American Journal of Pathology, Vol. 141, No. 4, October 1992

Glomerular Macrophages and the Mesangial Proliferative Response in the Experimental Nephrotic Syndrome

Jonathan R. Diamond,*† Guohua Ding,* Jeffery Frye,* and Ivona-Pesek Diamond*

From the Departments of Medicine and Cellular and Molecular Physiology,† Milton S. Hershey Medical Center, Hershey, and Pennsylvania State University College of Medicine, Hershey, Pennsylvania*

Mesangial cell proliferation, which is a harbinger of glomerulosclerosis, occurs in both immune and nonimmune glomerulopathies. The proximity of infiltrating glomerular macrophages to the contractile mesangial cells during acute puromycin aminonucleoside (PA) nephrosis suggests the possibility of a paracrine effect on mesangial cell growth. To test this, three maneuvers to either raise or lower the glomerular macrophage number during acute PA nephrosis (2 weeks after PA) were employed: 1) an essential fatty acid-deficient (EFAD) diet; 2) a cholesterol-supplemented diet (CSD); and 3) a single dose (600 rad) whole-body X-irradiation (XI) given to CSD-fed PA rats. Both the glomerular macrophage number and proliferation within the mesangium were evaluated immunohistochemically with ED-1, a mouse monoclonal anti-rat macrophage label, and 19A2, a mouse monoclonal anti-proliferating cell nuclear antigen (PCNA)/cyclin antibody, respectively. Immunohistochemical detection of 5'-bromo-2'-deoxyuridine (BrdU) incorporation confirmed that proliferation was occurring within the mesangial zones. The EFAD diet significantly reduced both the glomerular macrophage and PCNA/cyclin-positive cell number at 2 weeks after PA with a positive correlation ($r = 0.89$, $P < 0.05$). The CSD maneuver significantly increased both the glomerular macrophage and PCNA/cyclin cell number with a strong degree of correlation ($r = 0.95$, $P < 0.01$). X-irradiation administered to CSD-fed PA rats significantly lowered both the glomerular macrophage and PCNA/cyclin-positive cell number at 2 weeks. In all groups, the glomerular tufts did not express muscle actin using HHF 35, a specific immunolabel, suggesting that the proliferation in this model is not related to direct mesangial cell injury. This study shows that maneuvers that modulate the glomerular macrophage number are also associated with corresponding changes in the number of proliferating cells within the mesangium, suggesting a paracrine growth stimulation by the infiltrating macrophage during acute PA nephrosis. The infiltrating glomerular macrophage may be an effector mechanism for the propagation of initial glomerular injury to glomerulosclerosis by augmenting mesangial cell proliferation early in the course of this nonimmune progressive glomerulopathy. (Am J Pathol 1992, 141:887–894)

Proliferation of resident glomerular cells accompanied by the accumulation of excess mesangial cell matrix often constitute the initial glomerular structural aberrations in a number of different clinical and experimental renal disorders.[1,2] Although much insight has been gleaned from *in vitro* studies that have examined the regulatory factors for cultured mesangial cell growth, the precise pathobiologic stimuli for this aberrant process *in vivo* remain to be completely clarified.

Mesangial cell proliferation is observed during the course of many experimental immune glomerulopathies[3,4] as well as in biopsies of patients with focal sclerosis[5] and early during the course of the nonimmune progressive renal ablation model.[6] In immune-related glomerulonephritis models, *in vivo* studies have shown an important role for monocytes and activated macrophages,[4,7] as well as platelets,[3] in mediating mesangial cell proliferation. In nonimmune experimental models, however, which usually have a more chronic tempo, mesangial cell proliferation is often low-grade in intensity, segmental in location, and without an identifiable stimulus.

Presented in part at the 24th Annual Meeting of the American Society of Nephrology, November 17–20, 1991, Baltimore, Maryland.

Supported by USPHS grants DK 38394 and DK 40839 and a Research Grant-in-Aid from the American Heart Association (Pennsylvania Affiliate). Dr. Diamond is an Established Investigator of the American Heart Association.

Accepted for publication April 16, 1992.

Address reprint requests to Dr. J. R. Diamond, Division of Nephrology, Milton S. Hershey Medical Center, P.O. Box 850, Hershey, PA 17033.

It has been recently demonstrated in the rodent models of aminonucleoside nephrosis,[8,9] renal ablation,[6] and cholesterol feeding[8,10] that there is an increase in the number of infiltrating glomerular and periglomerular macrophages soon after the specific perturbation is initiated. In our model of chronic aminonucleoside nephrosis,[11,12] a single intrajugular venous bolus injection of puromycin aminonucleoside (PA), administered to Sprague-Dawley rats, results in an overt nephrotic syndrome peaking at 2 weeks after delivery of the visceral epithelial cell toxin. This initial phase is accompanied by reversible acute renal insufficiency and an increase in the number of infiltrating glomerular macrophages as well as a prominent periglomerular interstitial macrophage infiltrate.[8,9] Thereafter, an intermediate recovery phase occurs, during which the nephrotic syndrome abates spontaneously, and albuminuria falls to near-normal levels by 4 weeks after the previous PA injection. After another interval of approximately 3 to 4 weeks, a final phase emerges, during which albuminuria recurs, without further PA injection. This last phase is accompanied by the development of increased mesangial extracellular matrix accumulation and, ultimately, glomerulosclerosis affecting 15% to 20% of glomeruli by the end of the 18-week model. Other laboratories[13,14] have confirmed this early surge in infiltrating renal macrophages during acute PA nephrosis with a gradual waning in both macrophage number and *ex vivo* interleukin-1 (IL-1) generation from glomeruli isolated from rats with evolving glomerulosclerosis.

