A MONONUCLEAR CELL COMPONENT IN EXPERIMENTAL IMMUNOLOGICAL GLOMERULONEPHRITIS*

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Immunological damage to the glomerular capillary wall may result from an antigen-antibody interaction produced either by glomerular deposition of circulating immune complexes bearing no relationship to glomerular antigens or by fixation of antibodies with specificity for antigens of the glomerulus (reviewed in references 1 and 2). There has been extensive investigation of humoral and/or cellular mediators that may be responsible for the proteinuria and histological damage following these immunological reactions. So far, however, the only clearly defined immunological mediator is the complement (C)[1] system (3). C results in glomerular injury by producing a neutrophil infiltrate in a mechanism similar to that previously found in Arthus reactions (4). This was shown in the model of nephrotoxic serum nephritis (NTN) in which heterologous antibodies to glomerular basement membrane antigens injected into experimental animals bind to glomeruli within minutes, proteinuria becoming evident in a few hours; by 2–4 h, there is extensive glomerular infiltration of neutrophils (4). The absence of serum C (5, 6) or of circulating neutrophils (4) reduces proteinuria. The C-dependent lesions, however, appear to be short-lived, at least in the experimental models so far examined. In NTN, proteinuria extends for days without further infiltration of neutrophils beyond the first 6 h. Furthermore, injury can also be produced by antibodies to glomeruli that do not fix C (5–8). These observations suggest that there may be other factors and/or other mediation systems of glomerular injury responsible for neutrophil-independent injury. Intravascular coagulation can result in a more severe glomerular injury (9), but it does not account for all instances of glomerular pathology.

We have chosen a modification of accelerated NTN as an experimental model for investigating factors that may contribute to glomerular pathology aside from the C-neutrophil systems. In NTN, glomerular injury proceeds in two well-defined states—an early or heterologous stage resulting from the fixation of the heterologous anti-kidney antibodies to the glomerular basement membrane (i.e., a rabbit IgG with antibody to rat glomerular antigens injected into rats [10]); and a late or autologous phase in which the host anti-IgG antibodies react with the heterologous Ig, now behaving essentially as a glomerular-planted antigen (11). In 1965, Unanue and Dixon (11) developed a modification of NTN consisting of preimmunization of the recipient with

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[1] *Abbreviations used in this paper:* C, complement; H & E, hematoxylin and eosin stain; NTN, nephrotoxic serum nephritis; NTS, nephrotoxic serum; PAS, periodic acid-Schiff stain.

heterologous Ig (i.e., rats preimmunized with rabbit IgG) insuring a consistent and high immune response and a prominent autologous phase. This model shows a spectrum of histological changes taking place within a few days as heterologous and autologous phases readily develop and blend with each other. We now report that this modification of NTN shows a conspicuous glomerular infiltration of blood-derived mononuclear cells following the early neutrophilic phase. Evidence is presented that monocytes contribute to the pathological features of this model and participate in the glomerular injury.

Materials and Methods

The basic experimental design was to inject rabbit anti-rat nephrotoxic serum into rats immunized with rabbit IgG several days before. After the injection of nephrotoxic serum, the rats were placed in metabolic cages; 24-h urine samples were collected and tested for proteinuria. Rats were sacrificed at various periods of time from 2 h to 7 days, and the kidneys examined by light, electron, and immunofluorescence microscopy.

Rats were male Lewis rats (Microbiological Associates, Bethesda, Md.), and in a few experiments, outbred Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 100–120 g. Nephrotoxic serum (NTS) was produced in rabbits by repeated intraperitoneal immunization with basement membrane-rich sediment of kidney (10). The NTS was extensively absorbed with rat erythrocytes and heated at 56°C for 30 min before use. The serum was tested for its nephrotoxic activity by determining the dose required to produce proteinuria during the first 24 h after intravenous injection. Proteinuria was determined by the sulfosalicylic acid method, reading the turbidity spectrophotometrically against albumin standards. Uninjected rats usually excreted less than 2 mg of protein/day. The NTS produced detectable proteinuria (about 50 mg/day) at doses of 1.2 ml/rat. We chose a dose of 0.5 or 0.8 ml which did not result in proteinuria in unimmunized rats. Results are expressed as milligrams per 24 h (± SD).

Rats were preimmunized with 1 mg of rabbit IgG in 0.5 ml of complete Freund's adjuvant intraperitoneally. The adjuvant was obtained from Difco Laboratories (Detroit, Mich.).

For light microscopy and autoradiography, kidneys were fixed in Bouin's fluid or Karnovsky's fixative (2% of glutaraldehyde – 2.5% paraformaldehyde in cacodylate buffer). 4- to 6-μm paraffin sections and 1- to 2-μm sections embedded in glycolmethacrylate were stained with hematoxylin and eosin (H&E) or periodic acid-Schiff stain (PAS). The thin methacrylate sections allowed a more precise assessment of the cell types and were used in all autoradiographic studies and most studies requiring glomerular cell counting. For electron microscopy, tissues were fixed by immersion fixation in Karnovsky's fixative, thin sections were stained with uranyl acetate and lead citrate, and examined in a Philips EM 201 microscope. Glomerular cell counts were made of glomeruli measuring more than 30 μm in their largest diameter. From each rat kidney, some 20–100 glomeruli were usually counted. For immunofluorescence, we used fluoresceinated antibodies to rat IgG, rat C3, and rabbit IgG. In all instances, the antibodies were monospecific. The fluoresceinated preparations were IgG fractions having molar fluorescein:protein ratios of 4 to 8.

Irradiation Experiments. In several experiments, we studied the effect of whole-body X-irradiation on proteinuria and renal histology. Rats were anesthetized, a lead cuff of 0.5-cm thickness placed around their waists to protect the kidneys from irradiation, and then irradiated with 800 rad. We had established in control experiments, placing a dosimeter in the abdomen of dead rats, that the lead shield stopped about 92% of the irradiation. Rats were irradiated in a Westinghouse-Coronado X-ray machine (Westinghouse Electric Corp., Pittsburgh, Pa.) at a dose of 76 rad/min.

