Isolation and Characterization of Inflammatory Leukocytes From Glomeruli in an In Situ *Model of Glomerulonephritis in the Rat*

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Inflammatory cell populations in glomerulonephritis (GN) are not well characterized. A method is reported for isolating leukocytes from glomeruli. GN was induced in rats by perfusing left kidneys (LKs) with cationized human IgG followed by intravenous rat anti-human IgG serum. Acute GN developed in LKs with proteinuria, deposition of human and rat IgG and C3, leukocyte infiltration, and capillary wall electron-dense deposits. Glomeruli (GL) isolated at 24 hours were digested with collagenase, trypsin, and DNase, and the resulting cells were as follows (mean ± SEM): LK, 354 ± 25/GL; RK, 214 ± 32/GL. Cells were labeled with monoclonal antibody MRCOX1 (anti-rat leukocyte common [LC] antigen) followed by FITC $F(ab')_2$ rabbit anti-mouse Ig: LK, 170 ± 11 leukocytes/GL; RK, 8 ± 2 leukocytes/GL ($P < 0.001$). Isolated cells were sorted by flow cytometry to 98% pure LC^+ cells with >80% viability (Giemsa staining: 86% mononuclear cells, 14% neutrophils); the ultrastructure was that of maturing macrophages and neutrophils. This method quantitates leukocyte infiltration and provides leukocytes from nephritic glomeruli suitable for *in vitro* studies. (Am J Pathol 1987, 126:126-136)

BLOOD LEUKOCYTES infiltrate the glomerulus in acute proliferative glomerulonephritis (GN). Inflammatory cell activation in GN is poorly understood largely because it has proved difficult to isolate well-characterized populations of inflammatory cells from glomeruli, as has been achieved at other inflammatory sites. Monocytes have been isolated from nephritic glomerular explants[1,2] in studies which have contributed to the recognition of this cell type in proliferative forms of GN. The limitations of this technique are that only small numbers of glomeruli can be individually plated out, usually less than 50% of plated glomeruli attach to the culture flasks, and attachment time is variable and normally commences only 2-3 days after plating. This means that very small numbers of inflammatory cells are obtained after several days under *in vitro* conditions. To learn more about the role of leukocytes and their state of activation *in vivo* requires methods for extracting adequate numbers of cells, independent of cell features that may change with maturation, which preserves cell structure and functional capacity.

Our aim, therefore, was to develop a method of isolating pure populations of inflammatory cells from nephritic glomeruli. Phagocytes have previously been extracted from normal rat glomeruli by enzyme digestion of isolated glomeruli,[3] and this technique has aided the identification of a resident glomerular macrophage bearing Ia antigens.[4,5] To study inflammatory cells in GN, we have separated leukocytes from a mixed cell population of glomerular and inflammatory cells obtained by similar enzymatic digestion of nephritic glomeruli, using a fluorescence-activated cell sorter (EPICS). Glomerular inflammation was induced by a passive *in situ* immune complex injury.[6] In this paper we report the sequential ultrastructural appearance of the glomerular injury and the results of

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leukocyte isolation by EPICS. Quantitation and isolation of leukocytes has been achieved, and we are currently using these methods to study the state of activation of inflammatory cells in GN. These studies should increase understanding of the cellular events following glomerular injury and allow elucidation of the changes in leukocyte metabolism in inflamed glomeruli.

Materials and Methods

Animals

Inbred Lewis rats were used. Operative procedures were carried out with halothane (May & Baker, Dagenham, UK) anesthesia.

Preparation of Cationized IgG

Human IgG (HuIgG) was prepared from plasma by sodium sulphate precipitation followed by ion exchange chromatography on DEAE-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). After concentration by ultrafiltration it was dialyzed against phosphate-buffered saline (PBS). Purity was checked by immunoelectrophoresis. HuIgG was cationized by a modification of the method of Danon et al[7] as described by Oite et al[6] Briefly, 1 ml of 3-dimethylaminopropylamine (BDH Chemicals, Poole, UK) was added to 20 ml distilled water and the pH adjusted to 6.5 with 1M HCl. HuIgG, 0.8 g, and 1.5 g 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (Sigma Chemical Co., Poole, UK) were added with stirring. The pH was maintained at 6.5 for 8 hours in an Autoburette ABU1c (Radiometer, Copenhagen, Denmark) with the use of 0.2 M HCl. The reaction mixture was left overnight, dialyzed against six changes of cold PBS, pH 7.4, filtered through a 0.45-μ filter (Flow Laboratories, Rickmansworth, UK) and stored at −70 C until required. The degree of cationization was estimated by isoelectric focusing on thin-layer polyacrylamide gel (Ampholine Pagplate, pH 3.5-9.5, LKB, Croyden, UK). Standards of pI 3.5-9.3 (Pharmacia were run in lanes adjacent to the IgG. Cationized HuIgG used in these experiments had a pI > 9.3.

Induction of Glomerulonephritis

Lewis rats were repeatedly immunized with native HuIgG in complete Freund's adjuvant. Antiserum was complement-inactivated at 56 C for 45 minutes and then stored at −70 C until used. All antiserum employed in the present study was from a single pool. Antigen binding capacity was compared in reverse radial immunodiffusion with that of standard rabbit anti-human serum and was >180 μg HuIgG/ml antiserum.

The left kidneys of rats, weighing 200-250 g, were perfused *in vivo* with cationized HuIgG by the method of Hoyer et al.[8] The kidney was first flushed with 1 ml of normal saline, then 200 μg cationized HuIgG in 0.5 ml normal saline was perfused through the kidney over 30 seconds. After 2.5 minutes the kidney was again flushed with 1 ml normal saline and the holes in the aorta and renal vein repaired with 7/0 silk. Total ischemia time was less than 10 minutes. Immediately after closure of the abdominal incision 1.5 ml rat anti-HuIgG serum was given intravenously via the dorsal penile vein. A group of control rats were perfused as above and received normal rat serum intravenously. The results for these rats have been reported in detail elsewhere.[9] Urine samples were collected from rats in metabolic cages, and urine protein was estimated by the sulfosalicylic acid method.[10] Renal biopsies were performed at intervals from 30 minutes to 4 weeks after induction of GN.

Microscopy

Tissue for light microscopy and immunoperoxidase staining was fixed in neutral buffered formal saline and embedded in paraffin. Sections were stained with hematoxylin and eosin, and periodic acid-Schiff (PAS). Immunoperoxidase staining for human and rat IgG and for rat C3 were carried out on paraffin sections according to the method of Sinclair et al[11] with prior digestion for 45 minutes in 0.05% protease (Type VII, Sigma). Primary antibodies were rabbit anti-human IgG (Dako Ltd., High Wycombe, UK), rabbit anti-rat IgG (Sigma), and rabbit anti-rat C3 (Nordic, Maidenhead, UK). The secondary antibody was peroxidase-labeled swine anti-rabbit IgG (Dako). For electron microscopy 2-mm blocks of cortex were fixed in phosphate-buffered 4% glutaraldehyde (pH 7.3) at 4 C, postfixed in 2% phosphate-buffered osmium tetroxide, and stained with uranyl acetate and lead citrate. Glomeruli were selected from 0.5-μ sections, and ultrathin sections were examined on a Philips EM300 electron microscope.

