and AO (RTI^b) rats were specific pathogen-free adults from the MRC Cellular Immunology Unit. Conventional adult female BN (RTI^b) rats were bred at the Princess Margaret Hospital. For genetic studies, male, age-matched conventional PVG/c, DA, [PVG/c × DA]F₁ and PVG/RTI^a (congenic with PVG/c) rats were kindly provided by Dr. J. C. Howard (Agricultural Research Council Institute of Animal Physiology, Babraham, U.K.).

Preparation of Tissues. Kidneys were removed under ether anesthesia. For fresh frozen sections, a 2–3-mm thick transverse section including the renal pedicle was embedded by freezing in OCT medium (Tissue-Tek II, Miles Laboratories, USA) using liquid nitrogen-cooled isopentane. 5- μ m cross-sections of the kidney were cut using a Bright cryostat, air dried at room temperature for 1–2 h, fixed in absolute ethanol at 4°C for 10 min, and rehydrated through 70% ethanol to PBS¹ (Dulbecco's phosphate-buffered saline, Solution A, Oxoid, U.K.) before incubation with antibodies.

For sections of fixed kidney, three methods were used. (a) For demonstration of acid phosphatase, 2–3-mm transverse sections of the cortex were fixed for 24 h at 4°C in 4% paraformaldehyde freshly prepared in 0.1 M cacodylate buffer containing 1% CaCl₂, pH 7.2. After washing in three changes of gum-sucrose medium (27) for 24 h at 4°C the blocks were transferred to a 2:1 mixture of OCT in distilled water for 18 h and then neat OCT for 3 h. The tissues were then frozen in fresh OCT as above. 5- μ m cryostat sections were cut and air dried for 1–2 h before staining.

(b) For antigen localization studies, 1–2-mm cubes of renal cortex were fixed at 4°C in 0.1 M cacodylate buffer containing 1% paraformaldehyde, 0.05% glutaraldehyde, and 0.1 M sucrose for 6 h and washed in three changes of 0.01 M cacodylate buffer containing 0.25 M sucrose (washing buffer) for 24 h. The tissues were then embedded and sectioned at 5 μ m as described above.

(c) For morphological studies, 1–2-mm cubes of renal cortex were fixed at 4°C for 4 h in 2% glutaraldehyde and 1% paraformaldehyde, washed in three changes of washing buffer and postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 4 h. The tissues were then washed in three changes of washing buffer for 2 h, dehydrated through an acetone series, embedded in an Epon-Araldite mixture and sectioned at 1 μ m.

Antisera. Mouse monoclonal IgG antisera raised against "framework" determinants of rat Ia molecules have been described elsewhere (28, 29). MRC OX4 and MRC OX6 antibodies cross-react with gene products of the I-A subregion of the mouse major histocompatibility complex (MHC) and MRC OX17 cross-reacts with an I-E/C subregion gene product. MRC OX18 detects Class I antigens of all rat strains (29). A mouse monoclonal anti-human HLA antibody (W6/32) was used as a negative control (26). The monoclonal antibodies were used as hybridoma culture supernatants containing ~50 μ g/ml of antibody, to which was added 10% normal rat serum to inhibit Fc binding of mouse IgG.

Horseradish peroxidase conjugated to rabbit F(ab')₂ anti-mouse IgG (RAM-HRP) has been described elsewhere (30) and was used at a concentration of 40 μ g protein/ml in the presence of 10% normal rat serum. In some experiments a sheep anti-mouse IgG conjugate (SHAM-HRP) was used. Hyperimmune sheep anti-mouse IgG was raised against mouse IgG (all subclasses) that had been purified by elution from a Staphylococcal protein A-Sepharose C1-4B column (Pharmacia, Sweden) by the method of Watanabe et al. (31). Antibodies cross-reacting with rat immunoglobulins were removed from the sheep antiserum by passage through a rat IgG-Sepharose 4B column. Immunopurified sheep IgG-anti-mouse IgG (SHAM) was then prepared by absorption to a mouse IgG-Sepharose 4B column and elution with 3 M NaSCN. The resultant antibody did not significantly react with rat IgG in a sensitive ELISA assay.

¹ *Abbreviations used in this paper:* DAB, Dulbecco's solutions A plus B; NBCS, newborn calf serum; PBS, Dulbecco's phosphate-buffered saline, solution A; RAM-HRP and SHAM-HRP, respective conjugates of horseradish peroxidase with rabbit F(ab')₂ anti-mouse IgG or sheep anti-mouse IgG.

Conjugation to horseradish peroxidase was as described for RAM-HRP. Fluorescein isothiocyanate-conjugated immunopurified rabbit F(ab')₂ anti-mouse IgG (32) was used at 20 µg/ml in the presence of 10% normal rat serum.

Staining of Tissues. Immunoperoxidase detection of antigens by an indirect technique was essentially as described elsewhere (26, 30). Incubations with antibodies were performed at 4°C for 1 h in the case of fresh-frozen sections and RAM-HRP was used to detect bound mouse antibodies. In the case of fixed-frozen tissue, sections were incubated with antibodies for 2 h at 4°C and SHAM-HRP replaced RAM-HRP. After washing, enzyme-linked antibody was revealed by reacting with 3,3'-diaminobenzidine and hydrogen peroxide for 10 min at room temperature. The sections were lightly stained with Harris' hematoxylin (30). In some cases sections were stained by the PAS method before hematoxylin. For qualitative comparisons of Ia antigen between various rat strains, sections were processed in batches to ensure identical treatment.

