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Temporal Expression of Autocrine Growth Factors Corresponds to Morphological Features of Mesangial Proliferation in Habu Snake Venom-Induced Glomerulonephritis

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Habu snake venom induces an accelerated mesangial proliferative glomerulonephritis that follows a predictable course from early capillary aneurysms to micronodules comprised of confluent mesangial cells within 72 hours. We examined morphologically the course of mesangial cell proliferation and correlated it with the expression of messenger (m)RNA encoding two peptide growth factors, platelet-derived growth factor (PDGF) A and B chains and transforming growth factor-β (TGF-β). Rats were uninephrectomized and 24 hours later injected with Habu snake venom or saline. Kidney cortex and isolated glomeruli were obtained 24, 48, and 72 hours later for histological assessment, preparation and Northern analysis of mRNA, and immunohistochemical localization of PDGF using a polyclonal antibody that recognizes A and B chains. Maximal expression of PDGF B chain mRNA occurred at 24 hours and before the onset of mesangial cell proliferation; whereas maximal expression of PDGF A chain and TGF-β mRNA occurred at 48 hours and during active mesangial cell proliferation. Expression of TGF-β mRNA persisted at 72 hours at a time when PDGF A chain declined and PDGF B chain was not expressed compared to uninephrectomy and saline controls and at a time when mesangial cells within lesions reached confluence and proliferation subsided. PDGF protein localized in glomerular lesions associated with platelets at 24 and 48 hours and within mesangial cells at 48 and 72 hours. These results agree with the known roles of PDGF and TGF-β as positive and negative modulators, respectively, of mesangial cell growth in vitro and suggest that a relative balance of the expression of these factors may operate in glomerular disease in vivo. (Am J Pathol 1993, 143:1366–1376)

Proliferative glomerular diseases are common causes of permanent kidney damage. *In vivo* and *in vitro* studies suggest an important role for proliferating intrinsic glomerular cells as well as infiltrating inflammatory cells and platelets in the pathogenesis of proliferative glomerular disease. Platelets and their secretory products have been implicated as mediators of immune complex localization and glomerular cell proliferation in glomerular disease. Platelets contain and secrete a variety of mitogens that can directly influence glomerular cell growth.[1–3] These include platelet-derived growth factor (PDGF), transforming growth factor (TGF)-α and β, platelet factor 4 (PF4), epidermal growth factor, and a variety of other biologically active products. Of these secretory proteins, PDGF is a highly potent stimulator of mesangial cell proliferation *in vitro*.[4–6] In addition, mesangial cells are capable of synthesis and secretion of PDGF in response to serum and a number of peptide mitogens released from platelets or activated macrophages, completing an autocrine loop in cell proliferation.[6,7] Conversely, another platelet secretory protein, TGF-β has been shown to be a potent inhibitor of mesenchymal cell,[8] including mesangial cell[9–11] proliferation *in vitro*. TGF-β is an ubiquitous protein that is synthesized by many cell types including mesangial

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cells.[11–14] Thus, following activation, mesangial cells may regulate their own proliferation through autocrine pathways involving PDGF. TGF-β may operate through a negative autocrine loop to terminate cell proliferation. Indeed, recent studies have emphasized the role of activation of autocrine pathways involving mesangial expression of PDGF and TGF-β messenger (m)RNA in experimental and human renal disease.[12–21] Studies to date, however, have not simultaneously examined the temporal expression of both of these modulating peptides in a model of proliferative glomerular disease.

The objective of the current study was to examine the temporal course of events, from early platelet activation to the stimulation of autocrine pathways involving expression of PDGF and TGF-β mRNA in a platelet-dependent model of mesangial proliferative glomerulonephritis induced by Habu snake venom (HSV). This model conveniently allows the study of such regulatory autocrine pathways due to the rapid proliferation of mesangial cells within HSV-induced lesions, resulting in a confined mass of mesangial cells forming micronodules by 3 days. The course of glomerular localization of PDGF protein and expression of mRNA encoding PDGF A and B chain isoforms and TGF-β were related to the onset and abatement of mesangial cell proliferation.

Materials and Methods

Induction of HSV-Induced Glomerular Lesions

Glomerular lesions were induced by injection of HSV in an identical manner as previously described.[22] A total of 49 male Sprague-Dawley rats weighing 200 to 250 g (Charles River, Wilmington, MA) were unilaterally nephrectomized to increase the total population of glomerular lesions after injection of HSV. The next day, the rats were divided into two groups, one group received HSV (3.5 mg/kg, intravenously) and the other group received saline vehicle. The animals were subdivided and sacrificed at 24, 48, and 72 hours after HSV or saline. Immediately after removal of the kidneys, cortical tissue was obtained for histological assessment of glomerular lesions and immunoperoxidase or immunofluorescence localization of cell phenotypic markers, and the presence of a specific platelet α granule marker (PF4) and the cytokine PDGF (see below). Attempts to detect TGF-β 1 protein in glomerular lesions were unsuccessful. The remaining kidney cortex from each group was pooled and glomeruli were isolated for preparation of total RNA for Northern analysis of mRNAs encoding the autocrine growth factors PDGF and TGF-β (see below). The unilaterally nephrectomized kidneys were processed in an identical fashion on the day of removal and served as additional controls.

