Localization of an Ia-bearing Glomerular Cell in the Mesangium

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ABSTRACT Using trypsin to render intact, isolated rat glomeruli permeable to antibody, and using an electron microscopic immunoperoxidase technique, we have localized a phagocytic immunologically-relevant cell bearing Ia determinants to the renal mesangium. Thus there are at least two functionally distinct cell types in the renal mesangium: one is a contractile smooth musclelike cell, and the other a phagocytic cell that bears immunologically-relevant surface determinants.

We have previously described (1) a subpopulation of rat glomerular cells that bear I region associated (Ia) antigens: membrane antigens, encoded in the major histocompatibility locus, that regulate the immune interactions between phagocytes and lymphocytes. This glomerular cell resembles a mononuclear phagocyte and constitutes 1-2% of the glomerular cell population. Tissue culture experiments have demonstrated that the Ia-positive glomerular cells are capable of processing antigen and activating specifically sensitized lymphocytes in a genetically restricted manner.

Although the Ia-positive cell was present in the normal and uninflamed glomerulus, its precise localization was unknown. In the present study, we have used a combination of ultrastructural immunoperoxidase technique and a staining method that permits the labeling of Ia-positive glomerular cells in situ. We report that the Ia-positive cell is situated within the mesangium and is also phagocytic in vivo. The coexistence of Ia-negative cells in the mesangium suggests that there are functionally distinct mesangial cell populations.

MATERIALS AND METHODS

Animals

Female rats, Lewis strain, weighing 100-175 g, and at least 5 wk old, were obtained from Microbiological Associates (Walkersville, MD).

Antisera

Two mouse monoclonal anti-rat Ia antibody preparations, MAS 028b and MAS 029b (Accurate Chemical & Scientific Corp., Westbury, NY), were employed to label Ia antigens of glomerular cells. Their specificities have been extensively characterized (1-3). Each recognizes separate Ia determinants of the Lewis haplotype (RT 1). Labeling with the “sandwich” technique was carried out with F(ab) rabbit-anti-mouse IgG conjugated to horseradish peroxidase (HRPO) (4), at a concentration of 100 µg per ml. This antisera was a gift from Dr. Donna Mendrick (Harvard Medical School). Control labeling consisted of deleting the anti-Ia antibody and substituting electrophoretically purified mouse IgG from the plasmacytoma line MOPC 195 (Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, MD). The second step of incubation with F(ab)-anti-Ig-HRPO was maintained as before.

Glomerular Isolation

Before harvesting glomeruli, both kidneys were thoroughly perfused to remove circulating cells with a technique modified from Griffith, et al. (5), with the additional step of opening the left renal vein to prevent the perfusate to drain. Fifty to 100 ml of a 0.9% NaCl solution was injected via the aorta into both kidneys, resulting in their complete blanching and eventually resulting in a clear perfusate. Mottled kidneys, indicative of incomplete perfusion, were discarded. Glomeruli were obtained by pressing slices of renal cortex through graded sieves (Tyler Inc., Mentor, OH) of 250, 150, and 75 µm with a rubber stopper (1).

Immunoperoxidase Labeling of Intact Glomeruli

Whole glomeruli were placed in a solution containing trypsin, 0.5 mg per ml, and DNAase, 0.01 mg per ml (Sigma Chemical Co., St. Louis, MO), for 30 min at 37°C in HBSS, pH 7.2, and, in some instances, 10⁻⁵ M chlorpromazine HCl. The glomeruli were then washed twice and incubated first in a 1/4 dilution of pooled anti-Ia antisera for 30 min at 4°C, washed twice, and then incubated in 100 µg per ml of F(ab)-anti-Ig-HRPO for an additional 30 min at 4°C. After washing two further times, the whole but partially digested glomeruli were then fixed and processed for electron microscopic examination after staining for peroxidase activity (see below). Two technical considerations must be mentioned. First, we found that the trypsinized glomeruli are much more permeable to F(ab)-HRPO than to whole IgG-HRPO. Second, trypsinization of glomeruli appeared to increase the frequency of Ia-positive mesangial cell pseudopodia over control nontryptsinized glomeruli; this phenomenon is under further study but was markedly inhibited by adding 10⁻⁵ M chlorpromazine HCl (Smith, Kline & French Laboratories, Philadelphia, PA) to the trypsin-containing solution. Controls included: (a) deleting preincubation with trypsin; (b) deleting incubation with anti-Ia but labeling with F(ab)-anti-Ig-HRPO; and (c) substituting for the anti-Ia antibody an equal concentration of mouse IgG from plasmacytoma line MOPC 195, detailed above. In none of the controls did we observe surface labeling of glomerular cells.