Acid phosphatase was demonstrated by a modified Gomori technique (33) or by the simultaneous azo-coupling method using naphthol AS-TR phosphate (34). Thin plastic sections (1 µm) of fixed kidney were stained with 1% Toluidine Blue 0 in 1% borax or with hematoxylin and eosin after etching with sodium ethoxide.

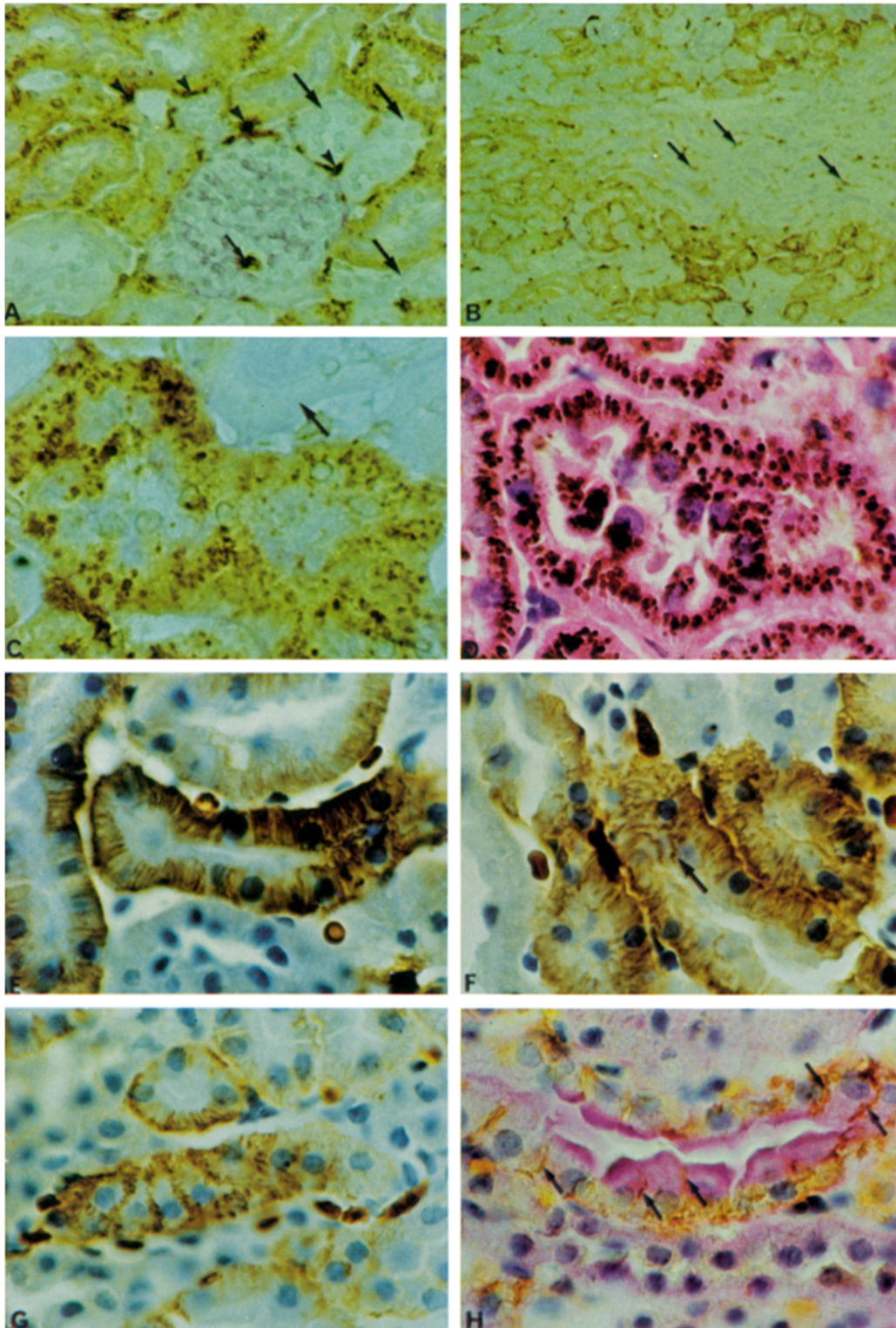
Analysis of Ia Antigen on Lymphoid Cells. Cells were obtained from submandibular lymph nodes by teasing with fine forceps in DAB/NBCS (Dulbecco's phosphate-buffered saline, solutions A plus B, containing 5% newborn calf serum). After washing with DAB/NBCS, aliquots containing 1×10^6 cells were pelleted in Dreyer tubes and resuspended in 50-µl aliquots of either MRC OX4 or W6/32 culture supernatants. This and all subsequent steps were conducted at 0°C in the presence of 0.01 M sodium azide. After incubation for 1 h the cells were washed three times in DAB/NBCS and incubated for 1 h with RAM-FITC. The suspensions were then diluted to ~1 ml with DAB/NBCS and analyzed using the Fluorescence Activated Cell Sorter (FACS II; B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA). Fluorescence profiles were obtained from analysis of 2×10^4 cells. Labeling by MRC OX4 antibody was significant when the fluorescence intensity exceeded that of 98% of cells incubated with W6/32.