Histological Assessment of Glomerular Lesions

Renal cortex was trimmed from each kidney and slices cut and snap-frozen in liquid nitrogen for subsequent preparation of frozen sections and immunohistological analysis. Additional slices of cortex were immersed in 10% neutral buffered formalin for routine processing and embedding in paraffin. Four-μ thick sections were cut and stained with hematoxylin and eosin or stained for immunohistological assessment of cell type, localization of PDGF and PF4 as described below.

Glomerular lesions were quantitated according to the morphological classification described previously.[23] Briefly, three general categories of lesions were observed by light microscopy over the course of development of glomerular proliferative lesions: 1) cystic, a lesion characterized by ballooning of glomerular capillary lumens forming dilated microaneurysms filled with blood elements, including large platelet aggregates, erythrocytes, and leukocytes (Figure 1A); 2) mixed proliferative lesions, characterized by the appearance of numerous mesangial cells as individual cells or clusters of cells distributed throughout the microaneurysm; cells were surrounded by blood elements and platelet aggregates (Figure 1B); and 3) micronodular lesions comprised exclusively of proliferative cells characterized by confluent masses of cells filling the microaneurysm (Figure 1C). The total number of glomerular lesions and the distribution of cystic, proliferative, and micronodular lesions were quantitated in histological sections of each kidney. At least 100 glomeruli were assessed in each section.

Characterization of Cell Type within Glomerular Lesions

Cell types within glomerular lesions were characterized by phenotypic identification by immunohistological analysis utilizing primary antibodies to the following marker proteins: mesangial cells—mouse anti-desmin (Dako Corp., Carpinteria, CA), mouse monoclonal anti-rat Thy 1.1 (Accurate Chemical &

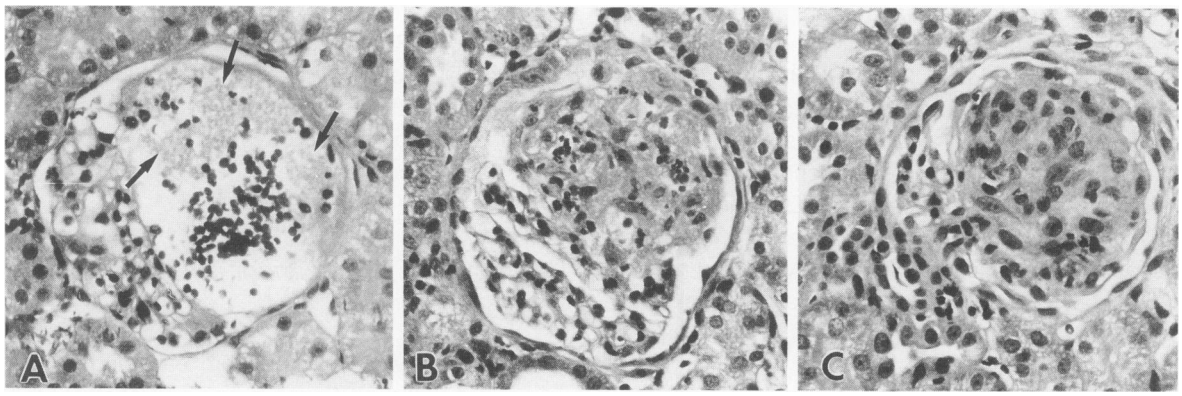

Figure 1. *Glomerular lesions 24, 48, and 72 hours after HSV. At 24 hours after HSV* (**A**), *lesions are characterized by microaneurysms filled with platelet aggregates* (*arrows*), *erythrocytes, leukocytes, and plasma proteins. At 48 hours after HSV* (**B**), *microaneurysms contain isolated or clusters of mesangial cells. Most lesions at 72 hours after HSV* (**C**) *are characterized by formation of micronodules comprised of confluent mesangial cells.*

Scientific Corp., Westbury, NY), and mouse monoclonal anti-α smooth muscle actin (Sigma Chemical Co., St. Louis, MO); endothelial cells—rabbit anti-human factor VIII-associated antigen (Axell, Accurate Chemical) and biotinylated Ulex Europaeus agglutinin 1 lectin (Vector Laboratories Inc., Burlingame, CA); epithelial cells—guinea pig anti-bovine keratin (Sigma); monocytes/macrophages—mouse monoclonal anti-rat myeloid cell (ED-1) (Sero-Tech, Bioproducts for Science, Inc., Indianapolis, IN) and mouse monoclonal anti-Ia surface antigen (Sera-lab, Accurate Chemical). Biotinylated secondary antibodies against their respective primary antibodies were goat anti-rabbit immunoglobulin G (IgG) (Vector), rat monoclonal anti-mouse IgG or IgM (Zymed Laboratories, Inc., San Francisco, CA) or rabbit anti-guinea pig IgG. Controls consisted of diluent without primary antibody or non-immune IgG or IgM of the appropriate species of primary antibody. Dewaxed paraffin or acetone fixed frozen sections were treated in a similar fashion as previously described[22] with minor exceptions accommodating the use of the avidin-biotin complex immunoperoxidase methods (Vector) as suggested by the manufacturer. In addition, formalin-fixed sections were incubated in 0.1 mol/L sodium acetate, pH 5.5, containing 1 mg/ml hyaluronidase and 0.85% sodium chloride before incubation with primary antibody. Sections were blocked with normal serum of the same species as the second antibody to block nonspecific antibody staining. Sections were also incubated with 0.6% hydrogen peroxide in methanol, to block nonspecific peroxidase activity, and 0.01% avidin, 0.001% biotin to block localization of endogenous biotin activity, respectively. Sections were evaluated and photographed utilizing a Zeiss Universal Research microscope.

