Virchows Arch (1995) 427:195–204

ORIGINAL ARTICLE

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Endothelial regeneration during the repair process following Habu-snake venom induced glomerular injury

Received: 20 March 1995 / Accepted: 18 July 1995

Abstract In this study, the kinetics of glomerular endothelial cells during the repair process following glomerular injury was investigated in a model of mesangial proliferative glomerulonephritis induced by Habu-snake venom (HSV) in rats. Intravenous injection of HSV led to a cystic ballooning type lesion at day 1. Subsequently a marked segmental proliferative lesion was observed in the cystic areas at day 5. Thereafter cellularity decreased and reconstruction of the glomerular tuft was gradually observed with time. The histological structure of the glomeruli had almost returned to normal 21 days following HSV injection. After prominent depletion at day 1, the number of endothelial cells increased rapidly and reached a plateau at day 7, not significantly different from that of the control group. Morphologically endothelial cell elongation from the vascular pole into the cystic lesion was seen together with premature capillary formation in the proliferative lesion. Accompanying the reduction of mesangial expansion, the endothelial cells gradually formed definite capillary lumens. We conclude that the mesangial proliferative glomerulonephritis induced by HSV recovers to its original structural state and that the migration and proliferation of endothelial cells with accompanying capillary formation are essential for the repair process, in addition to mesangial cell proliferation.

Key words Glomerular endothelial cells · Mesangial proliferative glomerulonephritis · Habu-snake venom · Tissue repair · Endothelial regeneration

Introduction

Recovery from proliferative glomerulonephritis has been seen in experimental and human glomerulonephritis. In proliferative glomerulonephritis, proliferation of glomerular mesangial cells is a central feature that results not only in glomerular hypercellularity but also in accumulation of extracellular matrix [21]. Recent papers have partially clarified the repair mechanism following glomerular injury, namely that mesangial hypercellularity is reduced by apoptosis [2, 32] and that accumulated extracellular matrix is remodelled by 72 kDa type IV collagenase [23]. In vitro studies have demonstrated that endothelial cells and their products might modulate mesangial proliferation [7, 15, 29]. However the precise mechanisms that lead to repair after glomerular injury remain to be clarified.

In general, the endothelial cell is thought to play a crucial role in wound healing [20]. Morphological studies of repair processes after tissue injury have shown that the essential basic events are the proliferation and migration of endothelial cells leading to vascular formation and anastmosis of newly formed vessels [14, 28, 36]. In this connection, attention is directed to the hypothesis that the migration and proliferation of glomerular endothelial cells may participate in the repair processes after glomerular injury. However little information is available on the kinetics and the role of glomerular endothelial cells in such repair processes.

In this study we have focused on the kinetics of the glomerular endothelial cell in repair processes using the well-established model of mesangial proliferative glomerulonephritis induced by Habu-snake venom (HSV). This model is characterized by rapid recovery back to its original structure [26].

Materials and methods

Male Sprague-Dawley rats were used (Saitama Experimental Animal Supply, Saitama, Japan) weighing 180–200 g and were held at constant room temperature with free access to standard rat chow and water.

Lyophilized venom from Habu pit viper, *Trimeresurus flavoviridis* (Wako Pure Chemical industries, Osaka, Japan) was dissolved in saline and injected into rats at a dose of 0.3 mg per 100 g