Labeling of Isolated Glomerular Cells

Isolated glomerular cells were obtained by subjecting sieve-purified glomeruli to sequential enzymatic digestion (1). Glomeruli were placed in a solution of trypsin, DNAase, and collagenase followed by incubation in EDTA followed by an incubation in collagenase and DNAase. After repeated washing, the suspension of single cells was incubated with anti-Ia antisera followed by F(ab)-anti-Ig-HRPO, as detailed above, for glomeruli.

Electron Microscopy

Cell suspensions and glomerular preparations were fixed in 1.25% glutaraldehyde in 0.1 M cacodylate buffer for 0.5 and 1.5 h, respectively. They were then...
diaminobenzidine (DAB) in Tris buffer for 20–30 min, and subsequently in 0.5% DAB containing 0.01% H2O2 for 20–30 min. Following further rinsing in Tris buffer, the preparations were fixed in 2% OsO4, dehydrated, and embedded in Epon. Some preparations were embedded in pellets of agar gel prior to dehydration. Thin sections were examined with a Philips 201 electron microscope either unstained or stained lightly with lead citrate.

In Vivo Phagocytosis

Soluble, heat-aggregated human γ-globulin (HGG) (Miles Laboratories Inc., Elkhart, IN) was prepared according to the method of Michael et al. (6) and conjugated to rhodamine isothiocyanate (RITC) by standard methods. Rats were injected intravenously with 8 mg of RITC-HGG and sacrificed 90 min later. The kidneys were perfused and glomeruli harvested and stained for Ia antigens with fluoresceinisothiocyanate-conjugated F(ab)2-anti-Ig, as detailed previously (1). Suitable filters were used and the percentage of Ia-positive cells containing RITC-HGG determined in 50 glomeruli.

RESULTS

In a previous report, we demonstrated that isolated, intact glomeruli can be rendered permeable to anti-Ia antibody by mild trypsinization, without distortion of overall cytoarchitecture (1). Because the Ia determinants under study are trypsin insensitive, trypsin-treated glomeruli can be stained for the presence of Ia-positive cells using labeled antibody in the sandwich method outlined in Materials and Methods. The membrane staining is sufficiently clear that the presence of a very small number of cells in an entire glomerulus can be detected. By light microscopy, whole glomeruli stained with the immunoperoxidase technique showed an average of 6–9 Ia-positive cells in different experiments. Fig. 1 demonstrates six Ia-positive cells in the same plane of focus; the membrane labeling with peroxidase produces a rim staining pattern, clearly distinguishable from the staining of endogenous peroxidase in occasional red cells that persisted after renal perfusion. Staining glomeruli after exposure to nonspecific mouse Ia followed by anti-Ig-HRPO results in the labeling of occasional erythrocytes but no other focal staining.

Trypsinization resulted in several morphological alterations on the ultrastructural level. The visceral epithelium was usually totally detached, and the mesangial matrix appeared expanded and partially digested. Nonetheless, the capillary lumens, endothelial cells, and basement membrane remained well-preserved, permitting clear localization of Ia-positive cells to the mesangial region (Fig. 2).

Fig. 2 shows a cell with heavy membrane staining for Ia. It is located within the mesangial region, identified by its relationships to the basement membrane and the urinary space stripped of its epithelial cells, with three adjacent capillary profiles. The cell has extended irregular, cytoplasmic processes through the mesangial matrix; one of the cytoplasmic projections has insinuated itself beneath the Ia-negative endothelial cell (arrows). The capillary lumens are empty and the typical fenestrated endothelial cells are not labeled.

Ia-positive cells are frequently seen in close apposition to Ia-negative cells in the mesangium. In Fig. 3, the Ia-positive cell has extended tortuous cytoplasmic processes that protrude marginally into a neighboring capillary lumen. The surface of Ia-negative cells in contact with Ia-positive cells shows slight staining due to the leaching effect of the reaction product (Fig. 3). Compared to the Ia-negative mesangial cell, the Ia-positive mesangial cells manifest a somewhat more open nuclear chromatin pattern and have many more peripheral vesicles and vacuoles, most of them stained for Ia.