The proximity of macrophages to contractile mesangial cells during acute PA nephrosis suggests the possibility of a paracrine mitogenic effect on the latter cell population. Previous investigators have demonstrated, in immune-mediated models, that depletion of circulating monocytes after irradiation prevents a heavy monocytic infiltrate in glomeruli as well as marked glomerular cell hyperplasia.[15] Because recent investigations[8,9,12] have placed early lipid-dependent glomerular macrophage accumulation in the pathway for the development of glomerulosclerosis, we tested whether increments or decrements in this parameter, induced by either dietary means (ie, cholesterol supplementation or essential fatty acid deficiency) or X-irradiation, likewise, affected proliferation within the mesangium, assessed immunohistochemically, in our customarily viewed nonimmune model of experimental nephrotic syndrome produced by PA in the rat.

Materials and Methods

Experimental Groups

Male Sprague-Dawley rats (125 to 150 g, Charles River Breeding Laboratories, Wilmington, MA) received PA (ICN Chemicals, Cleveland, OH, 50 mg/kg) through the right jugular vein as a single bolus injection over 3 to 4 minutes. The effects of three maneuvers on both the glomerular macrophage number (ie, ED-1–positive cells, *vide infra*) and proliferation within the mesangium *in vivo,* at 2 weeks after PA, were examined. First, an essential fatty acid-deficient (EFAD) diet or an isocaloric control diet, both purchased from Purina Test Diets (Richmond, IN), was fed to rats for 8 weeks before the induction of acute PA nephrosis and for 2 weeks after the delivery of the visceral epithelial cell toxin. The groups are designated PA/EFAD and PA/Control, respectively. We, as well as others,[16] have previously demonstrated that the EFAD diet for this 10-week duration produces a characteristic increase in 20:3 (n-9) eicosatrienoic acid (Mead acid) counterbalanced by a decrease in arachidonic acid (20:4), as well as other typical fatty acid changes on hepatic lipid analysis.[9] For the second maneuver, separate cohorts of rats made nephrotic with PA were fed either a 4% cholesterol/1% cholic acid-supplemented diet (PA/CSD, Teklad Diets, Madison, WI) or standard rodent chow (PA/Standard, Purina 5001, St. Louis, MO) for the 2-week acute nephrotic interval after PA injection. The third maneuver consisted of administering a solitary 600 rad dose of whole body X-irradiation (XI), using a cobalt source (Gammacell, Atomic Energy Ltd. of Canada) with bilateral kidney shielding, given at 3 days after PA delivery[12] *versus* sham-irradiation given to PA rat cohorts also fed the CSD (PA/CSD/XI and PA/CSD, respectively). Before killing, at 2 weeks after PA, albumin excretion by radial immunodiffusion,[17] and fasting serum total cholesterol and triglycerides, by standard colorimetric methods, were measured in all groups.

Additional cohorts of cholesterol-fed PA rats received 5′-Bromo-2′-deoxyuridine (BrdU), a thymidine analog that binds to single-stranded DNA and can be subsequently localized in tissue sections, to confirm that proliferation was occurring in the glomerulus by another immunohistochemical method. BrdU (Sigma Chemical Co., 500 mg/kg intraperitoneally) was administered 4 hours before killing as a "flash" labeling method. This protocol for BrdU administration minimizes the possibility of infiltrating leukocytes contributing to glomerular labeling.

Tissue Preparation and Immunohistochemistry

Both kidneys were perfused with 0.9% N NaCl through an infrarenal aortic cannula for 3 to 5 minutes to remove circulating blood cells. After removal, the kidneys were sectioned coronally and immersed in methanol-Carnoy's fixative for 16 to 24 hours. Midcoronal sections were embedded in paraffin and sectioned at ~4-μ thickness.