Localization of PF4 (Platelet Secretory Products) PDGF Protein and Type IV Collagen in Glomerular Lesions

Localization of platelet secretory products in glomerular lesions was assessed by immunodetection of PF4, a specific marker for platelet α granule constituents. Antiserum to rat PF4 was developed in rabbits as previously described.[22] Glomerular localization of PDGF protein was also examined by immunoperoxidase histochemistry utilizing a rabbit anti-human PDGF AB primary antibody (R&D Systems Inc., Minneapolis, MN) specific to PDGF A and B chains. The rabbit anti-human PDGF antibody was determined to cross-react with rat platelet releasate by dot-blot immunodetection as previously described.[22] The temporal synthesis of extracellular matrix was assessed by the glomerular localization of type IV collagen, using a rabbit anti-mouse antibody (Collaborative Biomedical Products, Becton Dickinson, Bedford, MA). Detection of primary antibodies was performed using biotinylated second antibodies and the avidin-biotin complex method as described above.

Analysis of Glomerular mRNA That Encodes Autocrine Cytokines

Kidneys from seven to nine rats in each group were pooled and glomeruli were isolated by the graded

sieving technique[24] using cold 2 to 4 C buffer (Hanks' balanced salt solution) containing 0.05% diethylpyrocarbonate to prevent RNA degradation. A sample of each glomerular isolate was fixed in 1.25% glutaraldehyde for subsequent morphological assessment, and the remainder was used for preparation of RNA utilizing guanidine thiocyanate extraction according to the method of Chirgwin et al.[25] Glomeruli were homogenized in 5.0 mol/L guanidine thiocyanate with a Kinematica polytron (Brinkman Instruments, Westbury, NY). Cesium chloride and sodium N-lauroyl-sarcosinate were added to the supernatant to make a solution containing 0.4 g/ml and 2.0% respectively. The samples were heated at 65 C for 15 minutes, then centrifuged at 35,000 RPM for 18 hours at 21 C through 5.7 mol/L cesium chloride, 0.1 mol/L ethylenediaminetetraacetic acid pads utilizing a SW 50.1 rotor (Beckman Instruments, Fullerton, CA). The pellet was resuspended in 5 mmol/L sodium citrate, 5 mmol/L ethylenediaminetetraacetic acid, and 1% sodium dodecyl sulfate, pH 7.5, followed by extraction with chloroform/butanol at a ratio of 4:1 volume/volume. The RNA was precipitated with 100% ethyl alcohol containing 3 mol/L sodium acetate, pH 6.0. The purity and concentration of glomerular RNA was determined from the absorbance at 260 and 280 nmol/L wave length. This method produced excellent nondegraded preparations of RNA as assessed by electrophoresis in 1% agarose gels and subsequent visualization of 28S and 18S ribosomal bands.

Total glomerular RNA (15 μg/lane) representing all time points was fractionated on 1% agarose-formaldehyde gels and transferred to GeneScreen (DuPont, NEN Research Products, Boston, MA). The transferred blots were hybridized with mouse PDGF B chain (CB8.A1A2) and mouse PDGF A (F9A5) complementary DNA probes cloned into pGEM-1 plasmids (gift from Dr. Charles Stiles) as described by Mercola et al.[26] The TGF-β 1 probe was a 1050-bp complementary DNA fragment cloned into SP 64 (gift from Dr. Rik Derynck, Derynck et al).[27] All probes were labeled by nick translation with [^{32}P]-UTP. Prehybridizations and hybridizations with radiolabeled probes were carried out as described previously.[7] The blots were stripped of radiolabeled probe by boiling then rehybridized with ^{32}P-labeled α tubulin complementary DNA probe as an internal control.[7] Relative levels of expression of mRNA were compared by densitometry. Glomerular expression of α-tubulin mRNA in Northern blots was slightly higher in HSV-injected animals (by approximately 10%) compared to controls, based on densitometric analysis of autoradiograms. Thus, all data were normalized to α-tubulin to accommodate changes in the expression of mRNA that encodes for other proteins besides cytokine and matrix mRNAs.