Autoradiographs. Autoradiographic experiments were performed to determine the origin of infiltrating mononuclear cells. Rats received [^{3}H]thymidine intravenously at a dose of 1 μCi/g of body weight. The [^{3}H]thymidine had a specific activity of 60 Ci/mM (New England Nuclear, Boston, Mass.). 2 μm glycolmethacrylate-embedded sections were processed for autoradiography by standard methodologies with Kodak NTB II liquid emulsion. The time of exposure was 8 wk. The exact experimental protocol is described below. Positive cells were those having more than five grains per nucleus.

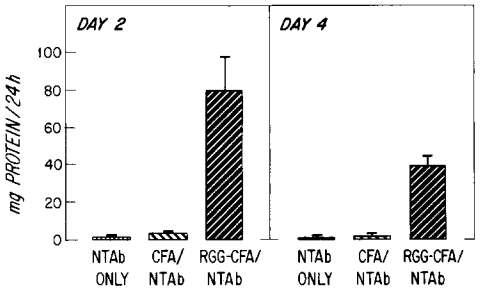


FIG. 1. Rats were injected with 0.8 ml of NTS and tested for proteinuria on days 2 and 4. The three groups (of six to eight rats each) received NTS only (NTAb only), complete Freund's adjuvant 5 days before NTS (CFA/NTAb), or rabbit IgG in adjuvant 5 days before NTS (RGG-CFA/NTAb). Value of proteinuria are in milligrams per 24 h (± SD).

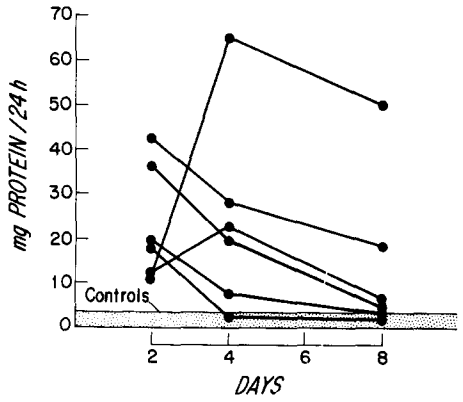


FIG. 2. The graph shows results of six individual rats given 0.8 ml of NTS 5 days after immunization with rabbit IgG in adjuvant.

Results

Effect of Immunization with Rabbit IgG. The experimental design followed in most experiments was to inject a subnephritogenic dose of NTS into rats immunized 5 days before with rabbit IgG. At this time, the rat's immune response to rabbit Ig is just detectable, and the preimmunization results in a consistent glomerular lesion. Thus, we found that rats injected with doses of 0.5 or 0.8 ml of NTS did not develop proteinuria. However, preimmunization with 1 mg of rabbit IgG, 5 days before the injection of NTS, resulted in moderate to severe proteinuria, present during the first 24 h and increasing markedly during the 2nd and 4th day. Although the excretion of protein varied from experiment to experiment, the averages were 15 mg during the first 24 h, and ranged from 20 to 80 mg by 2–4 days. In all experiments, the peak proteinuria occurred between days 2 and 4. Most rats showed a decrease in proteinuria from days 7 to 10. Figs. 1 and 2 show representative experiments examining for proteinuria at days 2 and 4 after NTS injection. Control experiments established that the development of proteinuria by days 2 and 4 required the incorporation of rabbit IgG in the adjuvant (Fig. 1). Preimmunization with adjuvant alone or administration of normal rabbit serum in rats

TABLE I

Proteinuria in Rats Immunized to Rabbit IgG

Group	Preimmunization with rabbit IgG	Proteinuria				Main histological changes
		Day 2	Day 4	Day 7	Day 14	
	Days before NTS	*mg/24 h*				
1	No immunization	2.9 ± 2.3	1.0 ± .46	2.8 ± 1.7	4.6 ± 2.5	Hypercellularity ±
2	1	1.3 ± .8	1.9 ± 2.6	6.5 ± 1.7	7.8 ± 1.0	Hypercellularity ±
3	3	2.1 ± 1.1	0.7 ± .24	3.9 ± .31	7.7 ± 3.0	Hypercellularity ++
4	5	21.9 ± 15.9	28.1 ± 25.7	6.7 ± 1.0	10.5 ± 3.9	Hypercellularity +++
5	8	116.9 ± 34.2	69.6 ± 44.8	40.9 ± 45.6	51.5 ± 64.7	Hypercellularity +++ Crescents
6	15	125.5 ± 98.6	24.3 ± 24.3	24.9 ± 8.4	Not done	Hypercellularity +++ Crescents

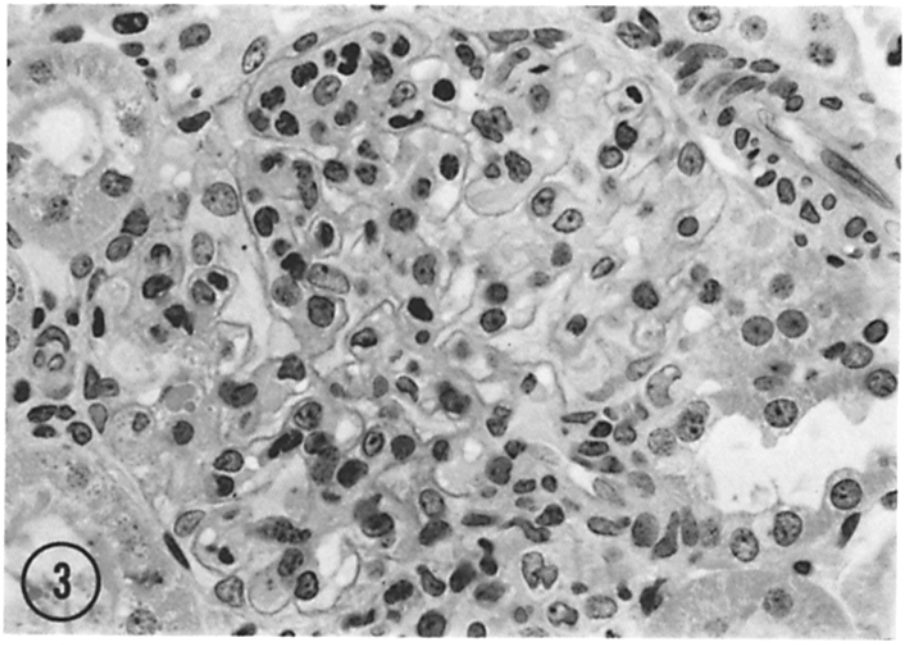
Each group consisted of four to six rats. Hypercellularity refers to increase in number of glomerular cells from day 2 on. Rats were not immunized or they were immunized with 1 mg of rabbit IgG in adjuvant from 1 to 15 days before injection of NTS. The volume of proteinuria in the groups not immunized or immunized 1 and 3 days before were not significantly different (groups 1, 2, and 3). Values of proteinuria between groups 4 and 5 and groups 1, 2, and 3 were all significantly different by t test ($P < 0.05$); group 6 was significantly different from groups 1, 2, and 3 at days 2 and 7 ($P < 0.001$).

preimmunized with rabbit IgG did not result in proteinuria. Most of the experimental observations were made during the first 4 days, a period in which the histological changes became prominent.