Results

Distribution of Ia Antigens in Fresh-frozen Kidneys of DA and BN Rats. Essentially similar results were obtained for both rat strains. Furthermore, MRC OX4 and MRC OX6 antibodies (detecting I-A gene products) produced identical results and MRC OX17 antibody (detecting an I-E/C gene product) also had the same distribution although the staining intensity was less than with either MRC OX4 or MRC OX6. There was no staining of any tissue components with W6/32 antibody. Only the results for MRC OX4 are illustrated.

(a) **RENAL TUBULES.** Many tubules in the cortex were densely stained by all anti-Ia antisera (Figs. 1a and 3a) but tubules in the medulla and renal pyramid were negative (not shown). Combined staining for Ia antigens and for the proximal tubule glycocalyx by the PAS method (Figs. 1a and 1b) showed that all Ia-positive tubules were also PAS-positive. PAS-negative tubules (i.e. distal tubules and collecting ducts) were Ia-negative. However, many PAS-positive tubules were Ia-negative and closer examination (Fig. 1b) revealed that as proximal convoluted tubules entered medullary rays, staining for Ia antigens became patchy and was then lost. Therefore, Ia antigen is restricted to the convoluted portion of the proximal tubule and is not expressed by either the parietal epithelium of Bowman's capsule (Fig. 1a) or the epithelium distal to the proximal convolutions.

(b) **GLOMERULI.** The vascular endothelium, the visceral epithelium of Bowman's capsule and most of the mesangium were Ia-negative. However, many cross-sections contained from one to four densely stained Ia-positive cells (Fig. 1a) that appeared to



be within the mesangium.

(c) **INTERSTITIUM.** Many densely stained Ia-positive cells with dendritic morphology were scattered throughout the cortex between the renal tubules (Figs. 2*a* and 3*a-c*). Dendritic cells were very numerous at the cortico-medullary junction (see below), rare in the outer medulla, and absent from the renal pyramid parenchyma and its epithelium.

(d) **PELVIS.** The transitional epithelium of the renal pelvis was not stained by any of the antibodies. However, its lamina propria contained many large dendritic cells. In some areas, Ia-positive dendritic cells were observed between the cells of the transitional epithelium, having presumably migrated from the underlying lamina propria (Fig. 2*b*).

(e) **BLOOD VESSELS.** No vascular endothelium was stained by anti-Ia antibodies. This included the endothelia of the glomerular capillaries (Fig. 1*a*), the vasa recta, and large intrarenal branches of the renal artery and vein.

Class I Antigens in Fresh-frozen Kidneys of DA Rats. The distributions of Class I antigens (MRC OX18 antibody) and Class II antigens (MRC OX4) were compared in DA kidney (not illustrated). In contrast to Class II antigens, none of the renal tubules were significantly stained for Class I antigens. However, the endothelia of the glomerular capillaries, the capillaries between tubules, and the large intrarenal blood vessels were strongly stained by MRC OX18.

Strain Variation of Ia Antigen Expression. Kidneys from the following rat strains were examined using MRC OX4 antibody: DA (RT1^a), BN (RT1ⁿ), PVG/c (RT1^c), and AO (RT1^u). Dendritic cells in all strains were strongly stained by MRC OX4 antibody, but strong staining of renal tubules was only observed in DA and BN kidneys. Weak, just detectable staining of proximal tubules was sometimes observed in PVG/c and AO kidneys.

The genetic control of Ia antigen expression in renal epithelium was examined in

FIG. 1. Ia antigens in rat kidney. *A-C*, fresh frozen sections of DA kidney showing Ia antigen detected with MRC OX4 antibody. The sections were also stained by the PAS technique and counterstained with hematoxylin. *A*, PAS-positive proximal tubules are strongly stained by peroxidase reaction product, indicating the presence of Ia antigen. PAS-negative distal tubules (*large arrows*) do not contain Ia antigen. The glomerulus is Ia-negative, with the exception of one strongly stained cell (*small arrow*). Dendritic cells between the tubules are also strongly stained (*arrowheads*). *B*, a medullary ray and adjacent cortex. Note that PAS-positive proximal tubules become progressively Ia-negative as they leave the cortex (*left*) and pass towards the medulla (*right*). Densely stained Ia-positive dendritic cells are present between tubules in the medullary ray (*arrows*). *C*, high power view of an Ia-positive proximal tubule. Note the granular appearance and predominantly basal distribution of the peroxidase reaction product in the epithelium. Some adjacent proximal tubules are Ia-negative (*arrow*). *D*, high power view of a proximal tubule in fixed-frozen kidney, stained for acid phosphatase to show lysosomes and counterstained with hematoxylin and eosin. Note the granular appearance of the lysosomes in the epithelial cells of proximal tubules. *E-H*, localization of Ia antigens in fixed-frozen sections of BN kidney, using MRC OX4 and MRC OX6 antibodies in combination. Note that fixed erythrocytes in capillaries between the tubules show pseudo-peroxidase activity. *E*, tubules in axial section. Note the appearance suggesting staining of closely stacked membranes that extend from the bases of the cells almost to the lumen. *F*, Ia-positive tubules in axial section and cross section. The stained interdigitating lateral cell membranes of cells viewed *en face* can be seen where the central tubule is obliquely sectioned (*arrow*). *G*, an oblique section of an Ia-positive tubule with epithelial cells viewed *en face*. Note the stained interdigitating lateral cell membranes outlining the cells. *H*, a stained Ia-positive tubule counterstained by the PAS method. Note reaction product at the bases of cells and along the lateral cell borders (*arrows*), but absence of reaction product in association with the PAS-positive luminal brush border. *A*, $\times 295$; *B*, $\times 74$; *C-H*, $\times 742$.