Analysis of Cell Proliferation

Proliferation of mesangial cells within lesions was assessed by identification of cellular incorporation of [^{3}H]thymidine into nuclear DNA using autoradiographic techniques. A separate group of 15 rats was prepared and given HSV as described above. One hour before sacrifice at 24, 48, and 72 hours after HSV, the rats were anesthetized and [^{3}H]thymidine, 6.7 Ci/mmol, (Dupont, NEN Research Products) was injected intravenously into a tail vein at a dose of 1 μCi/gm body weight. Kidney cortex was excised, trimmed, and processed for paraffin embedding as already described. Dewaxed paraffin sections (4 μ) were immersed, in the dark, in Kodak NTB-2 photographic emulsion (Eastman-Kodak, Rochester, NY) diluted with equal parts of 0.6 mol/L ammonium acetate and prewarmed to 45 C. The slides were kept in the dark, allowed to air dry, then exposed for a minimum of 2 weeks. The emulsion was then developed and stained with hematoxylin and eosin for subsequent light microscopical analysis. Twenty-five randomly selected glomeruli in each kidney section were evaluated for the total number of cells showing incorporation of labeled thymidine by identification of nuclei with at least five grains in the adjacent emulsion.

Quantitation of Macrophages within Glomerular Lesions

Because macrophages might contribute to the pathological events, including the increased expression of cytokine mRNA, the number of macrophages present within lesions throughout the course of HSV-induced glomerulonephritis was determined. Sections stained with ED-1 antibody, as above, were examined for positive staining cells and the number of macrophages per glomerulus recorded.

Results

Morphological Assessment of Glomerular Lesions

HSV induced a proliferative glomerulonephritis over the course of 3 days similar to those described previously.[22,23,28,29] HSV induced an average of 38% glomerular lesions (24 hours: 34 ± 5.6%; 48 hours: 43% ± 4.3% and 72 hours: 37% ± 3.7%) (mean ± standard error). At 24 hours after HSV injection, 100% of glomerular lesions were of the cystic type, characterized by formation of microaneurysms filled with large platelet aggregates, inflammatory cells, erythrocytes, and plasma proteins (Figure 1A). At 48 hours, the majority of microaneurysms (75%) contained numerous round or ovoid cells dispersed throughout the lesion (Figure 1B). A small percentage (17%) of the lesions were identical to those described at 24 hours. The remaining lesions were characterized by a replacement of the microaneurysm lumen with a confluent mass of cells forming micronodules. Approximately 75% of lesions at 72 hours were of the micronodular type (Figure 1C), whereas 25% and 1% of the lesions were of the mixed proliferative and cystic type, respectively.

Phenotypic identification of cells within lesions revealed that the cell type involved in the proliferative lesions was almost exclusively mesangial in origin, based on staining properties of desmin and Thy-1 antigen at 48 and 72 hours and α-smooth muscle actin at 72 hours (Table 1). Cells within lesions did not stain for factor VIII-associated antigen, Ulex Europaeus agglutinin 1, or keratin, excluding a significant contribution by endothelial and epithelial cells to the proliferative cell population. Monocytes/macrophages were identified in lesions at all time periods; but the presence of these cells was variable and when present occurred as a few isolated ED-1- or Ia-positive cells within the central aspects of microaneurysms at 24 and 48 hours or within micronodules at 48 and 72 hours. Occasional macrophages were observed in intact glomerular tufts; but, in most cases, glomeruli and nodular lesions were devoid of these cells consistent with previously published reports.[22] Quantitative studies revealed that glomerular lesions 24 hours after HSV had an average of 1.7 ± 0.2 (SEM) macrophages. This number doubled at 48 hours after HSV to 3.5 ± 0.3 macrophages per glomerular lesion. At 72 hours after HSV, the number of macrophages decreased to an average of 2.8 ± 0.2 macrophages per lesion.

Platelet secretory products as determined by localization of PF4 as a marker, were observed in glomerular lesions at all time periods. Intense staining for PF4 was observed in platelet aggregates and diffusely throughout the glomerular lesions at 24 and 48 hours after HSV (Figure 2). PF4 was also observed in many micronodular lesions, localized between cells, but weaker in intensity than at earlier periods. PF4 did not seem to stain cells within lesions. Control glomeruli did not stain for PF4 or PDGF (Figure 3A). Corresponding to PF4, PDGF localized to platelet aggregates and was present diffusely within glomerular lesions at 24 and 48 hours after HSV (Figure 3, B and C). In addition, numerous mesangial cells within 48-hour lesions (Figure 3C) and within micronodules at 72 hours after HSV (Figure 3D) stained for PDGF. Most staining seemed to be cellular, however, some PDGF localization to extracellular matrix cannot be excluded and

Table 1. *Phenotypic Identification of Cells within Glomerular Lesions after HSV*

Cell type	Proliferative lesions
Mesangial cells	
Desmin	+
Thy-1	+
α-SM actin	+*
Endothelial cells	
Ulex Europaeus agglutinin I	–
Factor VIII-associated antigen	–
Epithelial cells	
Cytokeratin	–
Monocytes/macrophages	
Ia surface antigen	+†
MCA (ED-1)	+†

* α-smooth muscle actin was observed in advanced micronodular lesions.

† Isolated cells in lesions. Most micronodules were comprised exclusively of mesangial cells.