The relationship between time of preimmunization with rabbit IgG and the development of renal disease was investigated in the experiment summarized in Table I. Rats preimmunized 1 or 3 days before showed minimal proteinuria by days 7 and 14. It should be noted that the rats preimmunized 3 days before contained many PAS-positive vacuoles in proximal tubules and definite hypercellularity in glomeruli (see below). The increased number of PAS droplets in tubular cells suggests that an increase in glomerular permeability to protein had most likely taken place. Rats preimmunized at days 5, 8, or 15 before NTS showed high proteinuria which was more marked in those preimmunized 8 or 15 days before.

Histological Changes. The experimental model examined most extensively was that in which a subnephrotoxic dose of NTS (0.8 ml) was injected 5 days after an injection of IgG. Such rats consistently showed moderate proteinuria and reproducible histological changes. The histological changes can best be divided into two stages: those noted during the first 2–4 h after injection of NTS, and those found by the 2nd to 4th day. The early 2- to 4-h changes consisted of extensive leukocytic infiltration with the vast majority of cells being neutrophils. The number of neutrophils per glomerulus averaged 75. Within 2–4 days after injection of NTS, further histological changes evolved. At these times, the neutrophilic infiltrate had largely disappeared. The glomeruli were hypercellular, and counts of glomerular cells, made on both 4-μm paraffin sections or 1-μm methacrylate sections, showed a 25–40% increase in total cell numbers.

By light microscopy, the increase in cells appeared to result from two processes: (*a*) infiltration by mononuclear cells which filled the capillary lumina (Fig. 3), and (*b*) proliferation of intrinsic glomerular cells, evidenced by the presence of mitotic figures in many glomerular cells. In preimmunized rats, the number of mitosis per 10 glomeruli ranged from four to nine, whereas, in contrast, rats not preimmunized showed less than 1 mitosis per 10 glomeruli.



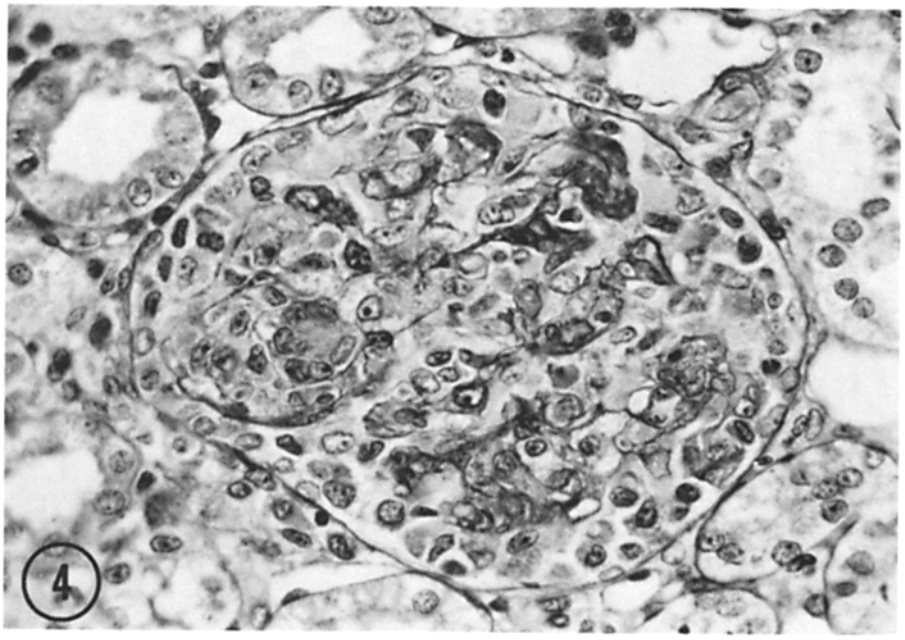


FIG. 3. Glomerulus from a preimmunized rat 4 days after injection of NTS. The glomerulus is hypercellular. Note the collection of mononuclear cells in capillary lumen (at 12 o'clock). Thin methacrylate section. PAS. × 400.

FIG. 4. This glomerulus is from a rat preimmunized 8 days before injection of NTS and sacrificed 4 days later. There is hypercellularity, destruction of basement membranes, and infiltration with mononuclear cells. PAS. × 400.

TABLE II
Glomerular Cell Counts and Proteinuria in Rats with Accelerated NTN

Immunization with rabbit IgG	Dose of NTS	Proteinuria	Glomerular cell counts
	ml	*mg/24 h*	*(No. ± SD)*
−	0.5	2.0	83.6 (± 11.02)
−	0.5	1.9	101.7 (± 14.30)
+	0.5	38.8	181.8 (± 27.20)
+	0.5	5.9	125.4 (± 20.60)
+	0.5	2.0	118.6 (± 18.30)
−	0.8	2.0	105.0 (± 8.00)
−	0.8	1.8	108.0 (± 16.10)
−	0.8	3.3	101.5 (± 16.30)
+	0.8	5.8	145.2 (± 20.90)
+	0.8	21.4	151.4 (± 23.60)
+	0.8	33.6	149.8 (± 15.10)

Table shows results of a representative experiment in which rats were administered 0.5- or 0.8-ml doses of NTS. Some rats were preimmunized 5 days previously with 1 mg of rabbit IgG in adjuvant (column 1). Proteinuria was measured every 2 days. Results of proteinuria are peak values obtained between days 2 and 4. Glomerular cell counts made on paraffin (4- to 6-μm) sections in 11 representative rats 4 days after the NTS injection. Range of glomerular cell counts in normal rats is 72 to 103.