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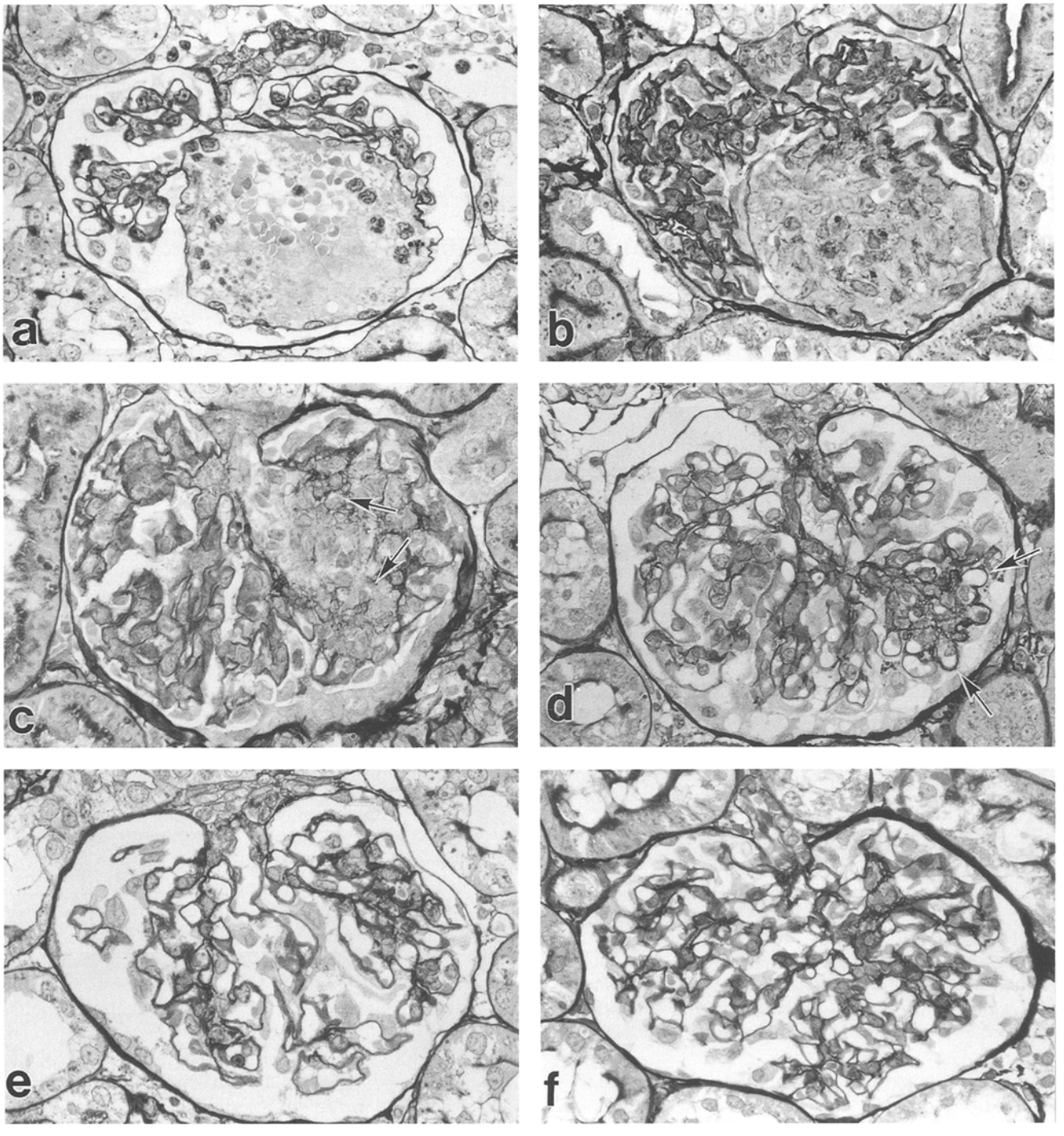


Fig. 1a–f Periodic acid methenamine silver stained tissue obtained at days 1 (**a**), 3 (**b**), 5 (**c**), 14 (**d**), 21 (**e**), and 42 (**f**) after Habu-snake venom (HSV) injection. At day 1 (**a**), glomerular lesion is characterized by segmental ballooning of glomeruli, the cystic lesion, filled with erythrocytes, neutrophils, platelets and fibrin. At day 3 (**b**), the cystic lesion reveals initial proliferative changes. At day 5 (**c**), lesions are filled with numerous mononuclear cells and slit-like spaces (*arrow*) are seen within the lesion. At day 14 (**d**), proliferative changes are reduced with definite formation of capillary lumina (*arrow*) and after 21 days, the glomeruli almost recover their normal structure (**e, f**). ×400

body weight via the tail vein. Control rats were given saline. Experimental and control rats were killed after an intraperitoneal injection of sodium pentobarbital (30 mg/kg) at 1, 2, 3, 4, 5, 6, 7, 14, 21 and 42 days after application of HSV or saline. Each experimental and control group consisted of two rats.

The tissue blocks were fixed in 20% formalin and embedded in paraffin for light microscopic examination. The deparaffinized sections were stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS) and periodic acid methenamine silver (PAM) stains for light microscopic examination. For electron microscopic study, small blocks were fixed in 2.5% glutaraldehyde solution in phosphate buffer (pH 7.4), post-fixed with 1% osmium tetroxide in phosphate buffer, dehydrated, and then embedded in Epon 812 mixture. Ultrathin sections were stained with uranyl acetate and

lead citrate, and examined in a Hitachi H-7100 electron microscope.