Isolation of Glomerular Cells

Kidneys from normal rats and from glomerulonephritic rats at 24 hours were perfused *in vivo* with 50 ml normal saline at room temperature and removed.

Cortical strips were placed in Tris-HCl buffer, pH 7.4, at 4 C. We pooled and sieved pairs of left and right kidneys from normal rats or two left and two right kidneys from nephritic rats to obtain isolated glomeruli.[4] Glomerular purity was >90%. Glomeruli were counted by light microscopy in three 10-μl aliquots from glomerular preparations. Whole glomeruli were enzymatically digested by a method previously described by Camazine et al[3] with the following modifications: For the first enzyme step glomeruli were incubated for 20 minutes in 10 ml Hanks' buffered salt solution (HBBS) containing 0.5 mg/ml trypsin (Type III, Sigma) 1 mg/ml collagenase (Type 1, Sigma), and 0.1 mg/ml deoxyribonuclease (DNase type III, Sigma). After washing in HBSS without calcium and magnesium, the partially digested glomeruli were incubated in 10 ml 2 mM EDTA, disodium salt (Sigma), in the same buffer for 30 minutes. The supernatant containing isolated cells was removed and kept on ice. For the second enzyme step glomeruli were incubated in 10 ml collagenase, 1 mg/ml, for 20 minutes. For final dissociation the cell preparation was passed three times through a 23-gauge needle, and the supernatant containing free cells was withdrawn after settling on ice for 5 minutes. This was pooled with the supernatant from the EDTA incubation. Cells were washed twice in HBBS with 5% fetal calf serum (HBSS–FCS). Viability of isolated cells was assessed as previously described[12] with the use of ethidium bromide in place of propidium iodide, and Cytospin preparations were examined by Giemsa staining.

The following primary monoclonal antibodies were used for labeling cell suspensions: MRC OX1 (ascitic fluid, Serotec, Blackthorn, UK), which recognizes rat leukocyte common antigen (LC),[13] and MRC OX3 and OX4 (culture supernatants, Sera-lab, Crawley Down, Sussex, UK), which recognize rat Ia determinants.[14] Cells at a concentration of $5-10 \times 10^6$ /ml were incubated with primary antibody in HBSS–FCS at 4 C for 45 minutes. Primary antibodies were used at 10 μl/ml for OX1 and 100 μl/ml for OX3 and OX4. As control ascitic fluid from mice bearing the IgG1-secreting monoclonal MOPC21 (gift from A. Boylston), 10 μl/ml was substituted for the primary antibody. After three washes in HBSS–FCS, cells were resuspended at the same concentration and incubated with fluorescein-conjugated rabbit $F(ab')_2$ anti-mouse Ig (Dako) with 5% normal rat serum at 100 μl/ml for 30 minutes at 4 C. The cells were washed twice in HBSS–FCS and finally suspended at approximately 1×10^6 cells/ml in protein-free HBSS for flow cytometry and fluorescence microscopy.

Fluorescence-Activated Cell Sorting

Analysis and sorting of antibody-labeled cells was carried out on an EPICS V (Coulter Electronics, Luton, UK). The 488-nm line of an argon laser run at an output power of 200 mW was used for excitation of both scatter and fluorescence signals. Fluorescence emission was measured distal to a 515-nm long-pass filter. Dot plots of log-integrated green fluorescence (LIGRN) versus forward angle light scatter (FALS) were generated; and from these, with suitable gating out of small and large particles, the percentage of fluorescent labeled cells could be determined and gates set for sorting LC^+ cells. Samples of final cell suspensions and of EPICS-sorted LC^+ and LC^- cells were examined by immunofluorescence microscopy (Leitz Laborlux 12), by light microscopy in Giemsa-stained Cytospin preparations, and by electron microscopy. For electron microscopy cells were processed as for tissues. Grids containing at least 50 cells were examined at ×800 magnification, and photomicrographs were taken of representative cells types at higher magnifications.

Statistics

Results are expressed as mean ± SEM. Comparisons of example means are by the two-tailed Student t test.

Results

Glomerulonephritis developed in the left kidneys (nephritic kidneys) of all rats unilaterally perfused with cat HuIgG, followed by intravenously administered rat anti-HuIgG. Proteinuria developed within 12 hours (39 ± 4 mg/12 hr, n = 6; control rats, 1.8± 0.4 mg/12 hr, n = 6; $P<0.05$), was maximal at 18 hours (43 ± 6 mg/12 hr; controls, 2.1 ± 0.5 mg/12 hr, n = 6), and resolved by Day 7. Macroscopically, the cortices of nephritic kidneys appeared edematous. In the right kidneys of these rats there was no evidence of GN. In the control group treated with normal rat serum intravenously after unilateral cationized HuIgG, there was no proteinuria, no morphologic change, and no glomerular deposition of rat Ig or C3.[9] The results of immunoperoxidase staining, light microscopy, and electron microscopy below are therefore confined to the left kidneys of nephritic rats.

Light Microscopy

Glomeruli showed a diffuse increase in cellularity which was apparent by 1 hour and maximal at 12 hours (Figure 1). Neutrophils and mononuclear cells

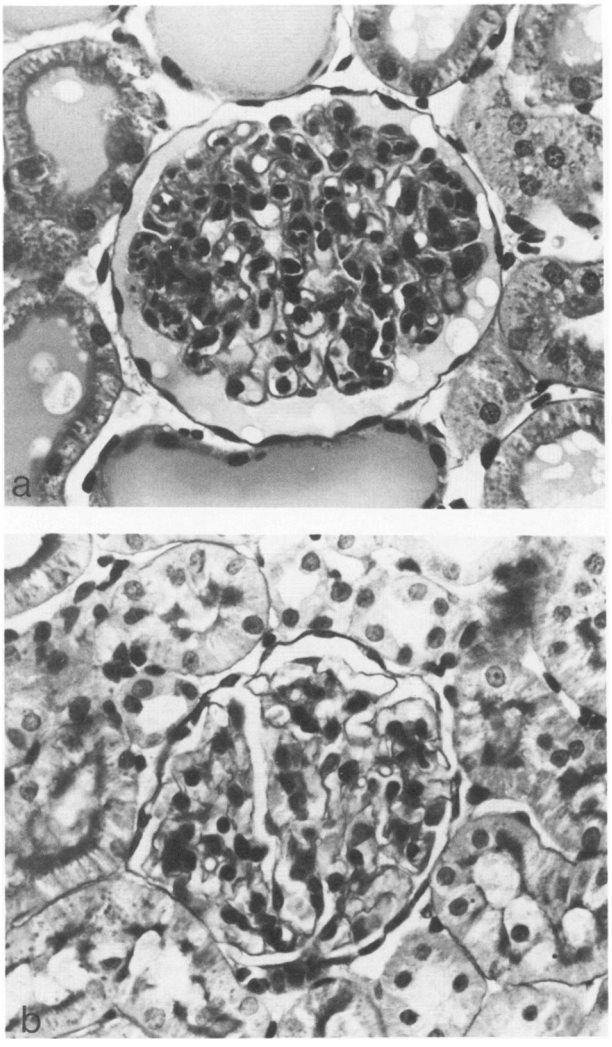


Figure 1a — Hypercellular glomerulus from the left kidney of a nephritic rat at 12 hours. There is proteinaceous fluid in Bowman's space and tubules. **b** — Normal glomerulus from the left kidney of a control rat. (Both PAS, ×500)