Determination of glomerular macrophage number, proliferation within the mesangium, glomerular anti-muscle actin, and mesangial cell surface immunolabeling was accomplished with an avidin biotinylated horseradish peroxidase method.[18] Briefly, after sections were serially deparaffinized with xylene, endogenous peroxidase activity was quenched with 4:1 methanol-H_2O_2 solution and endogenous biotin blocked with avidin D and biotin blocking solutions (Vector Laboratories, Burlingame, CA). After incubation in either normal horse or goat serum (1:20, Vector) for 50 minutes at 25 °C, five different primary antibodies were used. ED-1,[19] (1:2,000, Serotec, Oxford, UK) is a mouse monoclonal IgG antibody obtained from ascitic fluid that recognizes cytoplasmic antigens in monocytes and macrophages. 19A2 (1:5,000, American Biotech., Inc., Plantation, FL) is a mouse monoclonal IgM that recognizes human proliferating cell nuclear antigen (PCNA)/cyclin, a 36-kd nuclear protein expressed predominantly by proliferating normal and transformed cells during late G_1, S, G_2, and M phases of the cell cycle.[20] HHF 35[21] (1:2,500, Enzo Diagnostic, New York, NY) is a monoclonal mouse IgG, anti-muscle actin. OX 7 (1:60,000 Accurate Chemical Co., New York, N.Y.) is a mouse monoclonal IgG anti-rat Thy-1 antibody that labels rat thymocytes as well as the surface of contractile rat mesangial cells. A polyclonal goat anti-rat fibronectin (ani-FN, 1:2,000, Calbiochem Corp., La Jolla, CA) was used to label mesangial matrix. Anti-BrdU (1:50, Becton-Dickinson, Mountain View, CA), a mouse monoclonal IgG antibody, was used to confirm that cells within the mesangial zones were proliferating by another immunohistochemical method.[6] To denature the DNA before the anti-BrdU monoclonal antibody incubation, sections were immersed in 4 N HCl for 20 minutes and then were rinsed in 0.1 mol/l (molar) sodium tetraborate, pH 8.5 for 5 minutes.[22] Biotinylated anti-mouse IgG (rat adsorbed) antibody (Vector, 1:150) or biotinylated anti-mouse IgM antibody were used as secondary antibodies for a 1-hour incubation at 25°C. An avidin-biotinylated horseradish peroxidase complex (Vector), for 1 hour at 25°C followed by a 0.1 mol/l solution of 3,3′-diaminobenzidine (DAB, Sigma) in 0.05 mol/l TRIS (hydroxymethyl) aminomethane buffer, pH 7.6 (5 minutes), to which had been added 0.75 ml 3% H_2O_2 for 5 minutes followed. Sections were rinsed in distilled water and counterstained with 1% methyl green solution for 20 minutes. A negative control consisted of substituting the primary antibody with normal serum IgG or IgM.

One hundred randomly chosen glomeruli were scored for the number of ED-1–positive cells observed within the glomerular mesangium. This number is referred to throughout this paper as the "glomerular macrophage number."[8,9] Proliferation was expressed as the mean number of cells within the mesangium per glomerular cross-section that exhibited positive nuclear staining for PCNA/cyclin[3] on 100 randomly chosen glomeruli. To determine if the mesangial cells had acquired a smooth muscle cell phenotype, the HHF 35 antibody was employed. Double immunostaining, using the combinations of either 19A2 and ED-1, 19A2 and OX 7, or 19A2 and anti-FN, was performed to further delineate which cells within the mesangium were proliferating. A nickel chloride color modification of DAB[18] was used for 19A2 with the reaction product for the mouse monoclonal IgM anti-PCNA/cyclin staining black. Negative controls for the double immunolabeling consisted of omitting one of the two primary antibodies.

Analytical

Statistically significant differences between the two groups for each maneuver was determined by Student's *t*-test.

Results

Normal rats on standard rodent chow have 1.6 ± 0.1 ED-1–positive and 0.5 ± 0.2 PCNA/cyclin-positive cells per glomerular cross section. As shown in Figure 1, the EFAD diet in PA rats produced a significant ($P < 0.001$) fall in the glomerular macrophage number during acute nephrosis from a mean of 10.5 ± 0.8 ED-1–positive cells per glomerular cross section to 2.1 ± 0.4 cells. Accompanying this decrement in the glomerular macrophage number was a corresponding significant ($P < 0.001$) decline in the number of glomerular PCNA/cyclin-positive cells from 2.0 ± 0.3 cells per glomerular cross section in

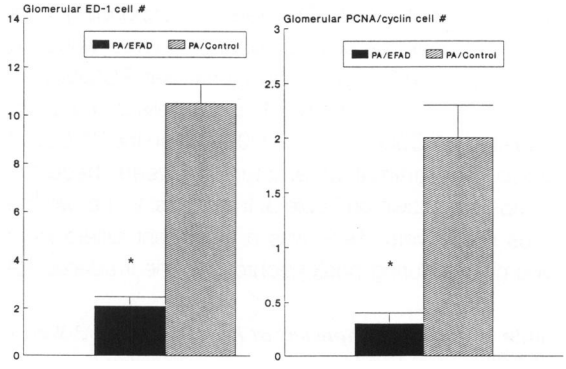

Figure 1. *The effect of an EFAD diet on the number of glomerular macrophages [(ED-1-positive cells) (left)] and proliferating cells within the mesangium [(PCNA/cyclin-positive cells) (right)] during peak PA nephrosis, 2 weeks after PA delivery. PA/EFAD comprises a cohort of (n = 5) which were fed a diet devoid of arachidonic and linoleic acids for 8 weeks prior to and for the 2 weeks after receiving PA. PA/Control (n = 5) is a group of rats which were fed an isocaloric control diet for the same time interval.* *P < .001 *vs. PA/control.*

PA/control rats to 0.3 ± 0.1 in the PA/EFAD group. Reducing the glomerular macrophage number, by means of the EFAD diet, correlated directly with the decline in proliferation with a significant correlation coefficient of 0.89 ($P < 0.05$). As shown in Table 1, despite the effects of the EFAD diet on significantly reducing both the glomerular macrophage and PCNA/cyclin-positive cell number at 2 weeks after PA, no significant differences in albuminuria, fasting total cholesterol, or triglycerides were noted.