Tissue blocks for immunohistochemical studies were fixed in 20% formalin and embedded in paraffin. After deparaffinization, the specimens were stained by the indirect immunoperoxidase technique following Hsu et al. [17]. Primary antibodies included a polyclonal rabbit anti-rat thrombomodulin (TM) antibody [16] (provided by Dr. D. Stern) diluted 1:400, which reacted with the surface of endothelial cells, a murine monoclonal anti α-smooth muscle actin antibody (SMA) diluted 1:500 (Dako, Glostrup, Denmark), and a murine monoclonal antibody against rat proliferating cell nuclear antigen (PCNA) diluted 1:50 (Dako). PCNA is an auxiliary protein to DNA polymerase delta which is expressed during the late G_1, S, G_2, and M phase cell cycles [22]. For all specimens, negative controls consisted of substitution of the primary antibody with an irrelevant murine monoclonal antibody, or with the normal rabbit IgG.

For immunoelectronmicroscopy, frozen sections of 4% paraformaldehyde-fixed tissue were incubated with 10% normal swine serum for 10 min, then incubated with anti-TM antibody or control normal rabbit IgG at a dilution of 1:100 overnight at 4° C, followed by incubation with peroxidase labelled anti-rabbit IgG antibody (Cappel Laboratories, West Chester, Pa., USA) at a dilution of 1:50 at 4° C for 2 h. After washing three times in PBS, sections were fixed with 1% glutaraldehyde solution in PBS, immersed in DAB solution for 30 min, and then treated with DAB-hydrogen peroxidase solution for 5 min. Finally, they were post-fixed with 2% osmium tetroxide in PB, dehydrated and embedded in Epon 812 mixture. Ultrathin sections were examined in a Hitachi H-7100 electron microscope without counter staining.

For each kidney sample, 20 glomeruli were sequentially examined and the number of cells counted. Glomerular cross-sections containing only a minor portion of the glomerular tuft (<20 discrete capillary segments/cross-section) were not included in the analysis. Cellularity was expressed as the total number of nuclei per glomerular cross-section in tissue sections stained with the PAS reagent. The number of TM positive cells and PCNA positive cells in a glomerulus was counted.

The results are expressed as the mean and standard deviation with statistical analysis performed by Student's t-test.

Results

Light microscopy

Injection of HSV into rats produced rapid mesangiolysis leading to segmental ballooning of glomerular capillary loops, the cystic lesion, in 30% of glomeruli at day 1. Such areas of cystic lesion were filled with erythrocytes, neutrophils, platelets and fibrin. However the glomerular basement membrane (GBM) remained intact (Fig. 1a). The cystic lesion revealed initiation of cell proliferation with scanty matrix expansion 2 to 3 days after HSV administration (Fig. 1b). At day 5, cystic lesions were filled with large numbers of mononuclear cells, the numbers of infiltrating inflammatory cells were decreased, and morphologically the picture mimicked severe proliferative glomerulonephritis (Fig. 1c). In the peripheral region of the cystic lesion, particularly between points of attachment of cellular clusters to the basement membrane, capillary like lumina were occasionally detected and in the centres of proliferation, slit-like spaces were observed. PAM-positive matrix components were detected in the areas of proliferative lesions. At day 14, mesangial expansion was reduced and the slit-like spaces replaced the definite capillary lumina (Fig. 1d). From day 21 onwards, the glomeruli almost recovered their original normal structure, cell number and matrix components decreased, although mild segmental sclerotic lesions persisted in 1–2% of glomeruli (Fig. 1e, f). In control rats, the glomeruli showed no noteworthy histological changes throughout the experimental period.