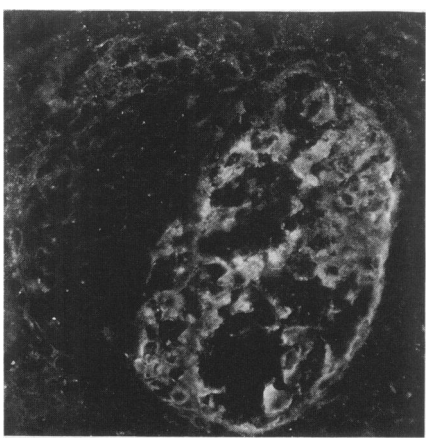

Figure 2. *Glomerular lesion 48 hours after HSV, illustrating immunofluorescence localization of PF4. This specific α-granule secretory protein localizes throughout the lesion but not in cellular locations.*

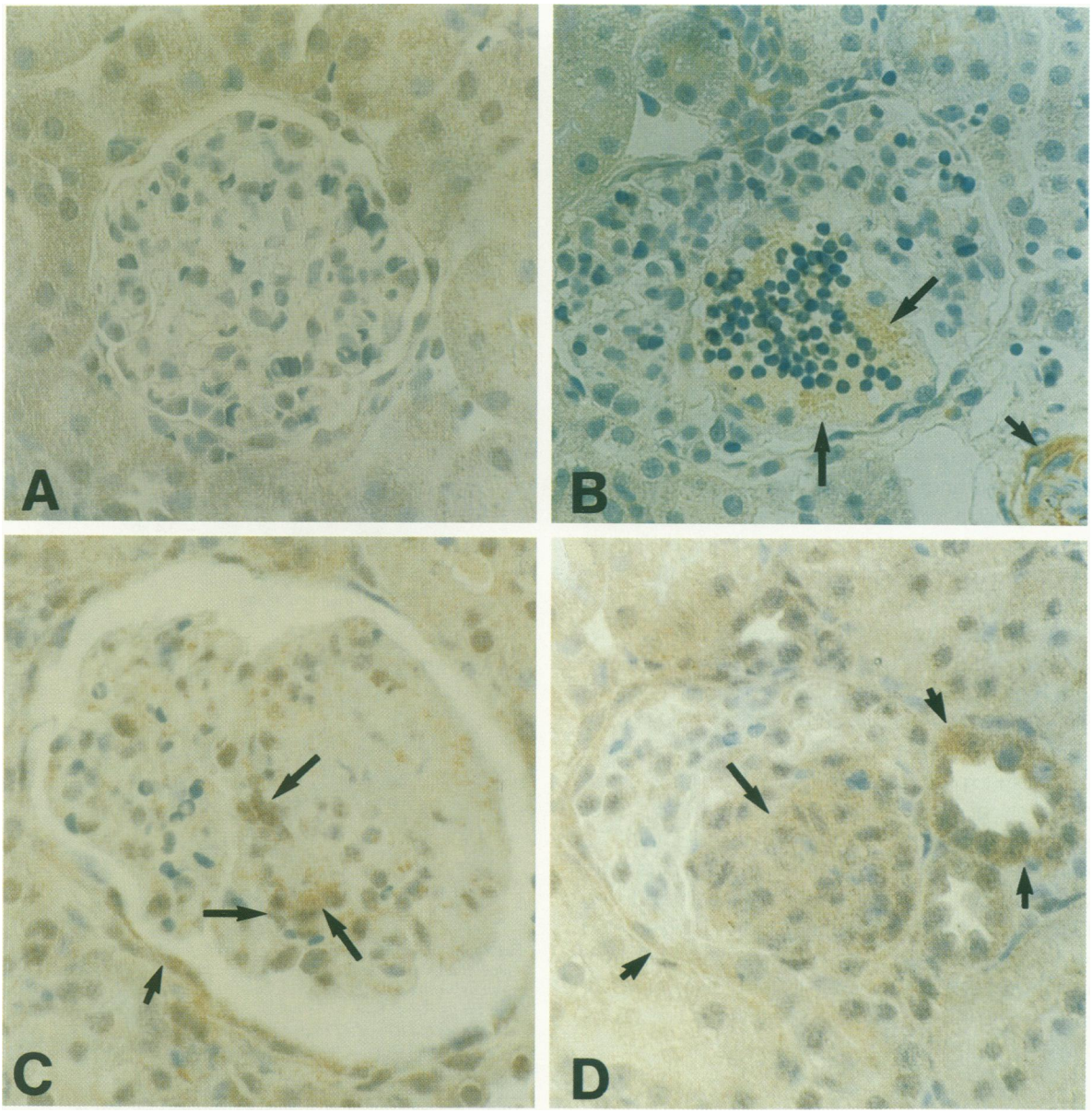

Figure 3. *Immunoperoxidase localization of PDGF in glomeruli from a saline control* (**A**), *and from rats at 24* (**B**), *48* (**C**), *and 72* (**D**) *hours after HSV. PDGF localizes only to the platelet aggregates* (*arrows*) *at 24 hours. In addition, PDGF can be observed in isolated mesangial cells* (*arrows*) *in lesions at 48 hours and in cells forming micronodules at 72 hours after HSV. Small arrows point to increased PDGF protein in Bowman's capsule, distal tubules, or arteries after HSV.*

may require higher resolution studies with immunoelectron microscopy. Parietal epithelial cells lining Bowman's capsule also showed increased expression of PDGF protein, particularly at 48 and 72 hours (Figure 3, C and D) after HSV. Cortical structures of controls did not stain for PF4; however, PDGF was present, albeit in weak intensities, in the arterial microvasculature and distal tubules and collecting duct cells. PDGF protein was present at higher intensities in these same structures after HSV (Figure 3, C and D).