Figure 2 shows the effect of a CSD on both glomerular macrophage and PCNA/cyclin-positive cell number. The glomerular macrophage number was significantly ($P < 0.01$) elevated from a mean value of 2.8 ± 0.2 cells per glomerular cross section in standard chow-fed PA rats to a value of 6.8 ± 0.8 cells in the cholesterol-fed nephrotic group. There was also a significant ($P < 0.01$) increase in the number of PCNA/cyclin-positive glomerular cells from a mean value of 1.4 ± 0.2 cells per glomerular cross-section in PA/Standard rats to 2.3 ± 0.2 cells in the PA/CSD group. Increasing the glomerular macrophage number correlated directly with enhanced proliferation with a significant correlation coefficient of 0.95 ($P < 0.01$). As shown in Table 2, PA rats fed the cholesterol-supplemented diet had no significant differences in either albuminuria or fasting triglyceride values when compared with PA/standard rats. Only the fasting total cholesterol parameter was significantly elevated between the two groups.

Figure 3 demonstrates the values for glomerular macrophage number and proliferation within the mesangium for both sham-irradiated and X-irradiated PA rats fed the CSD. The solitary 600-rad XI dose given at 3 days after PA delivery significantly ($P < 0.01$) lowered the glomerular macrophage number from a mean of 9.1 ± 0.8 cells per glomerular cross section in the PA/CSD group to 5.1 ± 0.3 cells in the PA/CSD/XI rats. Corresponding to the decline in the glomerular macrophage number was a significant ($P < 0.01$) fall in the glomerular PCNA/cyclin-positive cell number from 2.1 ± 0.3 cells in the sham-irradiated PA/CSD rats to 0.9 ± 0.1 cells in the PA/CSD/XI group. The animal parameters between these two groups are shown on Table 3. In contrast to the two previous maneuvers, there was a significant difference in albuminuria during peak nephrosis in the irradiated PA/CSD cohort. The XI maneuver, however, did not affect fasting circulating cholesterol or triglyceride levels.

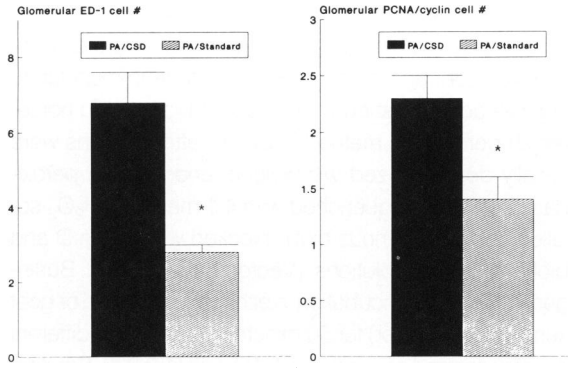

Figure 2. *The effect of a 4% cholesterol/1% cholic acid diet (CSD) on the number of glomerular macrophages [(ED-1-positive cells) (left)] and proliferating cells within the mesangium [(PCNA/cyclin-positive cells) (right)] during peak nephrosis, 2 weeks after PA. PA/Standard (n = 5) comprises a cohort of nephrotic rats maintained on standard rodent chow for 2 weeks after receiving PA. PA/CSD (n = 5) is another nephrotic group that was fed the CSD for the 2-week interval after receiving PA. *P < .01 vs. PA/CSD.*

Figure 4 is a composite photomicrograph of representative glomeruli from PA/CSD rats, 2 weeks after PA injection. The CSD in PA rats resulted in a pronounced mesangial infiltration of ED-1–positive macrophages, with many of these cells exhibiting a foam cell appearance (panel A). Proliferation within the mesangium, as evidenced by nuclear labeling with the mouse monoclonal IgM anti-PCNA/cyclin antibody, is shown in panel B. Double immunohistochemical labeling showed that the ED-1–positive macrophages and the PCNA/cyclin-positive proliferating cells within the mesangium constitute two separate populations of cells (panel C). Additional double immunohistochemical labeling studies showed that PCNA/cyclin-positive nuclei had surrounding surface staining with the mouse anti–Thy-1 monoclonal antibody (panel D). This observation further suggests that the proliferating cells within the mesangium are contractile mesangial cells. Incorporation of the thymidine analog, BrdU, confirms that proliferation is occurring within the mesangium in a representative glomerulus (panel E). In addition to the two nuclei incorporating the BrdU in the mesangial zones, proliferation of parietal epithelial cells along Bowman's capsule was also detected. All groups failed to express an altered phenotype for muscle actin, as evi-

Table 1. *Animal Parameters at Peak Nephrosis, 2 Weeks After PA*

Group (n)	UalbV (mg/day)	FTC (mg/dl)	FTG (mg/dl)
PA/EFAD (5)	206 ± 34	345 ± 70	316 ± 80
PA/Control (5)	206 ± 25	368 ± 60	281 ± 46
P value	NS	NS	NS

Table 2. *Animal Parameters at Peak Nephrosis 2 Weeks After PA*

Group (n)	UalbV (mg/day)	FTC (mg/dl)	FTG (mg/dl)
PA/CSD (5)	110 ± 27	1,209 ± 344	349 ± 90
PA/Standard (5)	106 ± 15	261 ± 26	408 ± 61
P value	NS	<.001	NS