were present within capillary lumens. Proteinaceous casts were seen in small numbers of tubules at 3 hours, and by 6 hours the majority of tubules were distended by casts. The increased glomerular cellularity began to resolve by Day 2, although some focal increase in intraluminal cells persisted at Day 7. At 4 weeks the glomerular changes had resolved, with no evidence of glomerular scarring, although there was focal tubular atrophy and focal infitration of the interstitium by mononuclear cells.

Immunoperoxidase

HuIgG (Figure 2) and rat IgG were present on glomerular capillary walls from 30 minutes to 4 weeks; at 30 minutes the distribution appeared continuous, but by 24 hours was finely granular and by 4 weeks was coursely granular. C3 staining was first seen at 6 hours and persisted at 4 weeks. (As previously stated, no Ig or C3 was seen in glomeruli from right kidneys of nephritic rats.)

Electron Microscopy

At 30 minutes (Figure 3) infiltrating leukocytes, predominantly polymorphs, were present in small numbers in glomerular capillary lumens, and rarely under the endothelium. There were small platelet aggregates with intact platelet granules. A fine line of electron-dense deposit was seen on the endothelial side of the lamina rara interna and along the epithelial side of the capillary wall bordering the epithelial cell foot processes. There were small areas of foot process obliteration. At 1 hour cell infiltration was more pronounced and many lumens were nearly occluded by neutrophils, with a few monocytes and occasional eosinophils. There were small areas of endothelial loss or partial detachment and more generalized loss of endothelial fenestrations and swelling. Some neutrophils were in contact with basement membrane. Platelets with pseudopods were present. Epithelial foot process obliteration had increased. The mesangium was normal. At 2 and 3 hours all these changes were more severe (Figure 4) and discrete subepithelial deposits were first seen, projecting between and displacing foot processes (Figure 5). Small mesangial deposits were seen between 2 and 24 hours. These changes were further advanced at 6 hours, when, in addition, many neutrophils were degenerating and neutrophils and monocytes were infiltrating the mesangium. Platelet aggregates had increased, and there

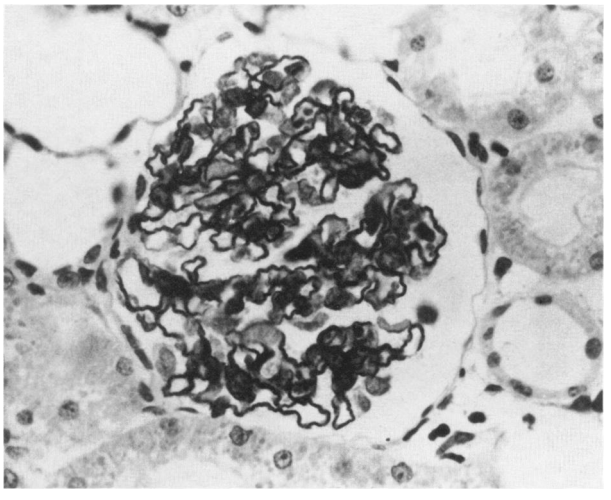


Figure 2 — Immunoperoxidase staining of HuIgG on glomerular capillary walls in the left kidney of a nephritic rat at 12 hours. (Peroxidase counterstained with hematoxylin, ×500)

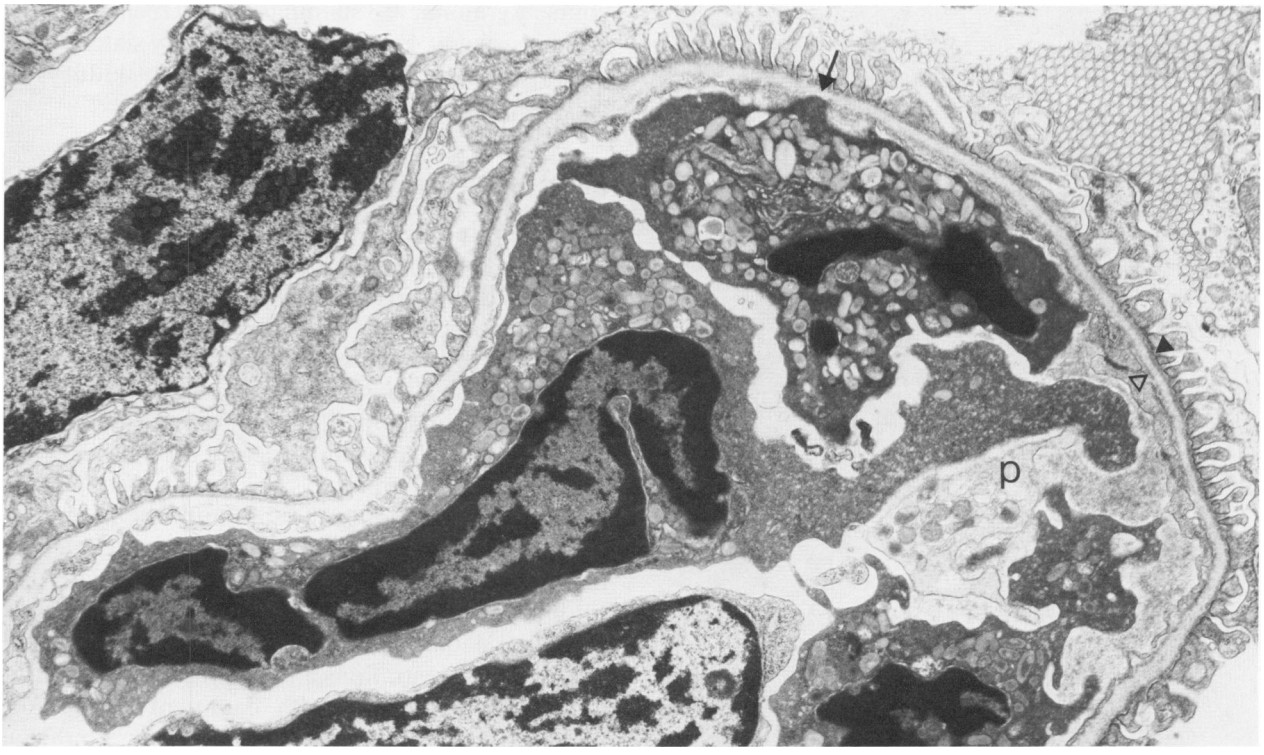


Figure 3 — Neutrophil accumulation in a glomerular capillary loop 30 minutes after induction of GN. A neutrophil process is in contact with the basement membrane *(solid arrow)*, and there are fine lines of electron-dense material beneath endothelium and epithelial cell foot processes *(arrowheads)*. *p*, platelet. (Uranyl acetate and lead citrate, ×10,800)