Glomerular Expression of mRNA Encoding Autocrine Growth Factors

We next examined the expression of mRNA encoding PDGF A and B chains and TGF-β in isolated

glomeruli. Morphological analysis of the glomerular preparations revealed a purity of approximately 90% showing structurally intact glomeruli from HSV-treated rats and controls. A sample of each of the glomerular preparations was pelleted and fixed in 1.25% glutaraldehyde, 0.1 mol/L cacodylate buffer, pH 7.4, dehydrated through graded alcohols and embedded in plastic. Subsequent examination of toluidine-stained sections revealed that the same average percentages of glomerular involvement as quantitated in tissue sections were observed in sections of pellets obtained from glomerular isolates, indicating that sieving was not preferential for either normal or injured glomeruli.

Northern analysis of glomerular RNA revealed differential increase in the expression of PDGF A and B chain and TGF-β mRNA over the course of the progression of HSV-induced glomerular lesions (Figure 4, Table 2). Message for all growth factors was expressed at 24 hours at levels higher than controls (Figure 4, A to C). PDGF B chain was maximally expressed at 24 hours showing levels of hybridization 1.5-fold higher than baseline nephrectomy controls (Figure 4B). By 72 hours, PDGF B chain mRNA was down compared to baseline. In contrast, PDGF A chain and TGF-β mRNA were maximally expressed at 48 hours after HSV, showing levels of approximately six- and 1.7-fold over controls, respectively (Figure 4, A and C). At 72 hours, expression of TGF-β mRNA persisted, whereas PDGF A chain was substantially reduced (Figure 4, A and C). Northern blots using pooled RNA from selected time periods obtained from another experiment showed similar profiles of PDGF and TGF-β mRNA expression verifying the reproducibility of these data.

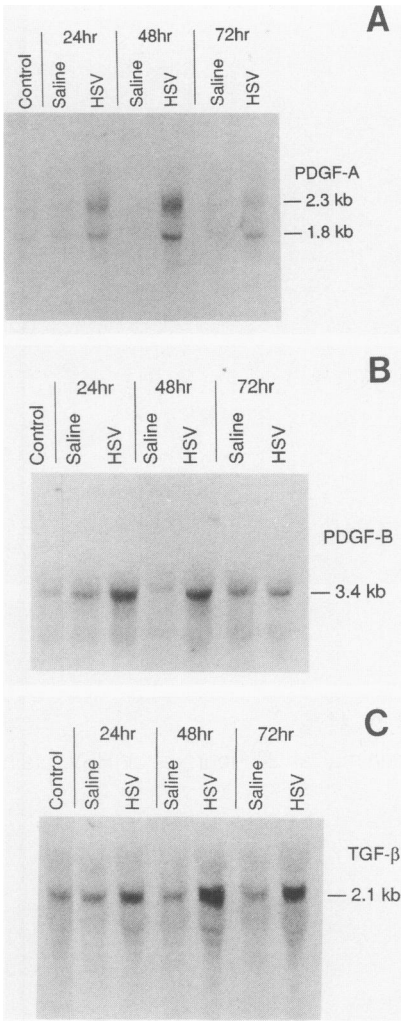

Figure 4. *Temporal expression of mRNA for PDGF A chain* (**A**), *PDGF B chain* (**B**), *and TGF-β* (**C**) *over the course HSV induced glomerular injury. PDGF B chain peaks at 24 hours, whereas PDGF A chain and TGF-β peak at 48 hours. At 72 hours after HSV, TGF-β remains elevated whereas PDGF A and B chains decline. Control = nephrectomized kidney, 24 hours before injection of saline or HSV.*

Table 2. *Expression of Glomerular mRNA Encoding PDGF A and B Chains and TGF-β1 in HSV and Saline-Treated Rats Relative to Controls*

	24 hours		48 hours		72 hours	
	Saline	HSV	Saline	HSV	Saline	HSV
PDGF A	0.9*	4.4	0.8	6.1	0.8	2.2
PDGF B	1.1	1.5	0.8	1.3	1.0	0.8
TGF-β_1	1.1	1.4	1.1	1.7	1.0	1.4

* Values represent densitometric measurements obtained from Northern blots and expressed relative to baseline controls (saline or HSV ÷ baseline nephrectomy control).