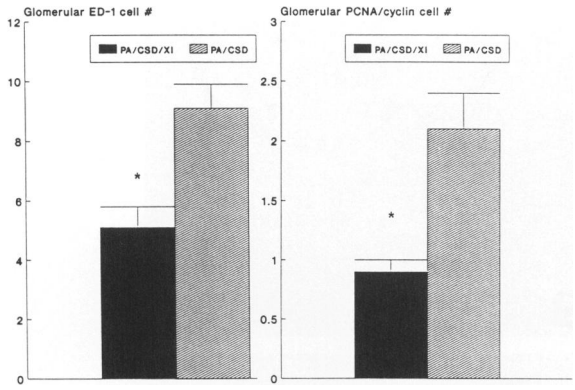

Figure 3. *The effect of a solitary XI dose (600 rads), given 3 days after PA delivery to CSD-fed nephrotic rats, on the number of glomerular macrophages [(ED-1-positive cells) (left)] and proliferation within the mesangium [(PCNA/cyclin-positive cells) (right)] during peak nephrosis, 2 weeks after PA. PA/CSD/XI comprises the CSD-fed nephrotic rats (n = 7) which received the XI while PA/CSD (n = 7) is a sham-irradiated cohort of CSD-fed PA rats.* *P < .01 *vs. PA/CSD.*

denced by the absence of immunolabeling of the tuft with HHF 35. Large blood vessels as well as glomerular arterioles (as shown) were positive for this smooth muscle actin label (panel F), however. As recently observed by Johnson and colleagues,[23] the absence of HHF 35 immunolabeling of muscle actin within the glomeruli, in this model of visceral epithelial cell injury, suggests that direct mesangial cell injury is not occurring. Panels G and H demonstrate double immunolabeling with 19A2, directed against PCNA/cyclin, and the anti-FN antibody. The PCNA/cyclin-positive nuclei are within the mesangial matrix, the latter evidenced by the fibronectin labeling.

Discussion

This study examined the association between lipid-dependent glomerular macrophage accumulation and proliferation within the mesangium in a nonimmune model of experimental nephrotic syndrome, produced by PA. Using dietary maneuvers and XI to modulate the glomerular macrophage number, during peak nephrosis, we observed that corresponding alterations in the number of proliferating cells within the mesangium, on immunolabeling with an anti-PCNA/cyclin monoclonal antibody, also were produced. Significant correlation between the number of ED-1–positive cells and degree of PCNA labeling was noted on linear regression analysis for the two dietary maneuvers. The observation that cells within the mesangium were proliferating, in this customarily viewed nonimmune model of glomerular injury, was confirmed immunochemically by incorporation of BrdU, a thymidine analog, during the S phase of the cell cycle.

Table 3. *Animal Parameters at Peak Nephrosis 2 Weeks After PA*

Group (n)	UalbV (mg/day)	FTC (mg/dl)	FTG (mg/dl)
PA/CSD (7)	88 ± 14	471 ± 114	78 ± 8
PA/CSD/XI (7)	37 ± 7	281 ± 54	67 ± 8
P value	<.01	NS	NS

Two potential explanations exist for the difference in the glomerular macrophage number between the two separate dietary control groups, PA/control and PA/standard. First, these diets are not equivalent in their fat content. The control diet, which is an isocaloric control for the EFAD diet, contains ~4% lard and ~4% corn oil. The standard diet (ie, Purina 5001) contains ~5% fat. Secondly, the magnitude of albuminuria was greater in PA/control rats (206 ± 25 mg/day) than in PA/standard animals (106 ± 15 mg/day). Theoretically, more severe glomerular injury, as evidenced by greater albuminuria values, could be associated with a more intense chemotactic signal to recruit circulating monocytes into the glomerular compartment.

The specific cell target for PA is the glomerular visceral epithelial cell. We have previously shown[24] that only the glomerular visceral epithelial cell releases significant amounts of labeled chromium in response to different concentrations of PA (0 to 500 μg/ml) added to tissue culture medium. A rat cortical tubular epithelial cell line, a Manin-Darby canine kidney cell line, and cultured rat glomerular mesangial cells all failed to release the intracellular label above baseline. To further demonstrate that direct mesangial cell injury is not occurring as a result of PA administration, we conducted anti-muscle actin immunolabeling[23] in all of our experimental groups and found that glomerular tufts were always negative for the HHF 35 label, whereas arteries and arterioles within the same sections were positive. Thus, direct mesangial cell injury cannot account for the enhanced proliferation within the mesangium during acute PA nephrosis.

An important feature of this study was the use of a double immunohistochemical labeling method to differentiate proliferating resident glomerular cells within the mesangium from those derived from the bone marrow (ie, infiltrating macrophages). Furthermore, we demonstrated that PCNA/cyclin-positive nuclei within the mesangial zones were surrounded by a surface immunolabeling for the Thy-1 antigen, which is present on the contractile mesangial cell membrane. This provided additional immunohistochemical evidence that the proliferating cells within the mesangium are most probably the contractile mesangial cells and not proliferating leukocytes.