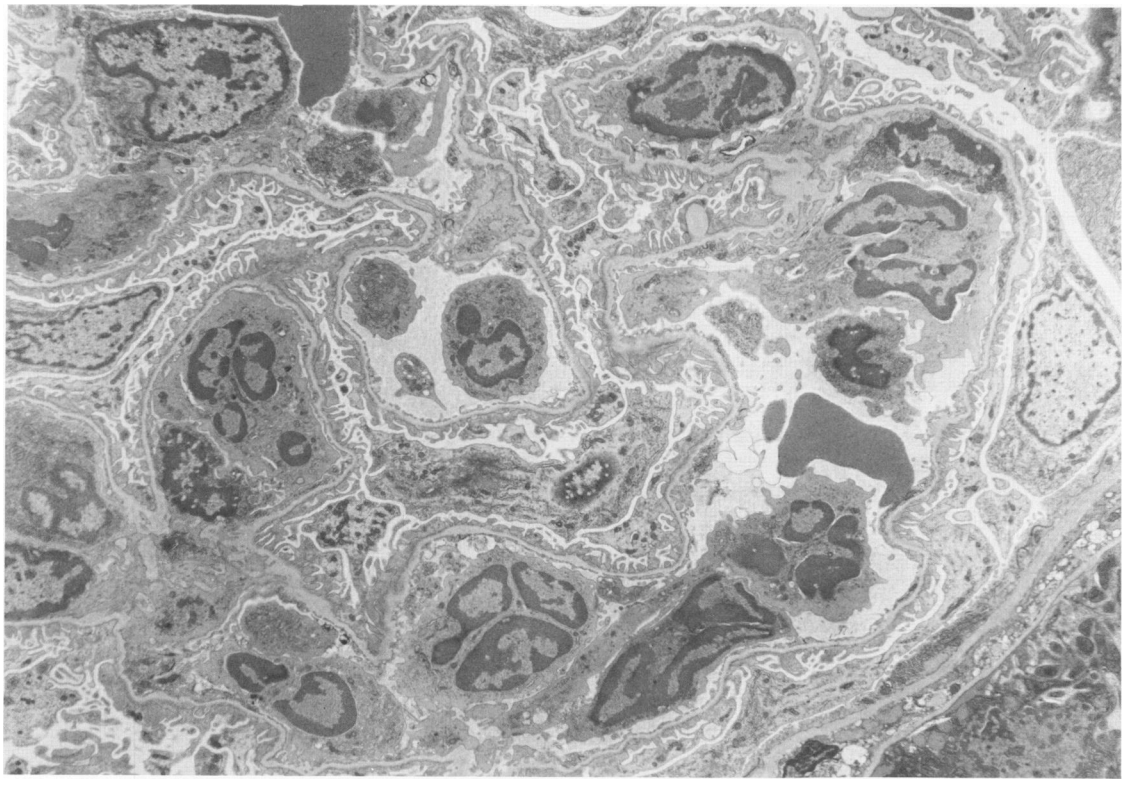


Figure 4 — Low-power electron micrograph at 3 hours demonstrating glomerular hypercellularity due to infiltrating leukocytes, predominantly neutrophils. (Uranyl acetate and lead citrate, ×3700)

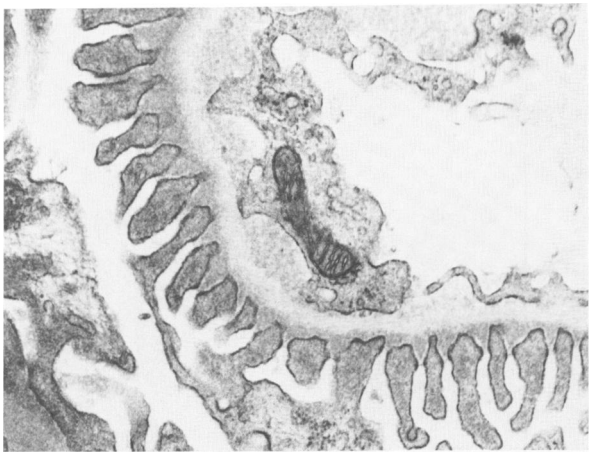


Figure 5 — Discrete subepithelial deposits projecting between epithelial cell foot processes at 3 hours. There is still subendothelial electron-dense material. (Uranyl acetate and lead citrate, ×18,900)

was fibrin in some loops. At 12–24 hours cellularity was greater with increased numbers of monocytes, and more mesangial infiltration (Figure 6). Some monocytes in the mesangium contained large electron-dense membrane-bound vacuoles. Subepithelial deposits were large, and all changes were more advanced, except for subendothelial deposits, which had disappeared. By 4 days leukocyte infiltration was resolving, but subepithelial deposits were larger. By 7 days (Figure 7) deposits were developing lucent areas, and few inflammatory cells were seen. Epithelial foot process obliteration remained extensive.

Isolation of Cells From Glomeruli

Table 1 shows the results of enzymatic digestion of glomeruli and antibody labeling of resulting cell suspensions of normal and nephritic rat kidneys at 24 hours after induction of GN. From normal kidneys $21.6 \pm 1.5 \times 10^3$ glomeruli per rat were isolated; the number of cells isolated by enzymatic digestion was 158 ± 17 per glomerulus. Viability was $79\% \pm 2\%$. There were 18 ± 2.7 LC^+ cells per glomerulus. In nephritic rats significantly more glomeruli and cells were obtained from nephritic kidneys, compared with right unaffected kidneys; the increase in glomerular numbers was presumably due to the edematous cortex sieving more easily. There were significant increases in the total number of cells (nephritic kidneys,