Cell Proliferation

Autoradiographic studies utilizing [^{3}H]thymidine showed that mesangial cell proliferation (defined as five or more grains in the emulsion adjacent to the cell) peaked at 48 hours and subsided at 72 hours after HSV (Figure 5). Glomerular lesions at 24 hours were essentially devoid of proliferating cells, showing an average of 0.1 ± 0.03 positive cells/lesion by autoradiography. On the other hand, 95% of the lesions at 48 hours demonstrated proliferating cells by autoradiography, with an average of 5.1 ± 0.6 positive cells/lesion. Those few lesions that did not contain positive cells were of the cystic type without proliferating cells in the microaneurysm. Lesions at 72 hours demonstrated fewer average number of proliferating cells 3.2 ± 0.4 positive cells/lesion. Also, 85% of the lesions showed cells positive for [^{3}H]thymidine. The remaining 15% of the lesions that did not show positive cells were of the advanced micronodular type of lesion.

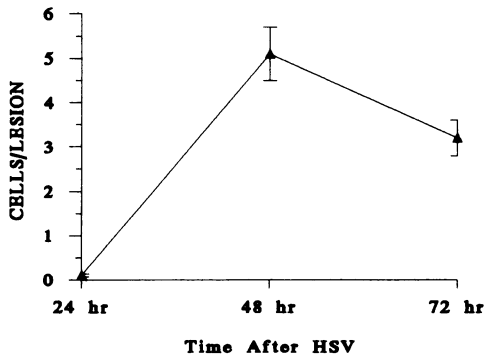

Figure 5. *Autoradiographic analysis of cell proliferation over the course of HSV-induced proliferative glomerulonephritis. Proliferating cells are rarely observed 24 hours after HSV; whereas glomerular lesions contain an average of 5.1 ± 0.6 (SEM) positive ([³H]thymidine) cells at 48 hours after HSV. Proliferation subsides at 72 hours after HSV showing an average of 3.2 ± 0.4 (SEM) positive cells/glomerular lesion.*

Type IV Collagen

The results showed that glomerular staining for type IV collagen correlated with matrix expansion and cellularity within lesions (Figure 6). Staining for collagen was minimal at 24 hours after HSV, staining few cells at the margins of lesions (Figure 6A). Staining became progressively more intense at 48 and 72 hours (Figure 6, B and C) after HSV, with the greatest intensity observed at 72 hours (Figure 6C).

Discussion

HSV induces a proliferative glomerulonephritis characterized initially by focal glomerular injury that leads to the formation of microaneurysms filled with platelet aggregates followed by the rapid proliferation of mesangial cells. These studies demonstrate a temporal relationship between the expression of mitogenic and matrix promoting peptides and glomerular morphological changes that follow a well-defined acute injury model. Previous studies demonstrated that glomerular lesions are associated with platelet secretory products (PF4) throughout the course of the disease.[22,23] We now demonstrate that PDGF, an α granule constituent, is also observed in glomerular lesions associated with platelets. In addition, PDGF was observed in mesangial cells within proliferative lesions, suggesting cellular synthesis of this autocrine cytokine.

Additionally, mRNAs encoding PDGF A and B chains and TGF-β were differentially expressed in isolated glomeruli over the course of study. Maximal expression of PDGF B chain mRNA occurred at 24 hours at a time point that precedes cell proliferation. This was followed by increased expression of PDGF A chain and TGF-β at 48 hours after HSV and during active proliferation. At 72 hours after HSV, when most lesions were of the nodular type and proliferation subsided, expression of TGF-β mRNA remained elevated, whereas PDGF A and B chain mRNA declined. Small increases of PDGF B chain were observed in glomeruli from saline-injected rats sacrificed 24 hours after injection of saline compared to the normal kidneys removed 48 hours earlier. It is not known if these changes are associated with the compensatory proliferation and hypertrophy that follow nephrectomy or if these minor changes reflect nonspecific effects of anesthesia and surgery. It is conceivable, however, that the nephrectomy primed the kidney, and specifically the glomeruli, to the HSV injection and therefore potentiated the increment in PDGF B chain expression.

These studies suggest that the primary source of PDGF in early lesions is derived from platelets because of the detection of platelet-associated staining and a lack of cellular staining at 24 hours after