Proliferation of resident mesangial cells, in the absence of a phenotypic change (ie, muscle actin expres-

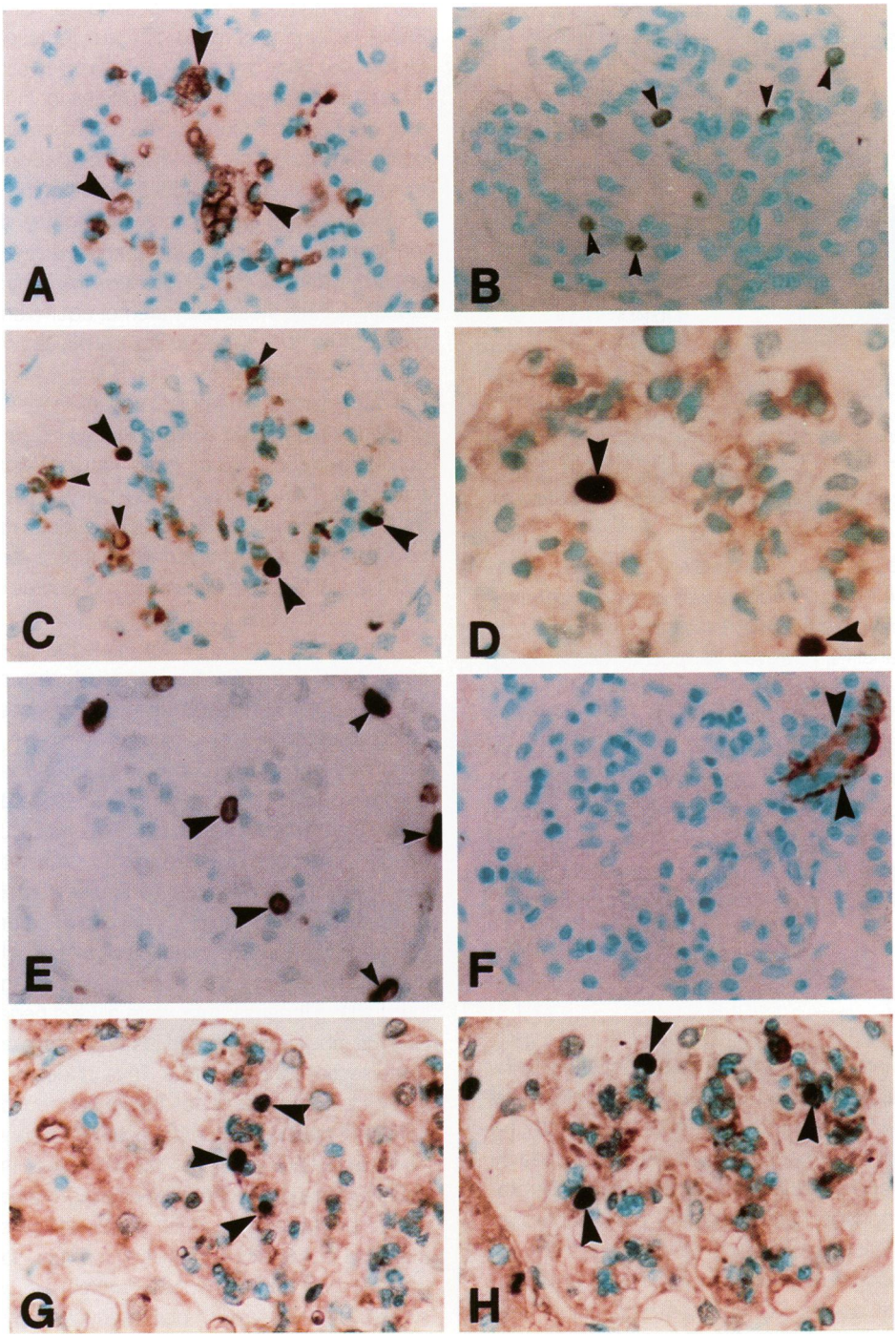

Figure 4. *Composite horseradish peroxidase immunohistochemistry photomicrograph of representative glomeruli from the CSD-fed PA rat cohort, 2 weeks after PA.* **(A)** *shows the pronounced influx of macrophages into the glomerular mesangium with the large arrowheads indicating foam cells with the typical vacuolated appearance. Sections were labeled with ED-1, 1:2,000 dilution, as the primary antibody and counterstained with methyl green (×440);* **(B)** *demonstrates proliferation within the mesangium utilizing 19A2, the mouse monoclonal IgM anti-PCNA/cyclin antibody (1:5,000 dilution). Small arrowheads indicate the darkly stained nuclei that are proliferating (×440);* **(C)** *is a double immunohistochemical stain for proliferation within the mesangium, utilizing 19A2 as the initial monoclonal antibody, with the reaction product color-modified by the addition of nickle chloride to the DAB. The large arrowheads indicate PCNA/cyclin-positive nuclei within the mesangium. Glomerular macrophages are labeled with ED-1 as the second monoclonal antibody (small arrowheads) (×440);* **(D)** *is another double immunohistochemical label for proliferating cells within the mesangium with 19A2 as the initial monoclonal antibody and OX 7, which identifies the Thy-1 antigen on the surface of contractile mesangial cells, as the second monoclonal antibody. The large arrowheads indicate two darkly stained PCNA/cyclin-positive nuclei with surrounding OX 7 labeling (×700);* **(E)** *also demonstrates proliferation within the mesangium (large arrowheads) as well as along the parietal epithelial layer of Bowman's capsule (small arrowheads) utilizing an anti-BrdU monoclonal antibody (1:50 dilution) (×440);* **(F)** *shows a representative glomerulus labeled with HHF 35, an anti-muscle actin monoclonal antibody (1:2,500 dilution). As is evident, the glomerular tuft is completely negative; however, the adjoining arteriole clearly demonstrates the positivity for smooth muscle actin (×440);* **(G)** *and* **(H)** *are representative glomeruli double immunolabeled with 19A2 and goat anti-rat fibronectin (1:2,000 dilution). The large arrowheads denote PCNA/cyclin-positive nuclei within the mesangial matrix, the latter as evidenced by the light-brown labeling for fibronectin (×440).*