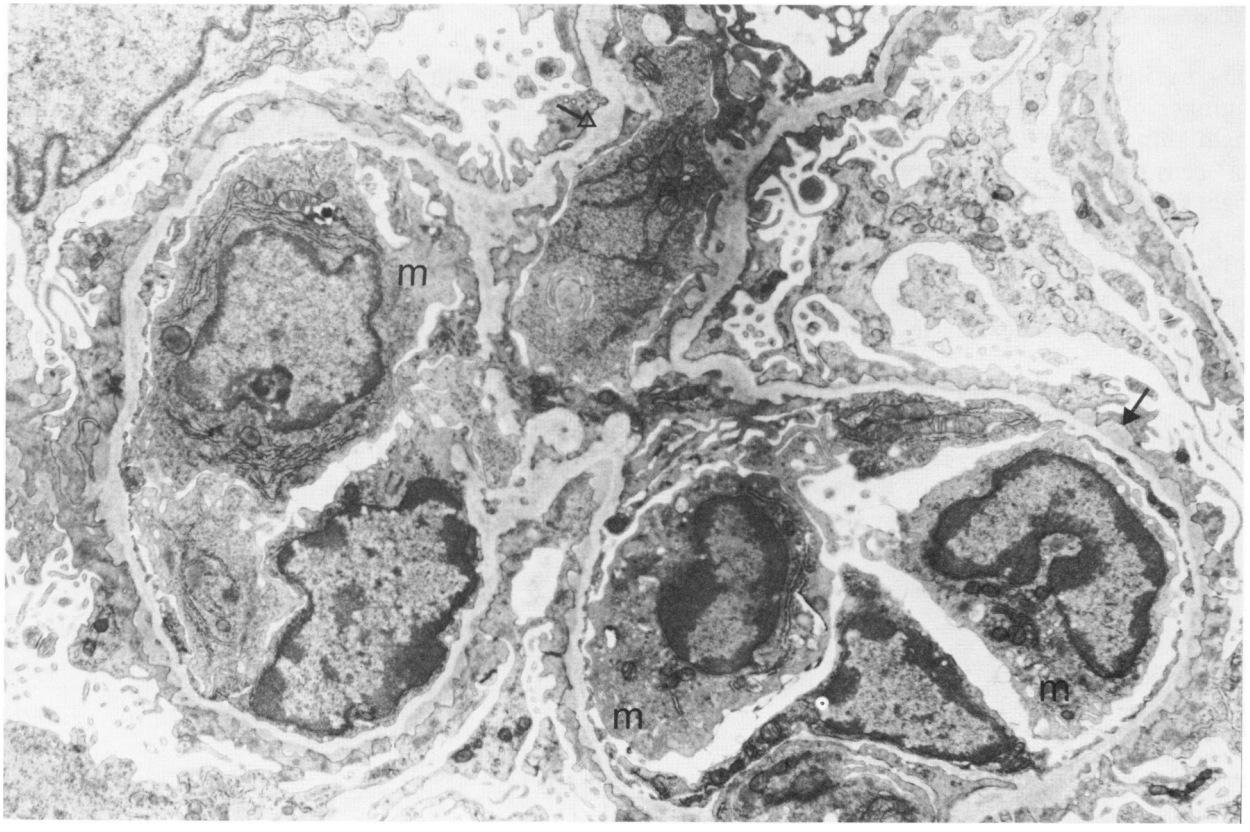


Figure 6 — Glomerular capillary loops and mesangium at 24 hours; the leukocyte infiltrate is now largely monocytic *(m)*. Subepithelial deposits have enlarged *(solid arrow)*; and there are tiny mesangial deposits *(open arrow)*. Foot process obliteration is extensive. (Uranyl acetate and lead citrate, ×6600)

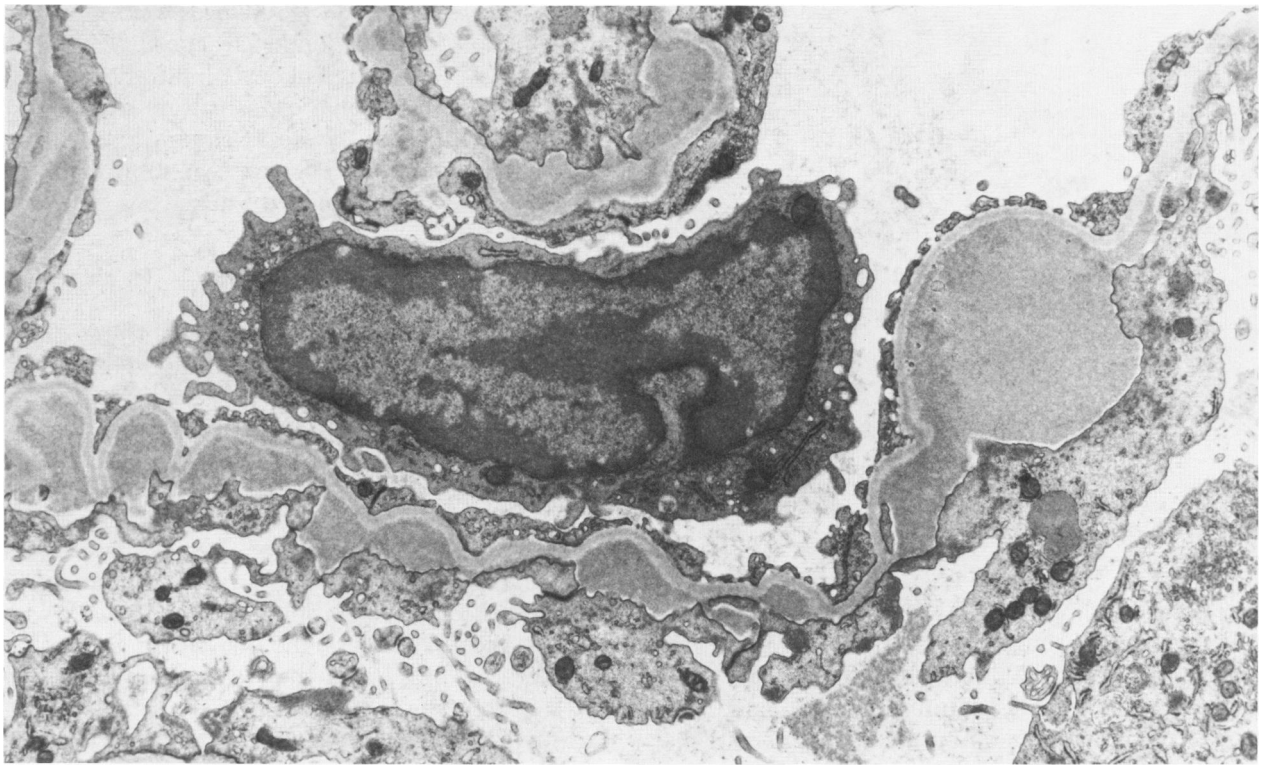


Figure 7—Part of a capillary loop at 7 days showing large subepithelial deposits, some of which have lucent areas. A single monocyte is present in the lumen. Foot process obliteration is still apparent. (Uranyl acetate and lead citrate ×9800)

354 ± 25: right kidneys, 214 ± 32; $P < 0.05$), the number of LC^+ cells nephritic kidneys, 170 ± 11; right kidneys, 8 ± 2; $P < 0.001$), and the number of Ia^+ cells per glomerulus (nephritic kidneys, 16 ± 3; right kidneys, 4 ± 1.5; $P < 0.05$). Viability of left kidney cell preparations was 79% ± 1% and of right, 73% ± 6%. In Giemsa-stained preparations from left kidneys, 14% ± 2% of cells were neutrophils and 33% ± 1% monocytes/macrophages, many with foamy cytoplasm.