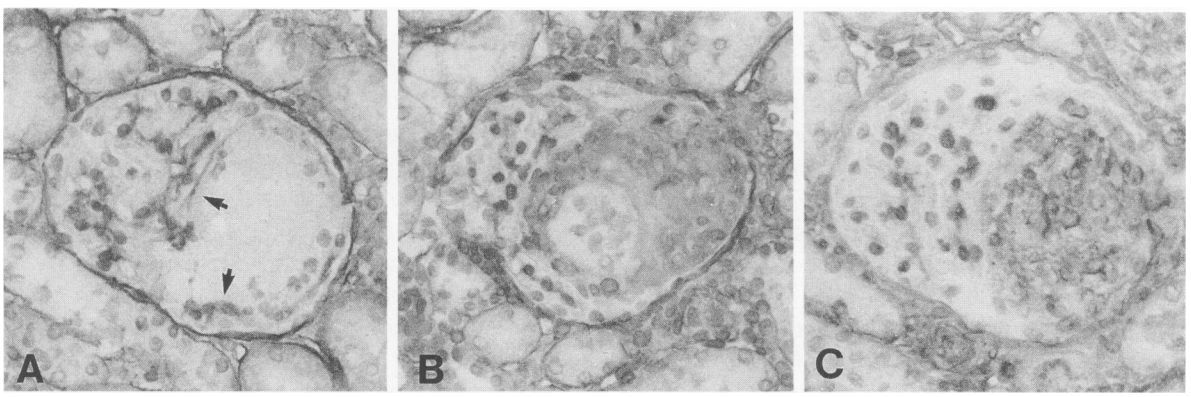

Figure 6. *Immunostaining for type IV collagen in glomerular lesions 24 (A), 48 (B), and 72 (C) hours after HSV. Type IV collagen is present in cells in the periphery of lesions (arrows) 24 hours (A) after HSV and increases in intensity at 48 (B) and 72 (C) hours after HSV corresponding to the degree of cellularity.*

HSV. Despite the absence of cell proliferation and cellular staining of PDGF at 24 hours, glomerular cells were activated as determined by the enhanced expression of PDGF A and B chains and TGF-β mRNA at this time. The source of enhanced levels of mRNA for PDGF and TGF-β at 24 hours after HSV is not precisely defined; however, a rapid proliferation of mesangial cells at later time periods and expression of PDGF message and translated PDGF protein indicate that mesangial cells are primarily responsible for increases in expression of these cytokines. In a separate study, we have shown that mesangial cells undergo a redistribution and margination in early glomerular lesions. This margination, interpreted as migration, was platelet-dependent and reflects stimulation of early events before cell division and proliferation[30] and is consistent with the ability of mesangial cells to migrate toward gradients of platelet secretory products *in vitro*.[31,32] Thus, it seems likely that these activated mesangial cells are the source of elevated expression of mRNA encoding PDGF and TGF-β at 24 hours after HSV; however, other sources of mRNA encoding these cytokines, such as a small contribution by macrophages, cannot be excluded. Platelets also contain and secrete PDGF BB[33] and contain very small amounts of mRNA-encoding secretory products.[34] However, mRNA for these products, unless they are abundant like PF4, usually cannot be detected by Northern analysis and require amplification, such as in the case of polymerase chain reaction methodology.[34,35] Thus, it seems unlikely that a significant amount of the cytokine mRNA detected in our experiments is derived from platelets. By 48 hours after HSV, mesangial cells themselves undergo translational expression of PDGF protein coinciding with a rapid cellular proliferation. Nearly all lesions at this time contained proliferating cells based on thymidine incorporation studies. Also, mRNA for PDGF A chain and TGF-β were maximally expressed, associating synthesis of these peptides with cell proliferation. At 72 hours after HSV, expression of mRNA for PDGF A and B chains declined and TGF-β message remained elevated, at a time when the majority of glomerular lesions were of a micronodular type with matrix expansion and when proliferation subsided. Cellular localization of PDGF protein was still detected at this time, perhaps reflecting residual posttranslational protein. Additionally, some PDGF may have localized to the extracellular matrix as recently reported by Nakajima et al.[36]

A role for platelets and their secretory products as initiators of cell proliferation has been suggested by the observations that platelet depletion or antiplatelet therapy inhibits mesangial cell redistribution and proliferation in glomerular lesions in this model.[29,30] Similarly, platelet-induced initiation of mesangial cell proliferation has been proposed in two additional models of mesangial proliferative glomerular disease induced by anti-thymocyte (Thy-1) antibody[18] and subtotal renal ablation.[16] In all three of these models, platelets were associated with mesangial cell proliferation and expression of PDGF, suggesting that mesangial cells may sustain cell proliferation through autocrine pathways.

The above data are consistent with the known effects of PDGF as a potent stimulator of mesenchymal cell proliferation.[13] Mesangial cells also respond vigorously to PDGF[4,6] and are known to produce PDGF through autocrine pathways *in vitro*[6,7] and *in vivo*.[13,16–18] On the other hand, TGF-β has potent antiproliferative effects on epithelial, endothelial, and most mesenchymal cells[8] and is a negative regulator of mesangial cell proliferation.[9,10] TGF-β, however, has been shown to have a biphasic effect on cultured mesangial cells, depending on the state of confluence.[10] TGF-β has been suggested to be involved in a switch from cell proliferation to differentiation and synthesis of extracellular matrix[8] and may be important in regulation of extracellular matrix synthesis in glomerulosclerosis.[12,14,21,37–39] Indeed, we observed that glomerular localization of type IV collagen was directly related to the degree of matrix accumulation and cellularity within lesions. The most intense staining occurred 72 hours after HSV and was associated with persistent elevations in TGF-β. Thus, the initiation, perpetuation, and termination of cell proliferation and onset of extracellular matrix synthesis may involve a complex balance between growth regulatory peptides derived from exogenous (platelets or other inflammatory mediators) and endogenous (autocrine) sources as well as the state and the cell population expressing PDGF and TGF-β. It should be pointed out that platelets and macrophages secrete and mesangial cells respond to and synthesize a variety of other biologically active peptides that may also be involved in growth regulatory activity. The specific roles that these growth factors have in the pathogenesis of mesangial proliferation in this model remain to be determined.

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