In addition to hypercellularity, occasional glomeruli in some animals exhibited foci of fibrin deposition and necrosis. The tubules showed numerous protein casts, reabsorption droplets, and, occasionally, foci of necrosis. There were also focal interstitial infiltrates consisting of monocytic and lymphoid cells. Very few neutrophils were present (less than one per glomerulus) in glomeruli. Table II shows a representative experiment in which glomerular cell counts were made.

The histological changes were similar in rats preimmunized 3 days before, although in general, they were less extensive.

Rats preimmunized 7 days before or longer had more severe changes (Fig. 4), with glomeruli exhibiting extensive hypercellularity, collapse and fragmentation of basement membranes, fibrinoid necrosis, capsular adhesions, and, occasionally, crescent formation. The latter were most prominent by day 7 after injection of NTS. In some glomeruli, binucleate and multinucleate giant cells were identified.

The main finding by immunofluorescence was the presence of rat IgG and rat C3 along glomerular capillary loops in preimmunized rats. The proteins, as well as rabbit IgG, were localized in a typical linear pattern. In rats preimmunized 5 days before or more, the rat Ig was already found by 2 h after injection of NTS, indicating the presence at this time of circulating antibody.

Electron Microscopy. Electron microscope studies of 2- and 4-day lesions were performed with the specific aim of identifying the cell types infiltrating the capillary loops, and the intrinsic glomerular cells exhibiting proliferative (mitotic) activity.

The glomerular capillary lumina contained large numbers of mononuclear cells (Fig. 5), which showed considerable heterogeneity in morphologic detail. Many were typical blood monocytes (Figs. 5, 6); a smaller number were lymphocytes; and other could not be definitely labeled in a single plane of section (e.g., Fig. 7). Others could not be definitely classified. Mononuclear cells were either free (Fig. 6) in the lumen or were attached to the basement membrane by cytoplasmic processes that bridged discontinuities across the endothelium (Figs. 5, 8).

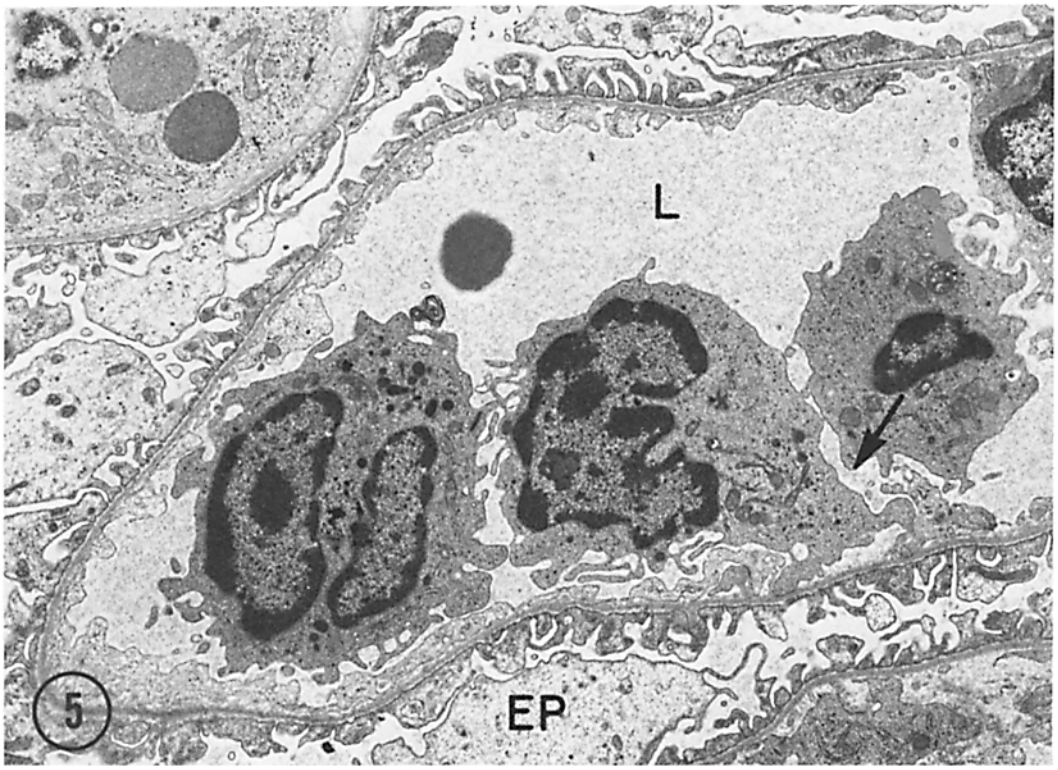
A very common cell type, accounting for much of the hypercellularity not confined to the lumen, was present between the endothelium and the basement membrane (Fig. 9). Such cells had a characteristic morphology: they were large, had a somewhat loculated nuclei, numerous phagolysosomes of variable density, and abundant numbers of cytoplasmic organelles. Although the most frequent localization of such cells was subendothelial, an occasional identical cell was present in the mesangium. It was not possible from examination of large numbers of micrographs to clearly determine whether these were activated monocytes migrating from the lumen into the capillary wall or mesangial cells migrating into a subendothelial site (see below).

By electron microscopy, mitotic figures were seen most frequently in the endothelial cells (Fig. 10). Indeed, many endothelial cells at 2 and 4 days exhibited other evidence of proliferative activity: enlargement of cytoplasm, increased numbers of organelles, dense bodies, and irregularity of surface contours. In addition, mitoses were seen, although more rarely, in mesangial cells (Fig. 11) and parietal epithelial cells (not illustrated).

Other findings included the presence of small subepithelial electron deposits, foci of platelet thrombi and intraluminal fibrin deposition, foci of endothelial cell detachments, focal loss of epithelial foot processes, increased numbers of membrane-bound dense bodies in visceral epithelial cells, expansion of mesangium, and hypertrophy of mesangial cells.