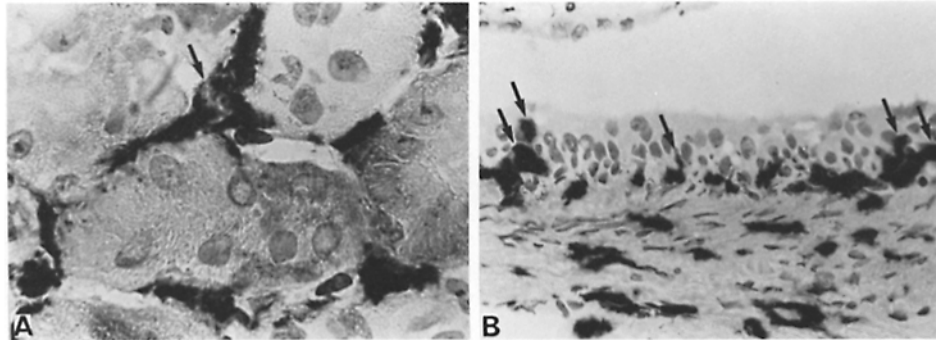


FIG. 2. Ia-positive dendritic cells in the kidney stained with MRC OX4 antibody. (A) Dendritic cells in the interstitial connective tissues between tubules in the cortex. Note the dense reaction product, the irregular shapes of the cells and the large nucleus in one of the cells (arrow). (B) Numerous dendritic cells stained in the lamina propria of the renal pelvis. Note that some cells appear to have entered or extended processes into the overlying transitional epithelium (arrows). A, $\times 714$; B, $\times 270$.

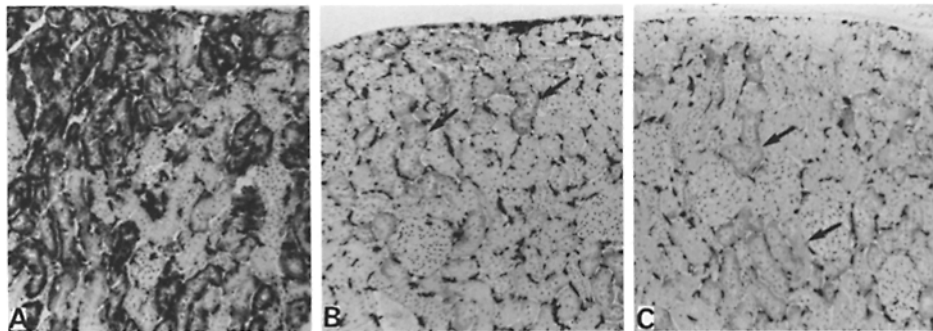


FIG. 3. Genetic regulation of Ia antigen expression in renal epithelium. Sections from age- and sex-matched rats stained with MRC OX4 antibody show dense staining of proximal tubules in DA kidney (A) but only faint staining of a few tubules (e.g. arrows) in PVG/C (B) and PVG/RT^a (C) kidneys. Photographed using a blue filter to enhance contrast of the peroxidase reaction product. All, $\times 71$.

two replicate experiments. In each, age- and sex-matched rats from the same conventional animal house were sacrificed and the kidneys processed in batches for fresh-frozen sections. Staining with MRC OX4 and MRC OX17 antibodies was compared in DA, PVG/c, (DA \times PVG/c) F_1 hybrid, and PVG. RT1^a kidneys. As the ranking of staining intensity was the same for both antibodies, only sections stained with MRC OX4 antibody are shown (Fig. 3). Staining of proximal tubules was dense in DA kidneys (Fig. 3a) but just detectable in PVG/c kidneys (Fig. 3b). The staining was intermediate in intensity in (DA \times PVG/c) F_1 hybrid kidneys (not shown) while in congenic PVG/RT1^a kidneys, it was comparable in intensity to staining in PVG/c parental kidney (Fig. 3c).

In contrast, staining of dendritic cells appeared to have equal intensity in all strains. Furthermore, counts of dendritic cells per unit area of cortex were not greatly different between DA and PVG/c rats. Counts were made in the subcapsular cortex, mid-cortex and cortico-medullary areas of the kidneys and are expressed as means of dendritic cells from 10 high power fields (the standard error of the mean did not exceed 2 in any instance). In the first experiment, respective counts were:- DA: 14, 15,

and 30; PVG/c: 22, 17, and 34, and in the second experiment:- DA: 17, 12, and 27; PVG/c: 15, 13, and 19.

Expression of Ia Antigen on B Lymphocytes. The differences in expression of Ia antigens in renal epithelium were not reflected in the levels of expression of surface Ia antigen by B lymphocytes. Fig. 4 illustrates the fluorescence profiles of lymph node cells from DA, PVG/c, [DA \times PVG/c] F_1 and PVG/RT1^a rats labeled with MRC OX4 antibody. A similar proportion of cells from each animal was labeled and the profiles of fluorescence intensity were indistinguishable.