Note that, whereas trypsinization is required to render the glomeruli permeable to antibody, it is not necessary for the detection of Ia on cell membrane. Ia determinants, recognized by the monoclonal antibodies, of membrane of peritoneal and splenic cells isolated without enzymatic digestion, did not change with pretreatment with trypsin (1).

Ia-positive mesangial cells were also examined in suspension after enzymatically digesting a preparation of isolated glomeruli. A representative Ia-positive glomerular cell is shown in Fig. 4. Its characteristics are similar to those observed in whole glomeruli. The densely labeled cell displays tortuous, elongated cytoplasmic processes and deep invaginations. There is considerable peripheral vacuolization, with some vacuoles stained for Ia and others negative for Ia. The nucleus is relatively large and has a fine chromatin pattern.

When intact glomeruli were stained for Ia antigens 90 min after in vivo administration of rhodamine-labeled aggregated HGG, 24% of glomerular Ia-bearing cells demonstrated uptake of the labeled protein when examined by fluorescent microscopy (Fig. 5). There was evidence of uptake by Ia-negative cells, albeit to a lesser extent, but such uptake could not be quantitated accurately in the absence of a membrane label.

DISCUSSION

We have demonstrated by electron microscopy that a recently-defined mononuclear phagocytic cell bearing Ia antigens is located within the normal glomerular mesangium. Previous experiments have demonstrated that these glomerular cells are phagocytic in vitro and here we show them to be phagocytic in situ. They are able to initiate immune reactions in vitro by presenting antigen to sensitized lymphocytes. Consistent with their display of Ia antigens, these glomerular cells strongly stimulate allogeneic lymphocytes in mixed cultures (1).

We have outlined a method for labeling glomerular cells by trypsinizing isolated glomeruli, a process that renders them
**FIGURE 2** Electron micrograph of a portion of glomerulus stained for $\text{Ia}$ antigen. The visceral epithelial cells have been digested off the urinary space (US), but the basement membrane (BM), endothelium (E), and mesangium (M) are relatively well-preserved. Note heavy surface labeling of cell in the mesangium. Mesangial cell processes (arrows) beneath the endothelium are also stained. Focal staining of the BM is due to leeching of reaction product, and staining of free organelles in capillary lumen (lower left) represents nonspecific adsorption of peroxidase to injured membranes. CL, capillary lumen. $\times 9,500$.

**FIGURE 3** The mesangial area contains an Ia-positive and Ia-negative cell, in close apposition. Note process (arrow), probably from Ia-positive cell around the Ia-negative cell. $\times 11,500$. 
permeable to antibody, which we employ in a sandwich-labeling technique. This method offers three advantages. First, it permits the detection of very small numbers of cells, in situ, in tissue fragments. With a mean of 6–9 Ia-positive cells per glomerulus, a range of 3–19 cells per glomerulus, and with an estimated total glomerular cell count of 600, one can accurately assess a population of cells comprising ~1–2% of total glomerular cells. Secondly, one can direct sectioning for electron microscopy for prior scanning under low power of stained, mounted, intact glomeruli, thus increasing the efficiency of electron microscopy for ultrastructural studies. Finally, although there is some distortion caused by loss or alteration of trypsin-sensitive structures, the morphology of the trypsinized, stained glomerulus is adequate for detailed study.