Effect of X-Irradiation. Our purpose here was to investigate whether or not the mononuclear cells in glomeruli, which we suspected on the basis of their morphology to be mononuclear phagocytes, could be derived from extrarenal sources. We reasoned that giving X-irradiation a few days after immunization should stop the output of monocytes from the bone marrow yet not affect significantly the levels of rat anti-rabbit IgG during the course of the experiment. After several trials, we found that the following protocol resulted in significant alteration in the mononuclear cell infiltrate without a marked change in the rat antibody deposited in glomeruli. We selected the 5-day interval between immunization and injection of NTS. Rats were immunized intraperitoneally with 1 mg of rabbit IgG in complete Freund's adjuvant, and 3 days later were given 800 rad of whole-body X-irradiation, protecting the kidney with a lead shield; 2 days later, NTS was injected. 24-h urine samples were collected 1, 2, and 4 days after the injection of serum. Rats were sacrificed at selected periods of time thereafter.

The proteinuria during the first 24 h in preimmunized rats was similar whether or not they were given X-irradiation. In contrast, rats not preimmunized did not show an increase in protein excretion (Table III). Representative rats were sacrificed at 2 h and examined histologically. Both groups of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preimmunized rats exhibited a marked increase in neutrophil infiltration over control not preimmunized (267% increase in nonirradiated and 71% increase in irradiated).

In contrast to observations at 2 h, we found marked changes between irradiated and nonirradiated groups of preimmunized rats at days 2 and 4. Proteinuria decreased markedly in the X-irradiated group but increased in the untreated. The mononuclear cell hypercellularity present in the nonirradiated rats was absent in the irradiated group (Table III).

Fluorescent antibody studies disclosed the presence of rat Ig in glomeruli of preimmunized rats whether or not they were irradiated. No rat Ig was found in glomeruli of rats not preimmunized. We attempted to estimate the amounts of rat Ig in glomeruli by testing different concentrations of the fluoresceinated rabbit anti-rat Ig. Glomeruli of irradiated and nonirradiated rats showed strong fluorescence at concentrations of rabbit IgG anti-rat Ig of 100 and 20 μg/ml. At 5 μg/ml the glomeruli of the nonirradiated rats showed distinct although weak linear fluorescence; the irradiated rats, although clearly positive, had less fluorescence and this was restricted to focal loops. At 1 μg/ml, there was no fluorescence in either of the two groups. We concluded, therefore, that the X-irradiated group contained rat Ig, but at a concentration somewhat lower than that found in untreated rats.

Autoradiographic Studies. The previous experiment with X-irradiation indicated that the glomerular hypercellularity was a radiosensitive phenomenon. If part of the glomerular hypercellularity were accounted for by mononuclear phagocytes, it should then be possible to trace these cells by radiolabeling their bone marrow precursors. We thus proceeded to examine whether the glomerular cells could be radiolabeled with [^{3}H]thymidine.

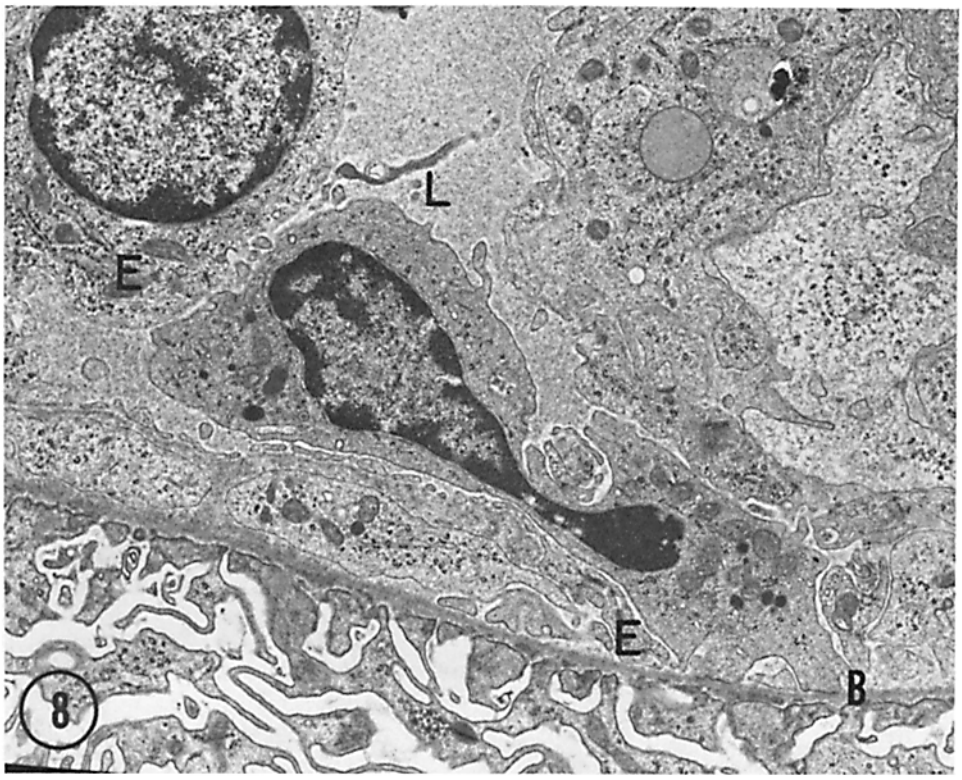
The experimental protocol was as follows: all rats were preimmunized by intraperitoneal injection of 1 mg of rabbit IgG in adjuvant. After 4 and 5 days, some received injection of [^{3}H]thymidine intravenously. NTS was injected at the 5th day; that is, to one group immediately after the thymidine pulse and to another 24 h after it. Pairs of rats were sacrificed immediately after the [^{3}H]thymidine pulse or 2 days after NTS injection, the earliest time where hypercellularity was well defined. Two groups of rats received 800 rads of whole-body irradiation with the kidney shielded on day 4, 1 h before the pulse; one of the two groups received NTS a day later. Kidneys were processed by autoradiography, and the number of labeled cells per glomerulus calculated. The results are shown in Table IV.

The glomeruli of the two rats that received thymidine at day 4 and were sacrificed 1 h later (group 1) contained very few labeled cells (less than one); a pair (group 2) sacrificed 72 h later contained a mean of 2.1 and 1.1 labeled cells per glomeruli. A third pair (group 3) labeled instead on the 5th day and

FIG. 5. Electron micrograph from a hypercellular glomerulus showing three mononuclear cells in capillary lumen (L). One of the monocytes extends a pseudopod (arrow), which is wedged between portions of endothelial cells and touches the basement membrane. × 8,000.