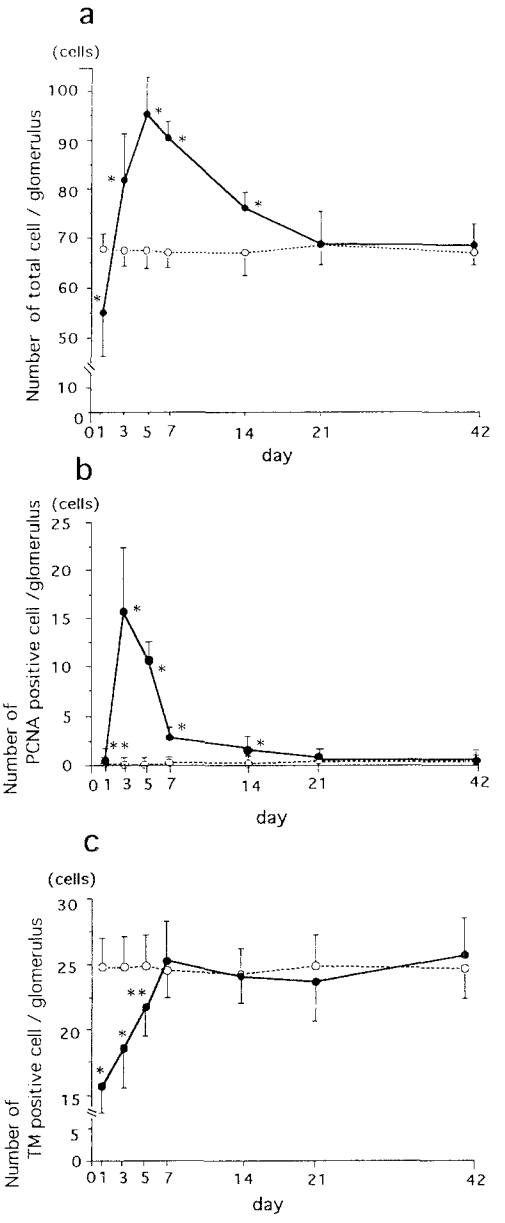


Fig. 2a–c The number of (**a**) total cells, (**b**) proliferating cell nuclear antigen (PCNA) positive cells, (**c**) thrombomodulin (TM) positive cells per glomerulus. Closed and open circles represent the numbers in experimental and control rats at various time periods. Values are expressed as mean ±SD. * $P<0.01$ when compared to control, ** $P<0.05$ when compared to control. Data derived from the analysis of 20 glomerular cross-sections

Figure 2a shows the total number of cells per glomerulus after HSV or saline injection. At day 1, in the experimental group the total number of cells was significantly decreased compared with the control group. Thereafter

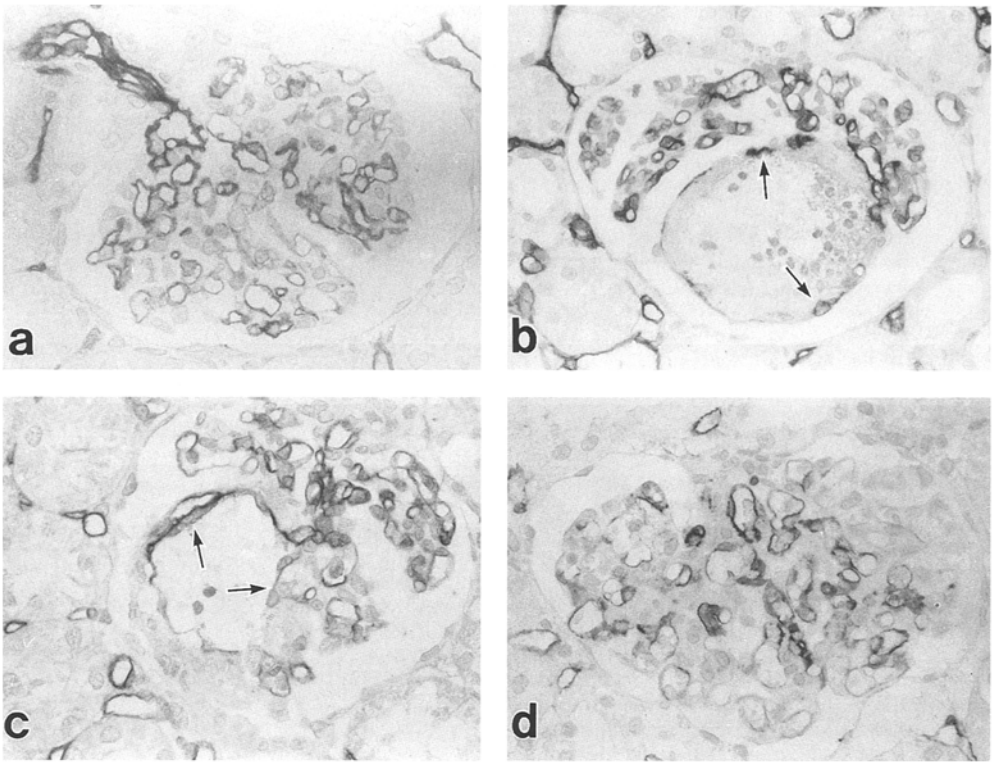


Fig. 3a–d Glomerular staining for TM in a control rat (**a**) and in experimental rats at days 1 (**b**), 2 (**c**), 14 (**d**). In the control rat, TM is localized on the exposed surface of the endothelial cell along the capillary lumina. At day 1 (**b**), the cystic lesion is devoid of TM positive cells, except for some focal peripheral regions (*arrow*). At day 2 (**c**), TM positive cells are seen inside the lesion (*arrow*). At day 14 (**d**), TM positive cells are more widely distributed with definite formation of capillary lumina. ×400