Cellular Distribution of Ia Antigens in Tubule Epithelial Cells. The appearance of the immunoperoxidase reaction product in fresh-frozen sections strongly suggested the presence of Ia antigen within the epithelial cells, with a mainly basal distribution (Fig. 1*c*). Furthermore, the granular reaction product in some tubules suggested possible association of Ia antigens with cytoplasmic organelles such as lysosomes, which are plentiful in the proximal tubule epithelium (Fig. 1*d*). These appearances, and the coincidental distribution of the antigen and lysosomes in the proximal tubules, suggested that Ia antigens might be components of the phagolysosome system.

Antigen localization has been attempted in fixed tissue to obtain improved preservation of structure. Adequate preservation of structure and lysosomal acid phosphatase localization, with retention of the antigenicity of Ia molecules, has been achieved in tissues fixed with 1% paraformaldehyde and 0.05% glutaraldehyde. Antigenicity was best preserved in the deeper regions of blocks and was lost after fixation for 24 h. The localization of antigens by a mixture of MRC OX4 and MR OX6 antibodies in fixed tissues is shown in Figs. 1*e-g*.

Ia antigen can be localized to the lateral cell membranes between the epithelial

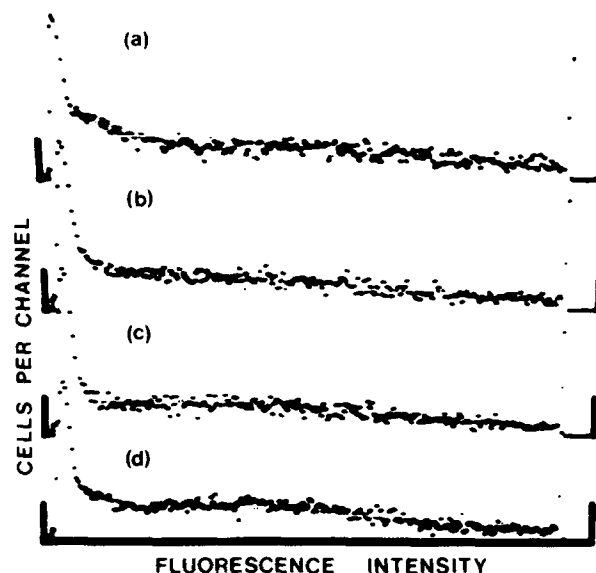


FIG. 4. Expression of Ia antigen on the surfaces of rat B lymphocytes. Lymph node cells were stained with MRC OX4 antibody using an indirect immunofluorescent technique and analyzed by flow cytometry. The fluorescence profiles were obtained from 1×10^5 cells in each case. *a*, PVG/RT1^a; *b*, [PVG/C \times DA] F_1 hybrid; *c*, DA; *d*, PVG/C. Vertical axis, logarithmic plot of cells per channel; horizontal axis, fluorescence intensity.

cells when viewed *en face*, the immunoperoxidase reaction product outlining the interdigitating cell membranes of adjacent cells (Figs. 1*f* and *g*). The antigens are not associated with the PAS-positive brush borders of the proximal tubular cells, but extend close to the luminal surface on the lateral cell membranes (Fig. 1*h*). In cells sectioned in a plane from lumen to base (Figs. 1*e* and *f*), a complex picture is seen suggestive of Ia antigens associated with vertically stacked intracellular membranes extending from the base of the cell almost to the lumen. No circular or granular profiles such as those seen in fresh-frozen sections (Fig. 1*c*) were found in fixed material. Fig. 5*a* and *b* illustrate the appearances of intercellular junctions and of the extensively infolded basal cell membranes in tubular epithelial cells in thin plastic sections of well-fixed kidney.

Discussion

Ia antigens in the kidney may be important in rejection of renal allografts (35), could be involved in immune responses to local infection and have as yet undefined function in tubular epithelium (16, 24). Their distributions on the various cellular components of rat kidneys have been investigated using an immunoperoxidase technique, thus allowing better identification of cells than is possible with immunofluorescence. Ia antigens have been localized to two cell types: proximal tubule epithelium and interstitial dendritic cells. In agreement with Hart and Fabre (24) and in partial agreement with Von Willebrand et al. (36), all vascular endothelium in rat kidney was negative for Ia antigens but was positive for Class I antigens. This finding contrasts with the presence of Ia antigens on human renal vascular endothelium (20, 37, 38).

Other immunochemical and serological studies have shown that kidneys from DA rats contain large amounts of Ia antigens when compared with kidneys from PVG/c rats (5, 39). The present study suggests that the differences cannot be accounted for by numbers of Ia antigen-bearing interstitial cells. However, the striking differences in expression of Ia antigens by the tubular epithelia of the two rat strains provide a