sion), suggests that the augmented *in vivo* proliferation noted in association with significantly greater numbers of glomerular ED-1–positive cells is not due to autocrine stimulation, but rather to a paracrine process. Our results suggest that the likely cell related to this enhanced proliferation within the mesangium is the infiltrating macrophage. In fact, van Goor et al,[6] using the renal ablation model, have recently demonstrated a temporal association between increased glomerular macrophage number, hypercholesterolemia, and mesangial cell proliferation *in vivo,* which preceded the development of any detectable mesangial expansion and the ensuing glomerulosclerosis. Specific macrophage products are mitogenic for the contractile mesangial cell. Lovett and colleagues[25] have shown that glomerular mesangial cells proliferate, *in vitro,* in response to macrophage-derived IL-1. We have previously identified macrophage-derived IL-1-alpha in nephrotic rat glomeruli *in vivo,* at 11 days after PA by immunohistochemical means.[26] Clearly, other polypeptide mitogens (eg, platelet-derived growth factor, tumor necrosis factor, etc.), in addition to IL-1, also may be involved in the regulation of mesangial cell growth[27] in this model.

The infiltrating macrophage may represent one cell type that mediates the deleterious effects of hypercholesterolemia, and, thus, serves as a new target for therapeutic intervention in an attempt to arrest the progression of nonimmune glomerular disorders. Both our laboratory[9,12] and Harris et al[28] noted that the renal dysfunction of acute PA nephrosis was ameliorated by both essential fatty acid (EFA)-deficient diet and whole body X-irradiation in association with a reduction of infiltrating renal macrophages. Also, Matsumoto and Atkins[13] reported an increase in glomerular macrophage number, 10 days after PA delivery; and macrophage accumulation declined gradually along with IL-1 levels, as glomerulosclerosis developed. Saito and Atkins[14] demonstrated both glomerular and interstitial infiltrating leukocytes contributing to disease progression in a slightly different model of chronic PA nephrosis. Similar to our own data, these investigators noted a threefold increment in glomerular macrophages in nephrotic rats as early as 24 days after the initiation of subcutaneous PA injections. In the interstitium, all leukocyte classes were identified throughout the evolution of this disorder; however, interstitial macrophages were present early in the model, but not at the end of the process. This investigation examines an earlier pathobiologic event in the course of progressive glomerulopathy and extends observations made in previous studies using this model, which demonstrated a role for infiltrating renal macrophages. Recent evidence supporting the macrophage as an effector mechanism for the noxious effects of hypercholesterolemia is based on the salutary effect of the nonhypolipidemic XI maneuver at blunting progressive albuminuria and glomerulosclerosis in PA rats with both profound and sustained hypercholesterolemia.[29]

In summary, an EFAD diet produces a significant decrement in the number of PCNA/cyclin-positive cells, in concert with a significant fall in glomerular macrophage number. A CSD increases both PCNA/cyclin-positive cell number and glomerular macrophages during acute PA nephrosis. The XI maneuver in CSD-fed PA rats significantly reduces the number of proliferating cells within the mesangium as well as the glomerular macrophage number. These studies indicate that the glomerular macrophage, perhaps through certain macrophage-derived peptide growth factors, may exert a paracrine effect on proliferation within the mesangium in this customarily viewed nonimmune model of progressive glomerulopathy. These results provide a mechanism to explain how early glomerular and interstitial macrophage infiltration during acute PA nephrosis may propagate initial glomerular injury to glomerulosclerosis.