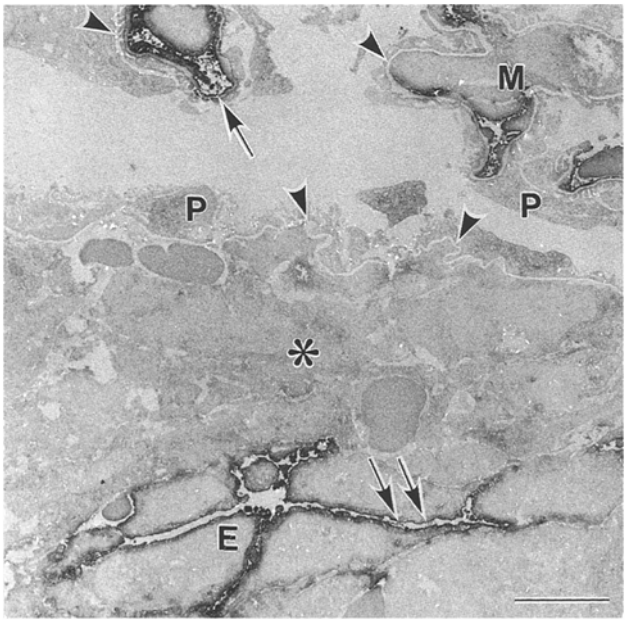


Fig. 4 Immuno-electron micrograph of a glomerulus at day 5 after HSV injection. The expression of TM is located along the exposed surface of endothelial cells (*arrow*) in non-affected tuft, and regenerating endothelial cells (*double arrow*) with tubular formation in the proliferative lesion (*asterisk*). It is not expressed in the mesangial or infiltrating cells. ×2,370; calibration bar=5 μm (▼: glomerular basement membrane; *M*, mesangial cell; *P*, podocyte; *E*, endothelial cell)

the total number of cells increased significantly, peaking at day 5, while the number of PCNA positive cells peaked at day 3 as shown Fig. 2b. From day 7, the total number of cells decreased slowly with time and returned to normal levels at day 21.

Immunohistochemistry

Tissue sections were stained by the immunoperoxidase technique with polyclonal anti-TM antibody for detection of endothelial cells. In the control rats, the expression of TM was confined to the exposed surface of the endothelial cells attached to the capillary loops (Fig. 3a). In experimental groups, TM antigen was not expressed in cystic lesions, except for some focal peripheral regions at day 1 (Fig. 3b). At day 2, TM positive cells now appeared inside the cystic lesions (Fig. 3c), and these TM positive cells in the cystic lesion connected with the afferent arterioles (Fig. 5a). At day 5, when intraglomerular cellularity was maximal, regions of proliferation included TM positive cells, which were endothelial cells as confirmed by immuno-EM. The expression of TM was localized on the exposed surface of the endothelial cell, but was absent from mesangial and infiltrating cells (Fig. 4). Analysis of serial sections of these glomeruli confirmed that TM positive cells in the proliferative lesions were connected to both TM positive cells in vascular pole and peripheral cystic lesions, and that these positive cells formed tubular structures with outgrowths (Fig. 7). At later stages, open capillary lumina with TM positive

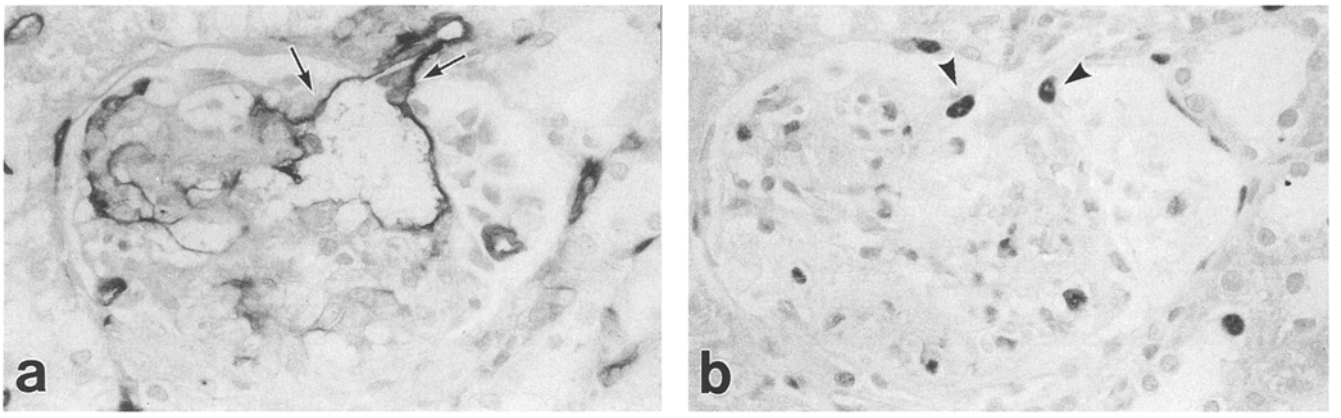


Fig. 5a, b Glomerular staining for TM (**a**) and PCNA (**b**) in serial sections in the experimental rat at day 2. It is evident that TM positive cells (*arrow*), elongated from the vascular pole, are also positive for PCNA (*arrowhead*). ×400