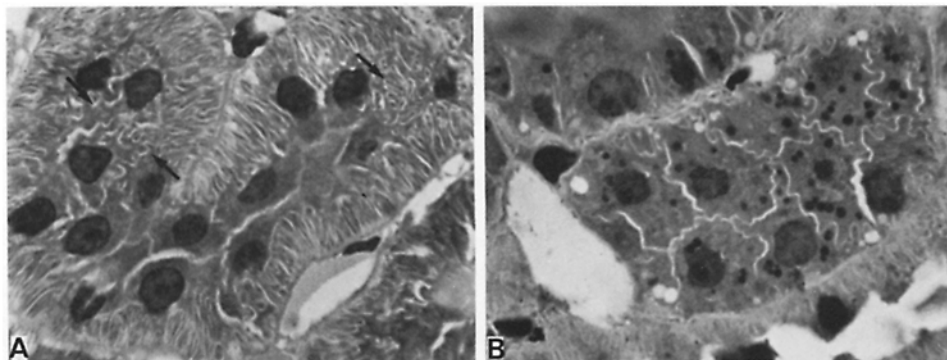


FIG. 5. Photomicrographs of 1- μ m sections from plastic-embedded kidney fixed with glutaraldehyde and paraformaldehyde and postfixed in Osmium tetroxide. (A) Mainly axial section of a tubule showing the extensively infolded basal cell membranes of the tubular epithelial cells. The section becomes oblique at both ends, revealing interdigitating lateral membranes around cells viewed *en face* (arrows). Note the similarities to Fig. 1*E* and *F*. (B) Section passing through a tubule in the plane of the epithelial cell nuclei. The interdigitating cell borders are similar to those in Fig. 1*g*. Both, $\times 1,000$.

ready explanation. The almost qualitative differences in expression between PVG/c and AO rats on the one hand and DA and BN rats on the other were found for gene-products of both I-A and I-E/C subregions. Studies on the intestines in DA and PVG/c rats (26) and AO and BN rats (unpublished results) show that the traits for high or low expression of Ia antigens concur in intestinal epithelium (endoderm-derived) and renal epithelium (mesoderm-derived). The Ia antigens in renal (24) and gut (26) epithelia have been shown to be endogenously synthesized and the concordant results with three monoclonal antisera directed at different "framework" epitopes on Ia molecules encoded by at least two separate loci makes remote the possibility that the staining observed is due to cross-reactions with non-Ia molecules in renal epithelium.

Genetic studies in [DA \times PVG/c] F_1 hybrids and PVG/RT1^a congenics allow the following conclusions. Firstly, high expression of I-A (MRC OX4) and I-E/C (MRC OX17) encoded antigens appears to be a dominant characteristic because staining was stronger in F_1 hybrid kidney than in the PVG/c parental strain. Gene dosage may explain the weaker staining in the F_1 hybrid as compared to the DA parent strain. Secondly, the low levels of both Ia antigens in the tubules of PVG/RT1^a kidneys show that expression of both loci is under control of non-MHC genes. However, these genes do not appear to affect the levels of expression of Class I antigens. Broadly similar conclusions have been reached for the control of Ia antigen expression in adult rat gut epithelium (26).

Thus the strain differences in Ia antigen expression in epithelia are not due to structural gene differences but to the actions of regulatory genes. This conclusion is supported by the findings in this and other studies (28) that constitutive expression of Ia antigens occurs at similar levels on B lymphocytes from all rat strains. Non-MHC genes therefore regulate expression of at least two spatially separate I region genes in two embryologically disparate epithelia while not affecting expression by B lymphocytes (and probably dendritic cells). Furthermore, in addition to genetic regulation, other factors such as maturity (26) and local immunological reactions (25) also affect the amounts of antigen expressed on intestinal epithelium. These findings suggest that regulated expression of Ia antigens in epithelia may have adaptive advantages for the organism and that Ia antigens could have specific functions in epithelia that may not necessarily be related to their functions in lymphoid tissue.

The distribution of dendritic cells observed in this study is similar to that described in rat kidney by Hart and Fabre (7, 24). However, in the present study small numbers of Ia-positive cells have also been identified in the glomeruli, where they do not appear to be intravascular leukocytes or mesangial cells. They may correspond to the Ia-positive glomerular cells described by Schreiner et al (40). A further new finding is the identification of dendritic cells in an intraepithelial position in the urinary epithelium of the renal pelvis. It is possible that these cells could have a function similar to Langerhans cells in skin (41), perhaps presenting antigen in urinary infections.

The subcellular distribution of Ia antigens in epithelia may provide important clues to its function. Its apparent intracellular localization by immunoperoxidase and immunofluorescence (24) techniques in fresh frozen tissue is therefore significant and is supported by morphological studies on rat intestine (26) and by reported absence of Ia antigens on the surfaces of dissociated renal tubule cells (36). The appearances of the immunoperoxidase reaction product in kidney and gut sections suggested

association with the phagolysosomes and possible involvement in macromolecule absorption (26), while its restriction to the proximal convoluted tubules where protein reabsorption and large lysosomes are found (42, 43) was consistent with this hypothesis.

However, when fixation precedes freezing and sectioning there is little doubt that the lateral and basal cell membranes bear Ia antigen, although it is undetectable on the luminal brush borders. The distribution is similar to that described by Parr and McKenzie (22) on mouse intestinal epithelium. The results also strongly suggest that Ia antigens are associated with the extensively infolded basal membranes of proximal convoluted tubule cells. No evidence of a granular reaction product or of circular profiles was seen, suggesting that the apparent intracellular distribution of antigen in poorly fixed material could be artifactual, perhaps resulting from disruption and rounding up of the stacked, infolded, basal membranes in unfixed tissue during freezing or sectioning. However, it remains possible that fixation has masked intracellular antigen (44), or that there are differences between DA and BN rats in the subcellular distributions of antigens. These possibilities are being examined.