Cell Sorting

Representative dot plots of FALS versus LIGRN for cells from a nephritic kidney stained with LC or with irrelevant antibody MOPC21 are shown in Figure 8. The FALS gives an estimate of cell size, and the lower panel (cells stained with OX1) shows that a population of large cells is staining positively with LC in this preparation. For sorting, gates were set around the population of LC^+ cells in order to minimize contamination by LC^- cells. At the same time LC^- cells were collected with the use of gates set with the same FALS parameters as for LC^+ cells and appropriate LIGRN gates. After sorting cells were examined by fluorescence microscopy; 98% ± 0.3% of cells collected in the positive sort were positive by microscopy and 3% ± 1.2% of those in the negative sort. In Giemsa-stained preparations of EPICS LC^+ cells, 14% ± 2% were neutrophils and the remainder were

Table 1—Cell Isolation From Enzymatically Digested Glomeruli (GL)

Kidney preparations	GL/kidney × 10^3	Cells/GL	Viability (%)	Immunofluorescent labeling	
				LC^+/GL*	Ia^+/GL†
Normal rats (n = 7)	21.6 ± 1.5	158 ±17	79 ± 2	18 ± 2.7	Not done
In situ GN rats Right nonnephritic (n = 3)	17.7 ± 2.6	214 ± 32	73 ± 6	8 ± 2	4 ± 1.5
Left nephritic (n = 3)	29 ± 2.5‡	354 ± 25‡	79 ± 1	170 ± 11§	16 ± 3‡

*MRC OX1-positive cells.
†MRC OX3/4-positive cells.
‡$P < 0.05$, compared with right nonnephritic kidneys
§$P < 0.001$, compared with right nonnephritic kidneys

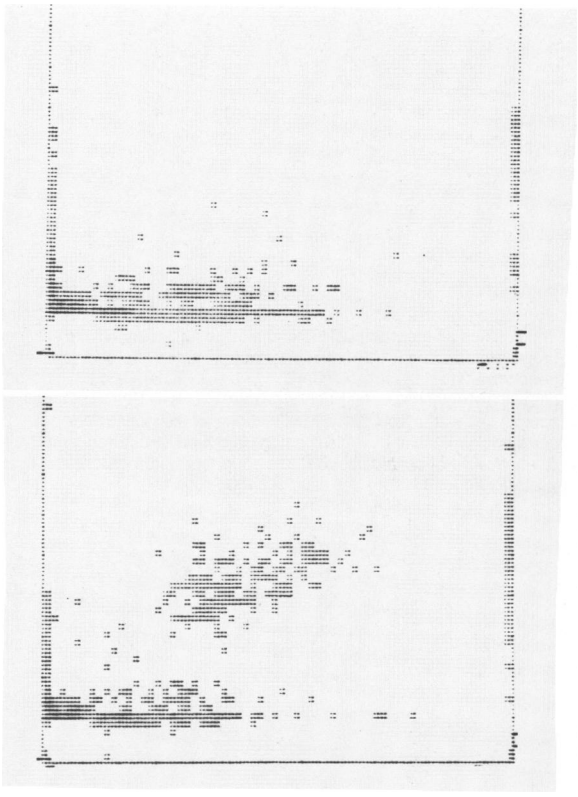


Figure 8 — EPICS dot plots of cells isolated from a nephritic kidney 24 hours after induction of GN. The preparation in the upper panel was stained with irrelevant antibody MOPC21 and that in the lower with OX1. There is a population of large cells showing positive fluorescent staining for LC antigen in the lower panel. x axis, FALS; y axis, LIGRN.

mononuclear cells, 92% of which had the appearance of monocytes/macrophages, as in the original left kidney preparations.

Electron Microscopy of Epics LC$^+$ Cells and Original Isolated Cell Preparations

EPICS LC$^+$ Cells: Infiltrating Leukocytes

In these samples a distinct cell type, rarely seen in isolated glomerular cell populations from normal kidneys, right nonnephritic kidneys or EPICS LC$^-$ samples, accounted for >90% of cells. These cells (Figure 9a) were large and rounded in shape, with discrete cytoplasmic projections around the surface. The nucleus was indented, often eccentric, with a large nucleolus and little heterochromatin. Membrane-bound structures containing electron-dense material and resembling phagolysomes were present in the cytoplasm. These are features of maturing macrophages. The remaining cells were a) smaller cells with central nuclei containing dense heterochromatin, no nucleoli, and no electron-dense membrane structures, resembling blood monocytes, and b) typical hypersegmented neutrophils.

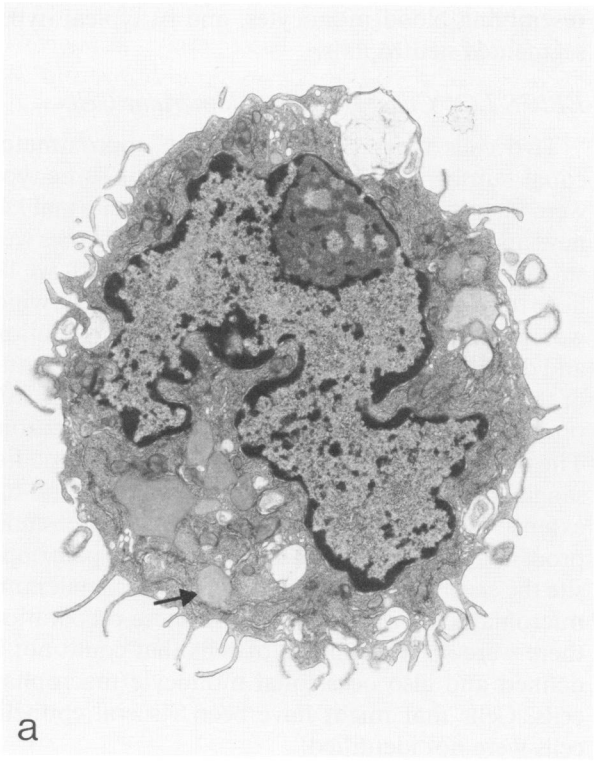
EPICS LC$^-$ Cells: Intrinsic Glomerular Cells