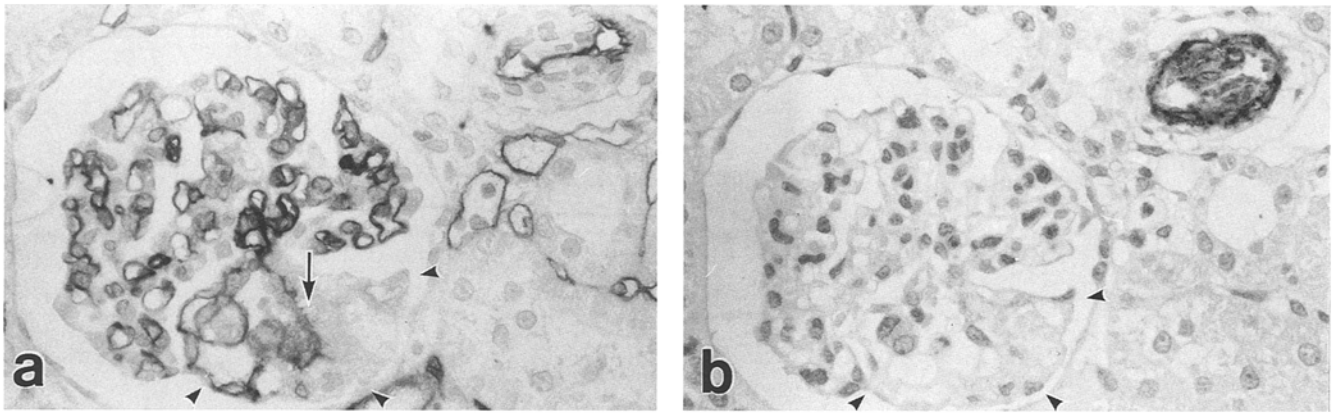
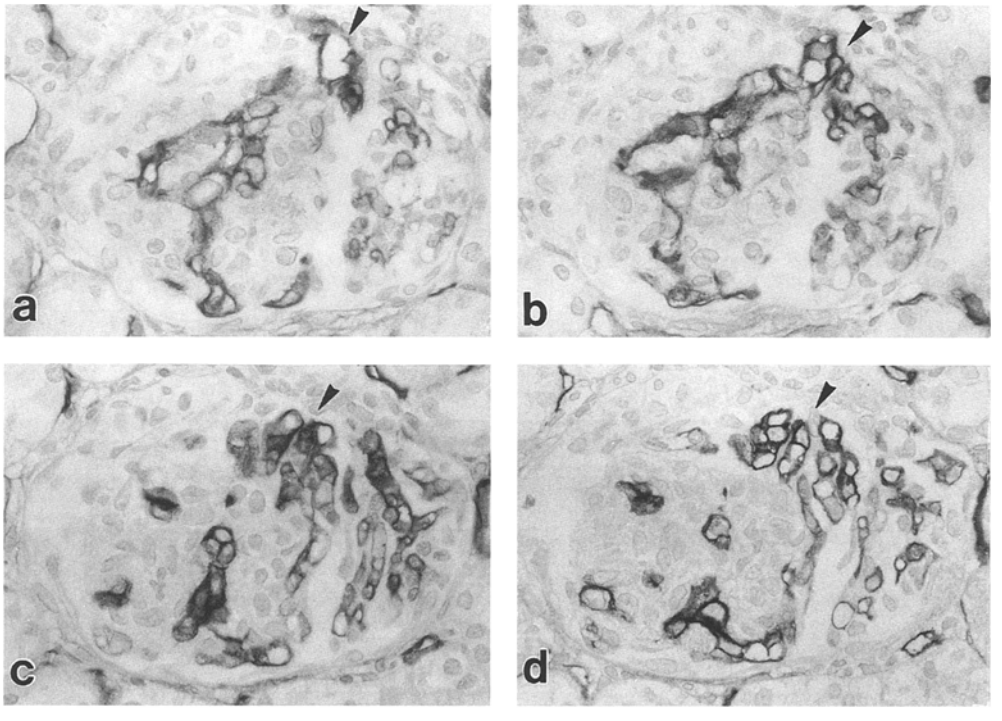