FIG. 6. A typical blood monocyte in capillary lumen. E, endothelium. × 5,000.

FIG. 7. A group of mononuclear cells in capillary lumen. P, platelet. × 3,500.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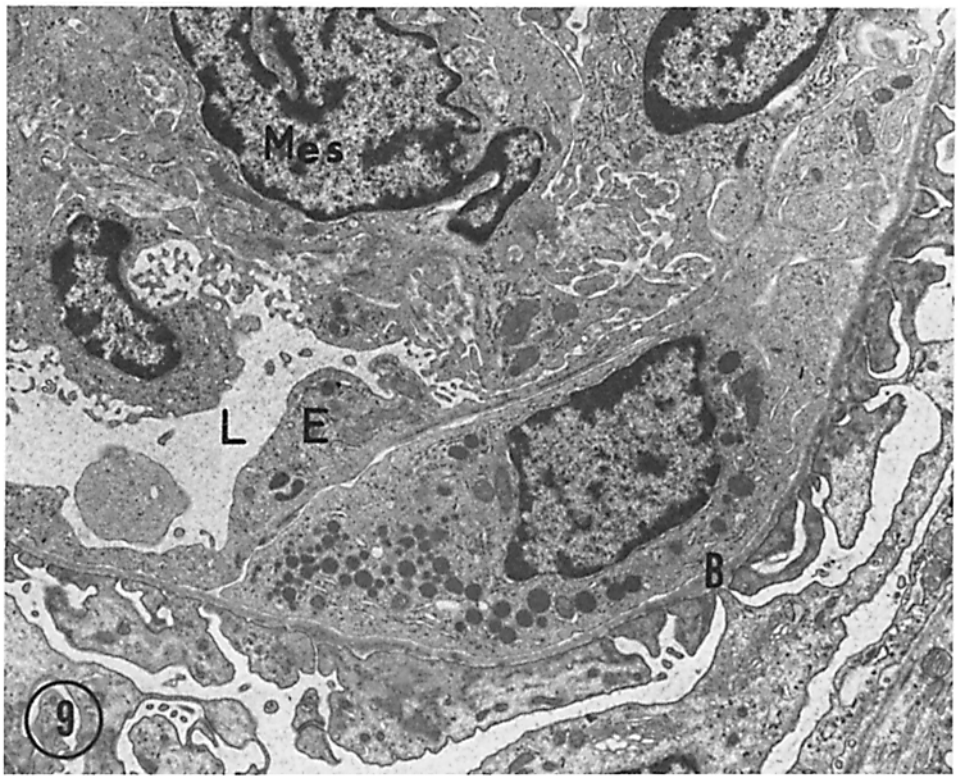
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examined 48 h later also contained a few labeled cells (0.8 and 1.7 cells per glomerulus). These three groups did not receive NTS.

Completely different results were found in rats that received the NTS. Groups 4 and 5 were injected with [^{3}H]thymidine the day before or 1 h before NTS and were sacrificed 48 h later. All contained substantial numbers of labeled cells. The largest number of labeled cells was found in group 4 pulsed with thymidine 72 h before (Table IV). The pair of rats given whole-body irradiation followed by NTS and thymidine contained very few labeled cells. We concluded, therefore, that the hypercellularity was, in part, explained by the entrance of circulating labeled cells into the glomeruli.

Discussion

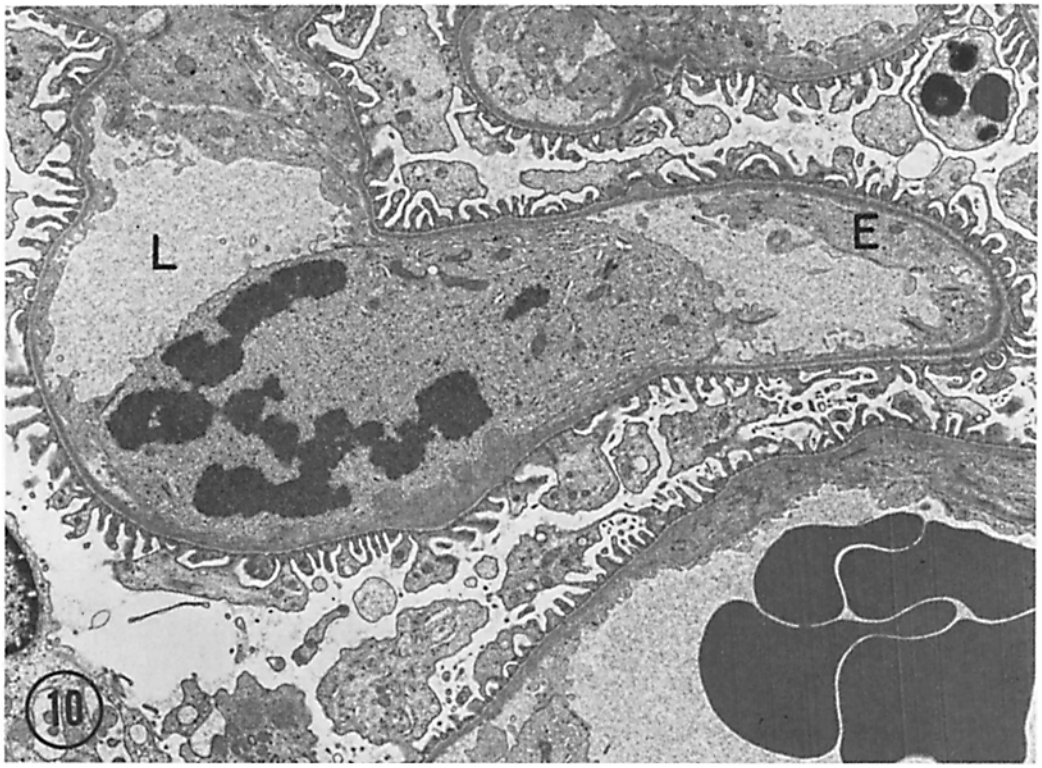
The present studies deal with an accelerated form of NTN in rats, consisting of immunization with rabbit IgG before injection of rabbit anti-rat NTS. The manipulation resulted in a rapid sequence of events in the glomeruli—first to an early neutrophil infiltrate accompanied by proteinuria and second to a prominent mononuclear response. Our studies indicated that a substantial part of the mononuclear cell response could be accounted for by cells that arose from dividing precursors localized outside the kidney, most likely mononuclear phagocytes. Finally, the results suggest that the mononuclear cell component may participate in the functional glomerular pathology.