The function of Ia antigens in epithelia remains unsolved. However, their absence from the luminal cell membranes and probably from intracellular organelles makes a role in uptake and transport of macromolecules appear less attractive. Rather, their display on the lateral and basal membranes could point to a function in the presentation of absorbed antigen in an immunogenic form as described for vascular endothelium (15). Alternatively, Ia molecules might have a role in tissue organization, as suggested by Barclay (6). Ultrastructural studies will be required to precisely define the subcellular distribution of these important molecules, while dynamic studies will be necessary to assign them a function. The question of expression of Ia antigens by human renal tubular epithelium (37, 38, 45) requires further consideration in view of the observed polymorphic expression of antigen in rats.

Summary

The distribution of Ia antigens in rat kidneys has been investigated by an immunoperoxidase technique, using monoclonal antibodies directed at framework determinants on molecules equivalent to gene products of the I-A and I-E/C regions of the mouse major histocompatibility complex (MHC). Antigens mapping to both loci were detected in proximal convoluted tubule epithelium and on scattered dendritic cells in the interstitial connective tissues, glomeruli, and mucosa of the renal pelvis. All other structures were negative. Genetic studies indicate that the levels of expression of Ia molecules from both genetic loci are controlled by non-MHC genes in epithelium, but not on lymphocytes. The subcellular distribution of Ia molecules has been investigated in tubular epithelium and is discussed in relation to their possible functions in epithelia.

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References

1. Hämmerling, G. J., B. D. Deak, G. Mauve, U. Hämmerling, and H. O. McDevitt. 1974. B lymphocyte alloantigens controlled by the I region of the major histocompatibility complex in mice. *Immunogenetics*. **1**:68.
2. David, C., T. Meo, J. McCormick, and D. Shreffler. 1976. Expression of individual Ia specificities on T and B cells. I. Studies with mitogen-induced blast cells. *J. Exp. Med.* **143**:218.
3. Hämmerling, G. J., G. Mauve, E. Goldberg, and H. O. McDevitt. 1975. Tissue distribution of Ia antigens: Ia on spermatozoa, macrophages and epidermal cells. *Immunogenetics*. **1**:428.
4. Steinman, R. M., G. Kaplan, M. D. Witmer, and Z. A. Cohn. 1979. Identification of a novel cell-type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers and maintenance *in vitro*. *J. Exp. Med.* **149**:1.
5. Mason, D. W., and G. G. Gallico. 1978. Tissue distribution and quantitation of Ia-like antigens in the rat. *Eur. J. Immunol.* **8**:741.
6. Barclay, A. N. 1981. Different reticular elements in rat lymphoid tissue identified by localization of Ia, Thy-1 and MRC OX2 antigens. *Immunology*. **44**:727.
7. Hart, D. N. J., and J. W. Fabre. 1981. Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. *J. Exp. Med.* **153**:347.
8. Unanue, E. R. 1981. The regulatory role of macrophages in antigenic stimulation. Part two: symbiotic relationship between lymphocytes and macrophages. *Adv. Immunol.* **31**:1.
9. Hoffman-Fezer, G., D. Göetze, H. Rodt, and S. Thierfelder. 1978. Immunohistochemical localization of xenogeneic antibodies against Ia^k lymphocytes on B cells and reticular cells. *Immunogenetics*. **6**:367.
10. Barclay, A. N., and G. Mayrhofer. 1981. Bone marrow origin of Ia-positive cells in the medulla of rat thymus. *J. Exp. Med.* **153**:1666.
11. Nagy, Z. A., C. N. Baxevanis, N. Ishii, and J. Klein. 1981. Ia antigens as restriction molecules in Ir-gene controlled T-cell proliferation. *Immunol. Rev.* **60**:59.
12. McDevitt, H. O. 1980. The role of H-2 I region genes in regulation of the immune response. *In Progress in Immunology*. M. Fougereau and J. Dausset, editors. Academic Press, London. **4**:503.
13. Benacerraf, B. 1980. Genetic control of the specificity of T lymphocytes and their regulatory products. *In Progress in Immunology*. M. Fougereau and J. Dausset, editors. Academic Press, London. **4**:419.
14. Werdelin, O. 1982. Immune response genes. *Allergy (Copenh.)*, **37**:451.
15. Hirschberg, H., O. J. Bergh, and E. Thorsby. 1980. Antigen-presenting properties of human vascular endothelial cells. *J. Exp. Med.* **152**:249s.
16. Wiman, K., B. Curman, U. Forsum, L. Klareskog, U. Malmnäs-Tjernlund, L. Rask, L. Trägårdh, and P. A. Peterson. 1978. Occurrence of Ia antigen on tissues of non-lymphoid origin. *Nature (Lond.)*. **276**:711.
17. Forsum, U., L. Klareskog, and P. A. Peterson. 1979. Distribution of Ia-antigen-like molecules on non-lymphoid tissues. *Scand. J. Immunol.* **9**:343.
18. Scott, H., B. G. Solheim, P. Brandtzaeg, and E. Thorsby. 1980. HLA-DR-like antigens in the epithelium of the human small intestine. *Scand. J. Immunol.* **12**:77.
19. Newman, R. A., M. G. Omerod, and M. F. Greaves. 1980. The presence of HLA-DR antigens on lactating breast epithelium and milk fat globule membranes. *Clin. Exp. Immunol.* **41**:478.
20. Natali, P. G., C. De Martino, V. Quaranta, M. R. Nicotra, F. Frezza, M. A. Pellegrino, and S. Ferrone. 1981. Expression of Ia-like antigens in normal human non-lymphoid tissues. *Transplantation*. **31**:75.
21. Scott, H., P. Brandtzaeg, B. G. Solheim, and E. Thorsby. 1981. Relation between HLA-