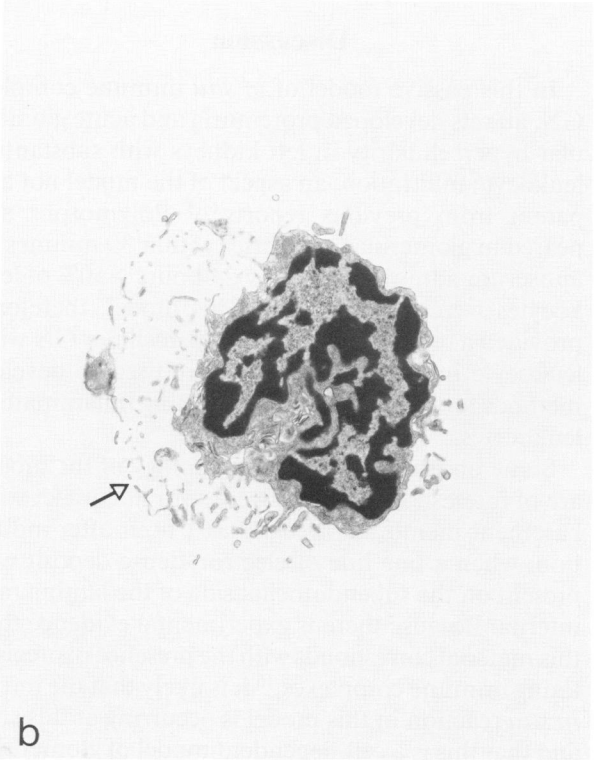
Two cells types predominated in approximately equal numbers in these samples, and the same types were identified in cell preparations from normal kidneys and right nonnephritic kidneys. Both types were smaller than EPICS LC$^+$ cells. In one, which we believe to be endothelial (Figure 9b), the nucleus, which was eccentric, had multiple irregular indentations and dense heterochromatin; the characteristic feature of these cells was a lacy network of thin cytoplasmic projections, many of which showed fenestrations. The other type, which appears to be an intrinsic mesangial cell (Figure 9c), lacked fenestrations and had extensive cytoplasmic protrusions and blunt-ended processes concentrated at the cytoplasmic pole opposite the eccentric nucleus. Discrete subplasmalemmal microfilaments were not seen. In these preparations there were small numbers of cells that could not be defined and also occasional monocyte/macrophage cells. Cells that might have been visceral epithelial cells were not identified.

Discussion

In this passive model of *in situ* immune complex GN, all rats developed proteinuria and acute glomerular hypercellularity in left kidneys with substantial leukocyte infiltration, an aspect of the model not apparent from previous reports.[6,15] Polymorphs appeared in glomerular capillaries within 30 minutes of antiserum administration; by 24 hours >50% of leukocytes were mononuclear. This model, therefore, provided a reproducible acute hypercellular GN with leukocyte infiltration that we have used to develop methods for quantitating and isolating inflammatory leukocytes.

Some observations on the pathology of the model are of interest. Leukocytes were seen in contact with basement membrane as early as 1 hour after induction, when a fine line of electron-dense deposit was present on the subendothelial side of the lamina rara interna. Because there is experimental evidence that this material corresponds with the presence of precipitating immune complexes,[16] it is likely that the initial *in situ* reaction in this model is occurring at this site, and that this is a cell-dependent model of glomerular injury.[17] There was a relationship between the onset and decline of proteinuria and leukocyte infiltration, but not with subepithelial deposits, which enlarged and persisted after proteinuria became undetectable. Other ultrastructural features were mesangial de-





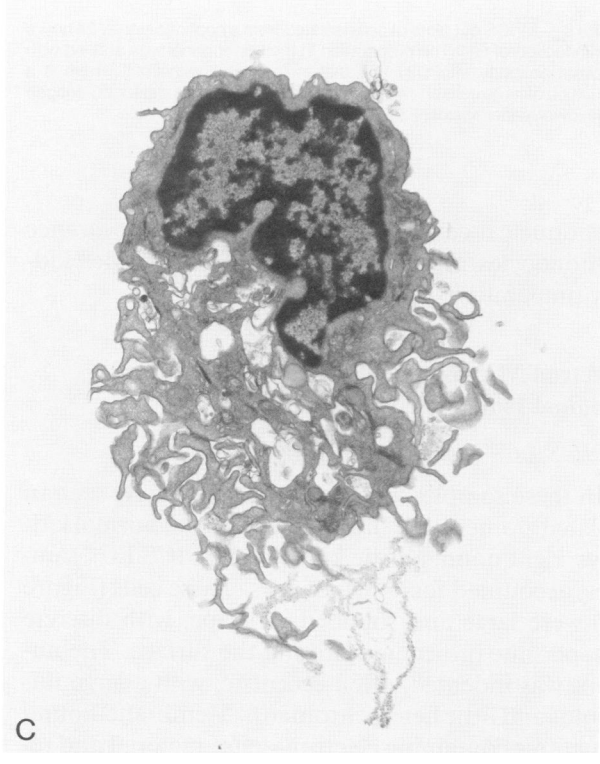


Figure 9—Electron micrographs comparing the three main cell types isolated by glomerular digestion and EPICS. **a**—EPICS LC^+ cell. The cell has an idented nucleus and a large nucleolus. There are membrane-bound structures containing electron-dense material *(arrow)* and peripheral cytoplasmic projections. **b**—EPICS LC^- cell. Endothelial cell with cytoplasmic fenestrations *(arrow)*. **c**—EPICS LC^- cell. Mesangial cell with cytoplasmic ramifications at the nonnuclear pole. The cell cytoplasm lacks multiple membrane-bound structures, and the nucleus, devoid of nucleolus, contains abundant heterochromatin. Uranyl acetate and lead citrate, (all cells, ×8400)

posits, infiltration of mesangium by leukocytes, and endothelial injury with platelets and fibrin.

The method of enzymatic digestion reproducibly produced cells from normal rat glomeruli with viability and cell numbers per kidney similar to those previously reported.[3] With the use of the monoclonal antibody MRC OX1 against the rat leukocyte common antigen, which is resistant to the enzymatic digestion used here,[5] small numbers of leukocytes were identified in these preparations from normal kidneys, as in the studies of Schreiner et al.[5] In nephritic rats the hypercellularity seen morphologically in left kidneys could be quantitated. The total cell isolates from left kidneys were significantly increased, compared with right kidneys, and MRC OX1 staining showed that this cell increase in glomeruli from nephritic kidneys was due to leukocytes with a mean of 170 cells per glomerulus extracted. This figure is similar to that obtained by Holdsworth et al[2] from glomerular explants of severe crescentic GN in sheep and far exceeds the numbers obtained from explants in crescentic GN in humans.[1] Because the present model was histologically only a moderately hypercellular GN, without crescent formation, we believe that the method extracted the majority of the leukocyte population, although mononuclear leukocytes were probably enriched, because polymorphs appeared to be more numerous in histologic sections than in isolated cell preparations.

Electron microscopy of EPICS LC^+ cells isolated at 24 hours identified a leukocyte population composed predominantly of maturing macrophages,[18,19] similar in appearance to those seen in electron micrographs of whole glomeruli, smaller numbers of less differentiated cells, probably newly arrived blood monocytes, and neutrophils. In tissue sections the proportion of neutrophils appeared higher than in the EPICS LC^+ cells, which suggests that neutrophils were lost during digestion, either because they are more susceptible to enzymic digestion or because *in vivo* they are already degenerating cells, as was seen in tissue sections from 6 hours onward.