The evidence indicating that part of the glomerular hypercellularity is accounted for by infiltration by mononuclear phagocytes comes from three observations: (*a*) many of the infiltrating cells were identical in morphology to classic blood monocytes, and furthermore, were found in peripheral areas of the glomerular capillary loops, well separated from mesangium; (*b*) the hypercellularity was sensitive to systemic irradiation, in conditions where the kidneys were shielded—in the same conditions, the rat antibody to rabbit IgG was still found in glomeruli, albeit in somewhat smaller concentrations; and (*c*) a great part of the hypercellularity was produced by the appearance of radiosensitive cells labeled with a DNA precursor 48–72 h before. It would thus appear that the glomerular hypercellularity has the same basic characteristics of chronic inflammatory type of reactions made up of mononuclear phagocytes. The phagocytes originate from rapidly proliferating precursor cells found in large numbers in bone marrow, which subsequently differentiate to blood monocyte and tissue macrophages (12, 13).

Are the mononuclear cells involved in the glomerular pathology? We believe the answer is yes. In the X-irradiation experiments, proteinuria readily developed in the first 24 h, and the typical early neutrophilic infiltrate occurred, although it was reduced (Table III). This first 24-h lesion is the classic antibody C-neutrophil lesion, well studied in the past (4). In experiments to be reported, we have found that, in fact, decomplementation by injection of cobra venom

FIG. 8. Portion of capillary loop showing a monocyte with pseudopod attaching to the basement membrane (B). E, endothelium; L, capillary lumen. × 10,000.

FIG. 9. Portion of capillary loop showing a mononuclear cell containing numerous membrane-bound granules and located between the endothelial cell (E) and the basement membrane (B). Mes, mesangial cell extending a pseudopod into the lumen. L, capillary lumen. × 9,000.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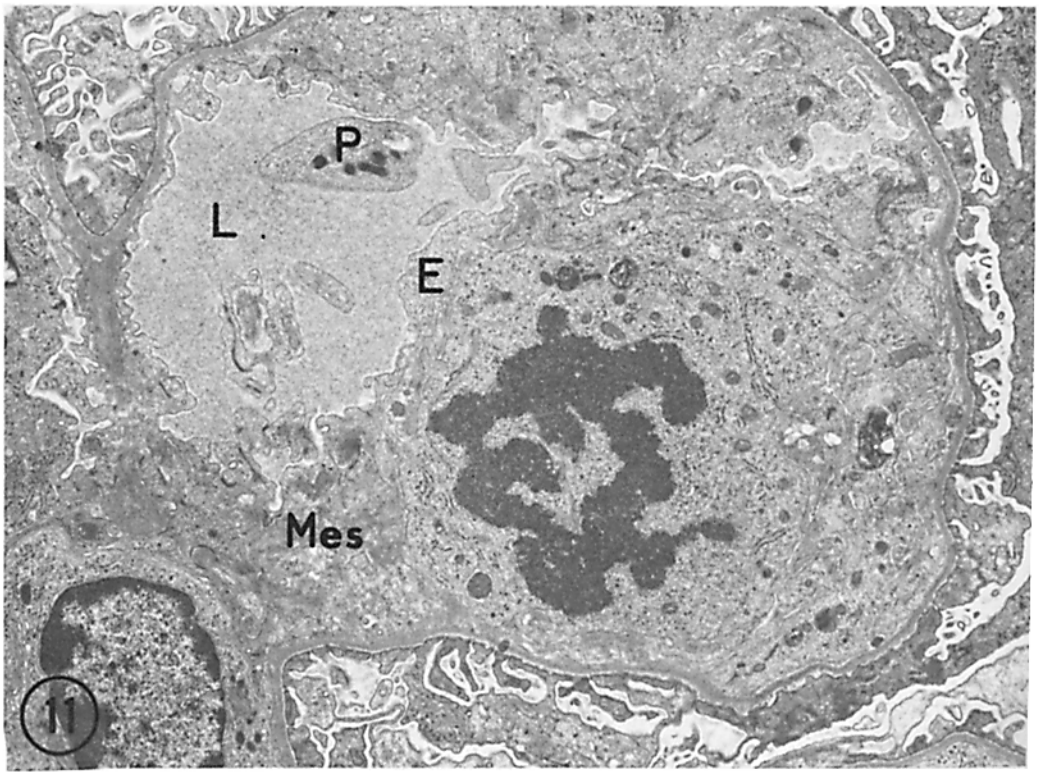
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TABLE III
Effect of X-Irradiation on Proteinuria

Group	Experimental manipulations			Proteinuria			Glomerular cell counts (day 2)*
	RGG-CFA	X-Ray	NTS	Day 1	Day 2	Day 4	
1	–	–	+	2.4 (± 5.2)	2.5 (± 4.5)	0.5 (± 2.3)	51.6 (± 7.4)
2	+	–	+	14.6 (± 3.3)	20.3 (± 3.2)	28.7 (± 5.5)	74.8 (± 13.8)
3	+	+	+	14.9 (± 3.4)	6.8 (± 3.9)	3.9 (± 4.8)	52.3 (± 5.8)

Each group consisted of four to six rats, some of which were immunized with 1 mg of rabbit IgG in adjuvant 5 days (RGG-CFA) before injection of 0.8 ml of NTS. 3 days after immunization, one group of rats received 800 rad of whole-body X-irradiation but with the area of kidney protected by a lead shield. Differences between proteinuria of groups 2 and 3 vs. 1 on day 1 and between group 2 vs. 1 and 3 on days 2 and 4 are all statistically significant ($P < 0.05$ by t test).

* Glomerular cell counts were made on two rats sacrificed on day 2. Counts were made on a 1- to 2-μm methacrylate section. Differences in glomerular cell counts of group 2 vs. 1 and 3 are also significant ($P < 0.05$).