- DR-like antigens and secretory component (SC) in jejunal epithelium of patients with coeliac disease or dermatitis herpetiformis. *Clin. Exp. Immunol.* **44**:233.
22. Parr, E. L., and I. F. C. McKenzie. 1979. Demonstration of Ia antigens on mouse intestinal epithelial cells by immunoferritin labeling. *Immunogenetics.* **8**:499.
 23. Curman, B., O. Kampe, L. Rask, and P. A. Peterson. 1979. Presence of alloreactive Ia antigens on murine intestinal epithelial cells. *Scand. J. Immunol.* **10**:11.
 24. Hart, D. N. J., and J. W. Fabre. 1981. Endogenously produced Ia antigens within cells of convoluted tubules of rat kidney. *J. Immunol.* **126**:2109.
 25. Mason, D. W., M. Dallman, and A. N. Barclay. 1981. Graft-versus-host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium. *Nature (Lond.)*. **293**:150.
 26. Mayrhofer, G., C. W. Pugh, and A. N. Barclay. 1983. The distribution, ontogeny and origin in the rat of Ia positive cells with dendritic morphology and of Ia antigen in epithelia, with special reference to the intestine. *Eur. J. Immunol.* In press.
 27. Holt, S. J., E. E. Hobbiger, and G. L. S. Pawan. 1960. Preservation of integrity of rat tissues for cytochemical staining purposes. *J. Biophys. Biochem. Cytol.* **7**:383.
 28. McMaster, W. R., and A. F. Williams. 1979. Identification of Ia glycoproteins in rat thymus and purification from rat spleen. *Eur. J. Immunol.* **9**:426.
 29. Fukumoto, T., W. R. McMaster, and A. F. Williams. 1982. Mouse monoclonal antibodies against rat major histocompatibility antigens. Two Ia antigens and expression of Ia and Class I antigens in rat thymus. *Eur. J. Immunol.* **12**:237.
 30. Barclay, A. N. 1981. The localization of populations of lymphocytes defined by monoclonal antibodies in rat lymphoid tissues. *Immunology.* **42**:593.
 31. Watanabe, M., T. Ishii, and H. Nariuchi. 1981. Fractionation of IgG1, IgG2a, IgG2b, and IgG3 immunoglobulins from mouse serum by protein A-Sepharose column chromatography. *JPN. J. Exp. Med.* **51**:65.
 32. Jensenius, J. C., and A. F. Williams. 1974. The binding of anti-immunoglobulin antibodies to rat thymocytes and thoracic duct lymphocytes. *Eur. J. Immunol.* **4**:91.
 33. Lojda, Z., R. Gossrau, and T. H. Schiebler. 1979. *Enzyme Histochemistry, a Laboratory Manual.* Springer Verlag, Berlin. 76-78.
 34. Barka, T., and P. J. Anderson. 1962. Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* **10**:741.
 35. Ting, A., and P. J. Morris. 1980. Powerful effect of HL-DR matching on survival of cadaveric renal allografts. *Lancet.* **2**:282.
 36. Von Willebrand, E., E. Parthenais, and P. Hayry. 1980. Expression of the major AgB histocompatibility complex antigens on different structural components of rat kidney and heart. *Cell Immunol.* **52**:313.
 37. Koyama, K., T. Fukunishi, M. Barcos, N. Tanigaki, and D. Pressman. 1979. Human Ia-like antigens in non-lymphoid tissues. *Immunology.* **38**:333.
 38. Hayry, P., E. Von Willebrand, and L. C. Anderson. 1980. Expression of HLA-ABC and -DR locus antigens on human kidney, endothelial, tubular and glomerular cells. *Scand. J. Immunol.* **11**:303.
 39. Davies, H. S., and G. W. Butcher. 1978. Kidney alloantigens determined by two regions of the rat major histocompatibility complex. *Immunogenetics.* **6**:171.
 40. Schreiner, G. F., J.-M. Kiely, R. S. Cotran, and E. R. Unanue. 1981. Characterization of resident glomerular cells in the rat expressing Ia determinants and manifesting genetically restricted interactions with lymphocytes. *J. Clin. Invest.* **68**:920.
 41. Green, I., G. Stingl, E. M. Shevach, and S. I. Katz. 1980. Antigen presentation and allogeneic stimulation by Langerhans cells. *J. Invest. Dermatol.* **75**:44.
 42. Straus, W. 1964. Occurrence of phagosomes and phagolysosomes in different segments of the nephron in relation to the reabsorption, transport, digestion, and extrusion of intravenously injected horseradish peroxidase. *J. Cell. Biol.* **21**:295.

43. Beard, M. E., and A. B. Novikoff. 1969. Distribution of peroxisomes (microbodies) in the nephron of the rat. A cytochemical study. *J. Cell Biol.* **42**:501.
44. Pignal, F., M. Maurice, and G. Feldmann. 1982. Immunoperoxidase localization of albumin and fibrinogen in rat liver fixed by perfusion or immersion: effect of saponin on the intracellular penetration of labeled antibodies. *J. Histochem. Cytochem.* **30**:1004.
45. Scott, H., P. Brandtzaeg, H. Hirschberg, B. G. Solheim, and E. Thorsby. 1981. Vascular and renal distribution of HLA-DR-like antigens. *Tissue Antigens.* **18**:195.