The ultrastructural appearance of the two major cell types in EPICS LC^- cells and cells from normal kidneys was essentially as observed by Camazine et al[3] in glomerular digests examined by electron microscopy immediately after dissociation. The presence of fenestrations in their study and ours appears to characterize the endothelial cells. The other cell type, which we believe to be mesangial, was largely differentiated from endothelial cells because it lacked fenestrations and had peripheral blunt processes that appeared similar to mesangial cells in tissue sections. Occasional cells with the appearance of monocytes/macrophages were seen in normal kidneys, which is in accord with our monoclonal antibody finding of 11% LC^+ cells in digests from normal kidneys, and with the presence of a population of resident mononuclear phagocytes in the rat glomerulus identified by Schreiner et al.[4] Camazine et al[3] also identified phagocytic cells in glomerular digests. These cells were heavily labeled after *in vivo* ferritin administration, adhered to glass, and expressed Fc and C3 receptors after 24 hours in culture. They suggested that these cells were a population of highly phagocytic mesangial cells. However, it is more likely that these cells were a mixture of resident macrophages and infiltrating monocytes that accumulated in the glomeruli after intravenous ferritin administration.[20-22] Epithelial cells were not identified. It has been noted previously that these cells were rarer in digested preparations, often appeared disrupted in isolated glomeruli before cellular dissociation,[3] and are removed by single-step enzymatic digestion.[23] It is probable that selective loss of epithelial cells partly accounts for the disparity between the mean number of cells extracted per glomerulus, compared with the morphometrically calculated number of cells in the normal adult rat glomerulus.[24]

In this study we reported the detailed ultrastructure of a model of passive *in situ* GN. The advantages of the model for our studies were the nonnephritic right kidneys were present to act as controls in leukocyte isolations and that by 24 hours, the majority of infiltrating leukocytes were mononuclear phagocytes. Using this model, we have demonstrated that digestion to single-cell suspensions, monoclonal antibody staining, and cell sorting allow quantitation and isolation of infiltrating leukocytes. With these methods, pure inflammatory-cell populations from nephritic glomeruli can be obtained in sufficient numbers for *in vitro* studies.

References

1. Atkins RC, Glasgow EF, Holdsworth SR, Thomson NM, Hancock WW: Tissue culture of isolated glomeruli from patients with glomerulonephritis. Kidney Int 1978, 17:515-527
2. Holdsworth SR, Thomson, NM, Glasgow EF, Dowling JP, Atkins RC: Tissue culture of isolated glomeruli in experimental crescentic glomerulonephritis. J Exp Med 1978, 147:98-109
3. Camazine SM, Ryan GB, Unanue ER, Karnovsky MJ: Isolation of phagocytic cells from the rat renal glomerulus. Lab Invest 1976, 35:315-326
4. Schreiner GF, Kiely J-M, Cotran RS, Unanue ER: Characterization of resident glomerular cells in the rat expressing Ia determinants and manifesting genetically restricted interactions with lymphocytes. J Clin Invest 1981, 68:920-931
5. Schreiner GF, Unanue ER: Origin of the rat mesangial

phagocyte and its expression of the leukocyte common antigen. Lab Invest 1984, 51:515–523
6. Oite T, Batsford SR, Mihatsch MJ, Takamiya H, Vogt A: Quantitative studies of *in situ* immune complex glomerulonephritis in the rat induced by planted cationized antigen. J Exp Med 1982, 155:460–474
7. Danon D, Goldstein L, Marikovsky Y, Skutelsky E: Use of cationized ferritin as a label of negative charges on cell surfaces. J Ultrastruct Res 1972, 38:500–510
8. Hoyer JR, Mauer SM, Michael AF: Unilateral renal disease in the rat: 1. Clinical, morphologic, and glomerular mesangial functional features of the experimental model produced by renal perfusion with aminonucleoside. J Lab Clin Med 1975, 85:756–768
9. Cook HT, Cattell V, Smith J, Salmon JA, Moncada S: Effect of a thromoxane synthetase inhibitor on eicosanoid synthesis and glomerular injury during acute unilateral glomerulonephritis in the rat. Clin Nephrol Clin Nephrol 1986, 26:195–202
10. Baker FJ, Silverton RE, Luckcock ED: An introduction to medical laboratory technology. London, Butterworths, 1966
11. Sinclair RA, Burns J, Dunnill MS: Immunoperoxidase staining of formalin fixed paraffin embedded human renal biopsies with a comparison of the peroxidase-antiperoxidase (PAP) and indirect methods. J Clin Pathol 1981, 34:859–865
12. Jones KH, Senft JA: An improved method to determine cell viability by simultaneous staining with fluorescein diacetate-propidium iodide. J Histochem Cytochem 1985, 33:77–79
13. Sunderland CA, McMaster WR, Williams AF: Purification with monoclonal antibody of a predominant leukocyte-common antigen and glycoprotein from rat thymocytes. Eur J Immunol 1979, 9:155–159
14. McMaster WR, Williams AF: Identification of Ia glycoproteins in rat thymus and purification from rat spleen. Eur J Immunol 1979, 9:426–433
15. Vogt A, Schmidt HU, Takamiya H, Batsford S: "In situ" immune complex nephritis and basic proteins. Proc EDTA 1980, 17:613–620
16. Lawrence YC, Agodoa V, Gauthier J, Mannik M: Precipitating antigen-antibody systems are required for the formation of subepithelial electron-dense immune deposits in rat glomeruli. J Exp Med 1983, 158:1259–1271
17. Salant DJ, Adler S, Darby C, Capparell NJ, Groggel GC, Feintzeig ID, Rennke HG, Dittmer JE: Influence of antigen distribution on the mediation of immunological glomerular injury. Kidney Int 1985, 27:938–950
18. Cohn ZA, Hirsch JG, Fedorko ME: The in vitro differentiation of mononuclear phagocytes: IV. The ultrastructure of macrophage differentiation in the peritoneal cavity and in culture. J Exp Med 1966, 123:747–756
19. Adams DO: The structure of mononuclear phagocytes differentiating *in vivo*: II. The effect of Mycobacterium tuberculosis. Am J Pathol 1975, 80:101–113
20. Farquhar MF, Palade GE: Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis of filtration residues by a distinctive "third" cell. J Cell Biol 1962, 13:55–87
21. Cattell V, Gaskin de Urdaneta A, Arlidge S, Collar JE, Roberts A, Smith J: Uptake and clearance of ferritin by the mesangium: 1. Phagocytosis by mesangial cells and blood monocytes. Lab Invest 1982, 47:296–303
22. Laohapand T, Smith J, Cattell V: Blood leucocyte infiltration after intravenous injection of ferritin in the rat. Br J Exp Pathol 1985, 66:475–482
23. Striker GE, Striker LJ: Glomerular cell culture. Lab Invest 1985, 53:122–131
24. Olivetti G, Anserva P, Melissari M, Loud AV: Morphometry of the renal corpuscle during postnatal growth and compensatory hypertrophy. Kidney Int 1980, 17:438–454