Fig. 6a, b Glomerular staining for TM (**a**) and α-smooth muscle actin (α-SMA) (**b**) in serial sections in the experimental rat at day 2. In the cystic lesion (*arrowhead*), TM positive cells are seen (*arrow*) whereas α-SMA is not expressed. ×400

Fig. 7a–d Immunohistochemical staining for TM on serial sections of the same glomerulus (**a, b, c, d**) in the experimental rat, with marked proliferative lesion at day 5. TM positive cells in the proliferative lesion, connect with both TM positive cells in the vascular pole (*arrowhead*) and in the peripheral region, and form a tube-like structure with outgrowth. ×400



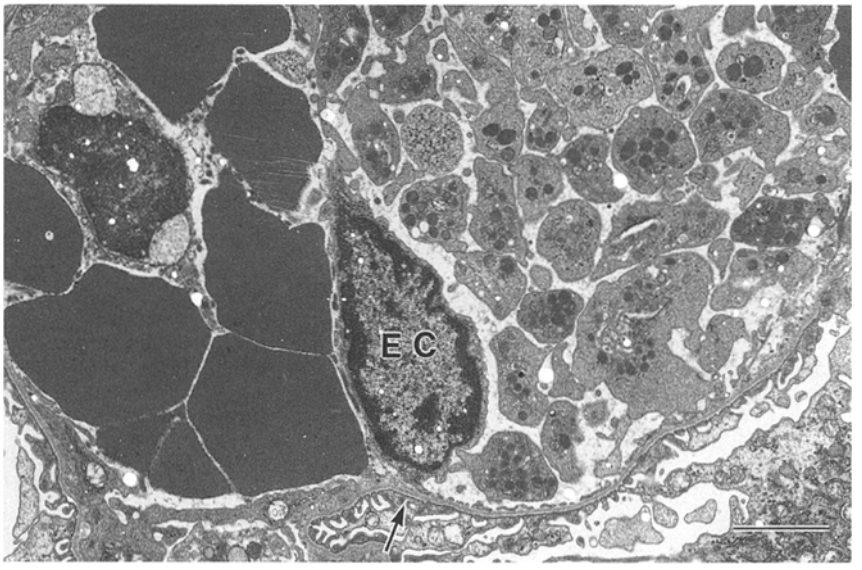


Fig. 8 In the cystic lesion, an electron micrograph shows that endothelial cells are extensively detached from the glomerular basement membrane (GBM) and the mesangium at day 1. However, a few endothelial cells with preserved attachment to GBM are present (*arrow*). Platelets full the cystic lesion. (*EC*, Endothelial cell) ×5,600; calibration bar=2 μm

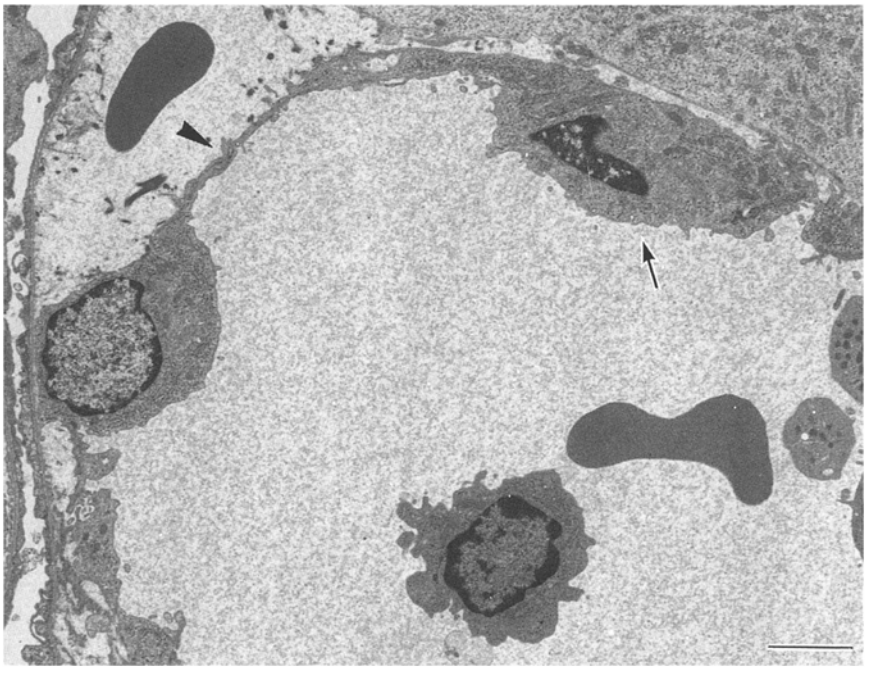


Fig. 9 Electron micrograph of an endothelial cell in the cystic lesion at day 2. The elongated endothelial cells (*arrow*) are connected with each other by junctional complexes (*arrowhead*). ×4,280; calibration bar=2 μm

