Localization of an la-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 la 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 (la) 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 la-positive glomerular cells are capable of processing antigen and activating specifically sensitized lymphocytes in a genetically restricted manner.

Although the la-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 la-positive glomerular cells in situ. We report that the la-positive cell is situated within the mesangium and is also phagocytic in vivo. The coexistence of la-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 la antibody preparations, MAS 028b and MAS 029b (Accurate Chemical & Scientific Corp., Westbury, NY), were employed to label la antigens of glomerular cells. Their specificities have been extensively characterized (1–3). Each recognizes separate la 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 antiserum was a gift from Dr. Donna Mendrick (Harvard Medical School). Control labeling consisted of deleting the anti-la 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-lg-HRPO was maintained as before.

Glomerular Isolation

Before harvesting glomeruli, both kidneys were thoroughly perfused to remove circulating cells with a technique modified from Griffiths, et al. (5), with the additional step of opening the left renal vein to permit the perfusate to drain. Fifty to 100 ml of 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-la antiserum for 30 min at 4°C, washed twice, and then incubated in 100 μg per ml of F(ab)-anti-lg-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 la-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-la but labeling with F(ab)-anti-lg-HRPO; and (c) substituting for the anti-la 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 sieved 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-la antiserum followed by F(ab)-anti-lg-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
rinsed three times in 0.05 M Tris buffer at pH 7.6, incubated first in 0.05 gm% 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 la antigens with fluoresceinisothiocyanate-conjugated F(ab)2-anti-Ig, as detailed previously (1). Spleen homogenate was prepared according to the method of Michael et al. (6) and conjugated to fluoresceinisothiocyanate-conjugated F(ab)2-anti-Ig. 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 la antigens with fluoresceinisothiocyanate-conjugated F(ab)2-anti-Ig, as detailed previously (1).

**RESULTS**

In a previous report, we demonstrated that isolated, intact glomeruli can be rendered permeable to anti-la antibody by mild trypsinization, without distortion of overall cytoarchitecture (1). Because the la determinants under study are trypsin insensitive, trypsin-treated glomeruli can be stained for the presence of la-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 la-positive cells in different experiments. Fig. 1 demonstrates six la-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 la 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 la-positive cells to the mesangial region (Fig. 2).

Fig. 2 shows a cell with heavy membrane staining for la. 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 la-negative endothelial cell (arrows). The capillary lumens are empty and the typical fenestrated endothelial cells are not labeled.

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

Note that, whereas trypsinization is required to render the glomeruli permeable to antibody, it is not necessary for the detection of la on cell membrane. la 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).

la-positive mesangial cells were also examined in suspension after enzymatically digesting a preparation of isolated glomeruli. A representative la-positive mesangial 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 la and others negative for la. The nucleus is relatively large and has a fine chromatin pattern.

When intact glomeruli were stained for la antigens 90 min after in vivo administration of rhodamine-labeled aggregated HGG, 24% of glomerular la-bearing cells demonstrated uptake of the labeled protein when examined by fluorescent microscopy (Fig. 5). There was evidence of uptake by la-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 la 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 la 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 la 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. × 9,500.

FIGURE 3  The mesangial area contains an la-positive and la-negative cell, in close apposition. Note process (arrow), probably from la-positive cell around the la-negative cell. × 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 la-bearing glomerular cells with regard to phagocytic capacity. We have shown that in culture there are functional subpopulations of la-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 la-negative mesangial cells, cultures of kidney cells contain phagocytic cells that are morphologically indistinguishable from la-positive phagocytes but do not display la antigens. Because such cells can be induced to express membrane la antigens in culture, they may represent a separate functional stage rather than a separate cell population. 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 monocyct infiltration. Additionally, circulating rat monocytes are la-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 la determinants. Assuming that one-third of glomerular cells are mesangial (1) and that 1-2% of glomerular cells bear la determinants, one can estimate that up to 12% of mesangial cells are a distinct subpopulation capable of phagocytosis, la determinant expression, 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 experiments utilizing bone marrow transplants indicate that the la-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 apparent 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 questioned (12), based on failure of the contractile mesangial cell to phagocytose in vitro, the difficulties in distinguishing pinocytosis in vivo studies, and the possible contribution of infiltrating blood monocytes to glomerular phagocytosis (7). Our approach has shown that there is at least one type of phagocytic mesangial cell type, the la-positive cell, but that one occurs sufficiently infrequently so as to render detection by other techniques difficult. Whether the la-negative blood monocytes that infiltrate the rat glomerulus under pathologic conditions (7) acquire la determinants in the renal mesangium is now under study.

Endogenous renal la 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 la-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 la-negative, as defined by the two monoclonal antibodies employed in this study which recognize the la 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 la antigens (15)) on glomerular 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 polyvalent antisera (16), and between heterologous antisera and alloantisera (17) have been noted. Whether the apparent difference in the distribution of I-region antigens between man and rat represents species differences, antisera differences, or undefined cross-reactive specificities uncovered in polyvalent antisera requires further clarification. It is possible that there is differential expression of I-subregion determinants among glomerular cells and that antisera recognizing la determinants other than those studied in this report may stain rat endothelium positively, as is the case in humans. Nonetheless, the la-positive phagocytic glomerular cell described herein is a mesangial cell and not a component of capillary endothelium.

It has been clearly established that marrow-derived mononuclear phagocytes, whether circulating cells or resident macrophages, 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 la antigens necessary for the induction of host immunity against transplanted kidneys.

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