The mesangial Ia-bearing cells are phagocytic in vivo, with respect to circulating aggregates of γ-globulin. The fact that, in
this assay, only 24% took up the aggregate may reflect three
considerations. The first is that there is heterogeneity among
Ia-bearing glomerular cells with regard to phagocytic capacity.
We have shown that in culture there are functional supopu-
tions of Ia-bearing cells, with ~35% demonstrating a phagocytic
capacity after 2 h in culture, that percentage doubling after the
activation of overnight in vitro culture (1). In addition to the
nonphagocytic spindle-shaped Ia-negative mesangial cells, cul-
tures of kidney cells contain phagocytic cells that are morpho-
logically indistinguishable from Ia-positive phagocytes but do
not display Ia antigens. Because such cells can be induced to
express membrane Ia antigens in culture, they may represent
a separate functional stage rather than a separate cell popula-
tion. Finally, we have negatively biased the assay by choosing
an extremely short time-course for glomerular isolation after a
single bolus phagocytic challenge. In a similar model, Striker
(7) found no evidence for infiltrating cells in the first 24 h after
the injection of immune complexes; we chose a time-course of
90 min to minimize the possibility of monocyte infiltration.
Additionally, circulating rat monocytes are Ia-negative.
Thus, the glomerular mesangium appears to contain at least
two, and perhaps more, subpopulations of cells. The first might
be termed the “classical” mesangial cell. It is the predominant
mesangial cell type, of renal origin and resembling smooth
muscle; it is contractile, and bears angiotensin II receptors (8,
9). This mesangial cell has been studied in culture by Kreisberg
et al. (8), who noted it is nonphagocytic, as we have. The
second mesangial cell type is much less common, phagocytic,
adherent to glass, and capable of immunologically specific
interactions with lymphocytes. Under in vitro conditions, 50–
60% of phagocytic glomerular cells bear Ia determinants. As-
suming that one-third of glomerular cells are mesangial (1) and
that 1–2% of glomerular cells bear Ia determinants, one can
estimate that up to 12% of mesangial cells are a distinct
subpopulation capable of phagocytosis, Ia determinant expres-
sion, or both. The phagocytic cell is located in approximation
to the contractile cell, raising the possibility of physiological
interactions between the two cell types. Preliminary experi-
ments utilizing bone marrow transplants indicate that the Ia-
positive cell originates outside the kidney, migrates into the
mesangium, and resides there for an unknown period.
These observations of phenotypically discrete mesangial cell
populations with different properties may, to some extent,
reconcile recent divergent opinions as to the nature of “the"
mesangial cell. Early ultrastructural studies had indicated ap-
parent phagocytosis by at least some mesangial cells of tracer
substances such as ferritin (10) and thorium (11). More recently,
the existence of a phagocytic mesangial cell has been ques-
tioned (12), based on failure of the contractile mesangial cell to
phagocytose in vitro, the difficulties in distinguishing pinocyto-
sis in in vivo studies, and the possible contribution of infiltrat-
ing blood monocytes to glomerular phagocytosis (7). Our ap-
proach has shown that there is at least one type of phagocytic
mesangial cell type, the Ia-positive cell, but one that occurs
sufficiently infrequently so as to render detection by other
techniques difficult. Whether the Ia-negative blood monocytes
that infiltrate the rat glomerulus under pathologic conditions (7)
acquire Ia determinants in the renal mesangium is now under
study.
Endogenous renal Ia antigens have been demonstrated via
absorption techniques in mouse renal homogenates (13), and by
immunohistologic methods in a dendriticlike cell, in the
renal interstitium of rats (14). We have observed a similar Ia-
positive cell in the renal interstitium after fluorescent antibody
labeling of frozen sections. Of particular importance is that in
both intact glomeruli and suspensions, endothelial cells are Ia-
negative, as defined by the two monoclonal antibodies em-
ployed in this study which recognize the Ia determinant. Recent
immunofluorescent studies on frozen sections of human kidney
or on isolated cells have suggested the presence of DR antigens
(thought to be analogous to rodent Ia antigens [15]) on glo-
merular endothelial cells, employing monoclonal antibodies
(16) or polyvalent, heterologous antisera (17). In human renal
tissue, differences in labeling between public and private DR
specifications (16), between monoclonal antibody and polyva-
 lent antisera (16), and between heterologous antisera and al-
loantisera (17) have been noted. Whether the apparent differ-
ence in the distribution of I-region antigens between man and
rat represents species differences, antisera differences, or un-
defined cross-reactive specificities uncovered in polyvalent an-
tisera requires further clarification. It is possible that there is
differential expression of I-subregion determinants among glo-
merular cells and that antisera recognizing Ia determinants
other than those studied in this report may stain rat endothelium
positively, as is the case in humans. Nonetheless, the Ia-
positive phagocytic glomerular cell described herein is a mes-
angial cell and not a component of capillary endothelium.
It has been clearly established that marrow-derived mono-
nuclear phagocytes, whether circulating cells or resident mac-
rophages, engage in reciprocal, modulatory interactions with
lymphocytes that are critical to a host’s immune reactivity to an
antigen (18). The observation that such cells can be found
within the mesangium may be significant with respect to possible
mechanisms of immunologically mediated injury to the
glomerulus. Their presence also suggests an accessible
source of endogenous donor Ia antigens necessary for the
induction of host immunity against transplanted kidneys.

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