TABLE IV
Labeled Cells in Glomeruli in Rats Given NTS

Group	Experimental manipulations				Labeled cells in glomeruli
	Day 0	Day 4	Day 5	Day of sacrifice	
					No. (range)
1	RGG-CFA*	TH3‡	–	½ h after TH3	0.20 (0–2) 0.25 (0–1)
2	RGG-CFA	TH3	–	Day 7	2.10 (0–4) 1.10 (0–3)
3	RGG-CFA	–	TH3	Day 7	0.80 (0–5) 1.70 (0–10)
4	RGG-CFA	TH3	NTS§	Day 7	11.55 (4–25) 8.40 (4–14)
5	RGG-CFA		TH3-NTS	Day 7	4.60 (2–9) 4.20 (1–4)
6	RGG-CFA	X-ray-TH3	NTS	Day 7	0.30 (0–3) 0.15 (0–1) 0.05 (0–1) 0.10 (0–2)
7	RGG-CFA	X-ray-TH3	–	Day 7	0.25 (0–2) 0.15 (0–1) 0.05 (0–1)

Labeled cells are those showing five or more grains in the nucleus. Each result represents values of one rat. Counts were made on thin glycomethacrylate sections. The exact protocol is detailed in the text.

* RGG-CFA, 1 mg of rabbit IgG in adjuvant intraperitoneally.

‡ TH3, [^{3}H]thymidine.

§ NTS, nephrotoxic serum, 0.8 ml.

FIG. 10. Mitotic figure in an endothelial cell 2 days after injection of NTS in preimmunized rat. CL, capillary lumen. E, endothelium. × 6,000.

FIG. 11. Mitotic figure in a mesangial cell 4 days after injection of NTS in a preimmunized rat. E, endothelium; Mes, mesangium; L, capillary lumen. × 7,000.

factor abrogates this early pathology, but leaves the subsequent 2- to 4-day lesion unaltered. The observation that X-irradiation prevented the proteinuria of days 2 and 4 suggests a pathogenic role of the mononuclear cell component. It can be argued, however, that the amounts of rat antibody in the kidney were somewhat lower in the X-irradiated rats, probably because the rat anti-Ig response was affected; and that, therefore, one cannot rule out the fact that the decrease in proteinuria may have resulted from a drop in glomerular-bound antibody. Against this argument are observations, to be reported separately, indicating that administration of rat anti-rabbit IgG antibodies, even at high doses (instead of preimmunization as done in the present study), followed by NTS will result in early, 24-h proteinuria, but not in proteinuria at 2–4 days. This indicates that the somewhat reduced fixation of rat antibody in the X-irradiated experiment (Table III) was not a significant factor in the abrogation of the day 2–4 proteinuria because the evidence indicates that the rat antibody will not directly produce injury. It seems logical to us that mononuclear phagocytes can produce glomerular lesions in a manner analogous to that produced by neutrophils. Indeed, there is evidence that the phagocytes release neutral proteases which might well affect collagenous structure (14).

In addition to mononuclear infiltration, the glomerular lesions exhibited considerable proliferation of intrinsic glomerular cells—endothelial, mesangial, epithelial cells. This proliferation might be a consequence of injury and subsequent regeneration of cells. However, in the irradiation experiments, it was noted that glomerular mitoses were also markedly reduced, although the kidneys were properly shielded from the irradiation. Macrophages have been shown to regulate the proliferation of other cell types, and in particular, factors capable of inducing fibroblastic (15) and vascular (16) proliferation have been identified. It is possible, therefore, that glomerular infiltration by macrophages might contribute to the local proliferative activity in glomerulonephritis.

Although mononuclear cells have been neglected as possible mediators of glomerular injury, their occasional presence in glomerular lesions has been mentioned in a number of experimental and clinical studies. In particular, Kondo et al. (17) showed that monocytes infiltrated the glomerulus in rabbit NTN and showed electron microscope images suggesting that they are transformed into epithelioid cells, which form part of the glomerular crescents characteristic of this model. Shigematsu (18) reported cells resembling monocytes in rat NTN, but their origin and role were not ascertained. Johnston and Latta (19) reported that monocytes contributed to the axial hypercellularity seen in focal glomerulonephritis induced by injections of yeast in rabbits. On the basis of their morphology and phagocytic capacity in culture, Atkins et al. (20) thought that cells emanating from crescents in human, rapidly progressive glomerulonephritis represent macrophages. Experiments now in progress will examine the contribution of monocytes to crescent formation in our model when preimmunization with IgG is carried out 8 and 15 days before injection of NTS.

The nature of the subendothelial, highly granulated mononuclear cell frequently seen in electron micrographs (Fig. 9) could not be clearly ascertained. Morphologically, the cells resembled activated macrophages, and it is tempting

to conclude that they represent such cells in the process of emigrating into the glomerular capillary wall. However, mesangial cells are also capable of proliferation and migration into a subendothelial site. It is hoped that kinetic studies with electron microscope autoradiography will resolve the identity of such cells.

Finally, what is the immunological process that induces the mononuclear cell infiltrate? One can speculate that either the cells are somehow attracted by the immune complex and/or by a directed process more akin to cellular immunity-type reactions. Alternatively, the infiltrate might be nonspecific, induced by local cellular injury and serving a "scanvenger" function akin to that occurring in wound healing. Experiments in progress will analyze these possibilities.

Summary

An accelerated form of nephrotoxic serum nephritis in the rat was examined. The experimental model consisted of preimmunization of the rat with rabbit IgG 5 days before injection of subnephrotoxic doses of rabbit anti-rat kidney serum. The immunized rats developed proteinuria during the first 24 h, increasing by 48–96 h. The early 24-h proteinuria correlated with a neutrophilic infiltration of glomeruli and with deposition of rat Ig and C. The 48- to 96-h proteinuria was associated with a glomerular infiltration by mononuclear cells and proliferation of intrinsic glomerular cells. Many of the mononuclear cells were morphologically identical to monocytes and macrophages. [^{3}H]thymidine labeling experiments indicated that the mononuclear cells originated from dividing precursors localized outside the kidney. Preimmunized rats given systemic irradiation (the kidney being protected by a shield) showed loss of the mononuclear cell infiltrate and absence of 48- to 96-h proteinuria. We conclude that mononuclear phagocytes can infiltrate the kidney in immunological glomerular disease and might contribute to the functional abnormalities.

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