References

1. Diamond JR, Karnovsky MJ: Focal and segmental glomerulosclerosis: analogies to atherosclerosis. Kidney Int 1988, 33:917–924
2. Klahr S, Schreiner G, Ichikawa I: The progression of renal disease. N Engl J Med 1988, 318:1657–1666
3. Johnson RJ, Garcia RL, Pritzl P, Alpers CE: Platelets mediate glomerular cell proliferation in immune complex nephritis induced by anti-mesangial cell antibodies in the rat. Am J Pathol 1990, 136:369–374
4. Sterzel RB, Pabst RB: The temporal relationship between glomerular cell proliferation and monocyte infiltration in experimental glomerulonephritis. Virchows Arch [B] 1982, 38: 337–350
5. Schwartz MM, Lewis EJ: Focal segmental glomerular sclerosis: The cellular lesion. Kidney Int 1985, 28:968–974
6. Van Goor H, Fidler V, Weening JJ, Grond J: Determinants of focal and segmental glomerulosclerosis in the rat after renal ablation. Lab Invest 1991, 64:754–765
7. Van-Diemen-Steenvoorde R, Lambers A, van der Waal A, van Rooyen N, Dijkstra C, Hoedemaeker PJ, de Heer E. Macrophages are responsible for mesangial cell injury and extracellular matrix expansion in anti-Thy-1 nephritis in rats. J Am Soc Nephrol 1991, 2:585A
8. Diamond JR, Pesek I, McCarter MD, Karnvosky MJ: Alterations in rat macrophage function during nephrosis: synergistic effects of hypercholesterolemia. Am J Pathol 1989, 135:711–718
9. Diamond JR, Pesek I, Ruggieri S, Karnovsky MJ: Essential fatty acid deficiency during acute puromycin nephrosis ameliorates late renal injury. Am J Physiol 1989, 257:F798–F807
10. Kasiske BL, O'Donnell MP, Schmitz PG, Kim Y, Keane WF:

Renal injury of diet-induced hypercholesterolemia in rats. Kidney Int 1990, 37:880–891
11. Diamond JR, Karnovsky MJ: Focal and segmental glomerulosclerosis following a single intravenous dose of puromycin aminonucleoside. Am J Pathol 1986, 122:481–487
12. Diamond JR, Pesek-Diamond I: Sublethal X-irradiation during acute puromycin nephrosis prevents late renal injury: Role of glomerular macrophages. Am J Physiol 1991, 260:F779–F786
13. Matsumoto K, Atkins RC: Glomerular cells and macrophages in the progression of experimental focal and segmental glomerulosclerosis. Am J Pathol 1990, 134:933–941
14. Saito T, Atkins RC: Contribution of mononuclear leukocytes to the progression of experimental focal glomerular sclerosis. Kidney Int 1990, 37:1076–1083
15. Schreiner GF, Cotran RS, Pardo V, Unanue ER: A mononuclear cell component in experimental immunologic glomerulonephritis. J Exp Med 1978, 147:369–384
16. Lefkowith JB, Schreiner G: Essential fatty acid deficiency depletes rat glomeruli of resident macrophages and inhibits angiotensin II-induced eicosanoid synthesis. J Clin Invest 1987, 80:947–956
17. Mancini G, Carbonaro AO, Heremans JF: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 1965, 2:235–254
18. Gown AM, Vogel AM: Monoclonal antibodies to human intermediate filament proteins: II. Distribution of filament proteins in normal human tissues. Am J Pathol 1984, 114:309–321
19. Dijkstra CD, Kopp EA, Joling P, Krall G: The heterogeneity of mononuclear phagocytes in lymphoid organs: Distinct macrophage subpopulations in the rat recognized by monoclonal antibodies to ED 1, ED 2, and ED 3. Immunology 1985, 54:589–599
20. Ogata K, Kurki P, Celis JE, Nakamura RM, Tan EM: Monoclonal antibodies to a nuclear protein (PCNA/Cyclin) associated with DNA replication. Exp Cell Res 1987, 168:475–486
21. Tsukada TM, McNutt MA, Ross R, Gown AM: HHF-35, a muscle actin-specific monoclonal antibody. Am J Pathol 1987, 127:389–402
22. Lacy ER, Kuwayama H, Cowart KS, King JS, Deutz AH, Sistrunk S: A rapid, accurate, immunohistochemical method to label proliferating cells in the digestive tract. Gastroenterology 1991, 100:259–262
23. Johnson RJ, Iida H, Alpers CE, Majesky MW, Schwartz SM, Pritzl P, Gordon K, Gown AM: Expression of smooth muscle cell phenotype by rat mesangial cells in immune—complex nephritis. J Clin Invest 1991, 87:847–858
24. Diamond JR, Anderson S: Irreversible tubulointerstitial damage associated with chronic aminonucleoside nephrosis: Amelioration by angiotensin I converting enzyme inhibition. Am J Pathol 1990, 137:1323–1332
25. Lovett DH, Ryan JL, Sterzel RB: Stimulation of rat mesangial cell proliferation by macrophage interleukin 1. J Immunol 1983, 131:2830–2836
26. Diamond JR, Pesek I: Glomerular interleukin-1 and tumor necrosis factor during acute aminonucleoside nephrosis: An immunolohistochemical study. Lab Invest 1991, 64:21–28
27. Jaffer J, Saunders C, Shultz D, Throckmorton E, Weinshell E, Abboud HE: Regulation of mesangial cell growth by polypeptide mitogens: Inhibitory role of transforming growth factor beta. Am J Pathol 1989, 135:261–269
28. Harris KPG, Lefkowith JB, Klahr S, Schreiner GF: Essential fatty acid deficiency ameliorates acute renal dysfunction in the rat after the administration of the aminonucleoside of puromycin. J Clin Invest 1990, 86:1115–1123
29. Pesek-Diamond I, Ding G, Frye J, Diamond JR: Macrophages mediate the adverse effects of cholesterol feeding in experimental nephrosis. Am J Physiol (in press)