lining gradually appeared and eventually TM showed almost the same distribution as in control rats (Fig. 3d). The number of TM positive cells increased rapidly, after marked initial depletion by mesangiolysis, reaching a plateau at day 7 that did not significantly differ to that of controls (Fig. 2c).

Mesangial cell activation, which was determined by the expression of α-SMA, was assessed in glomerular lesions throughout the experimental period. Glomeruli in control rats did not exhibit expression of α-SMA, although α-SMA could be observed in the afferent and efferent arterioles. In the experimental group in contrast, α-SMA expression in glomeruli was detected at day 3, when the initiation of cell proliferation was observed. The strongest expression of α-SMA was observed in mesangial areas in a nodular pattern at day 5 (data not shown). Thus at day 2 the cystic lesion showed no detectable expression of α-SMA as described above (Fig. 6b). However, TM positive cells were detected inside the cystic lesion at day 2 (Fig. 6a).

Figure 2b shows of PCNA positive cells in a glomerulus. A prominent peak was observed at day 3, thereafter the number of PCNA positive cells rapidly decreased. In order to determine whether TM positive cells were associated with intraglomerular cell proliferation, serial sections were examined. One serial section was immunostained with anti-PCNA antibody and a second serial section with anti-TM antibody. This proceeding demon-

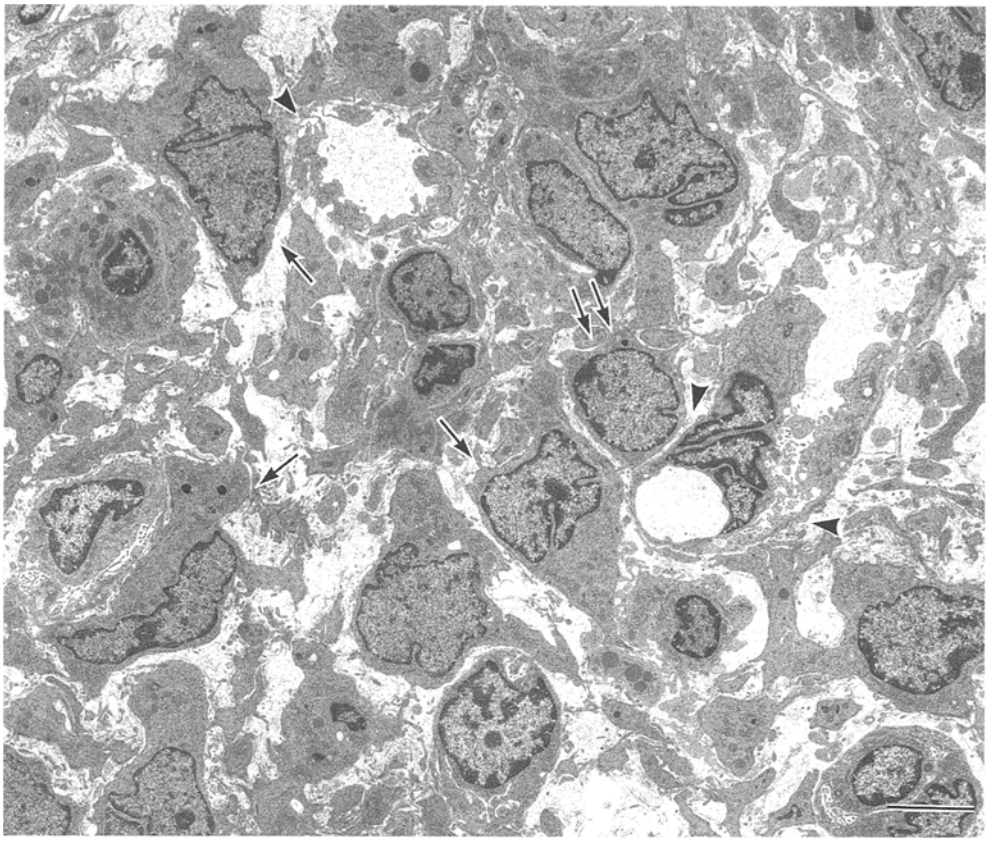
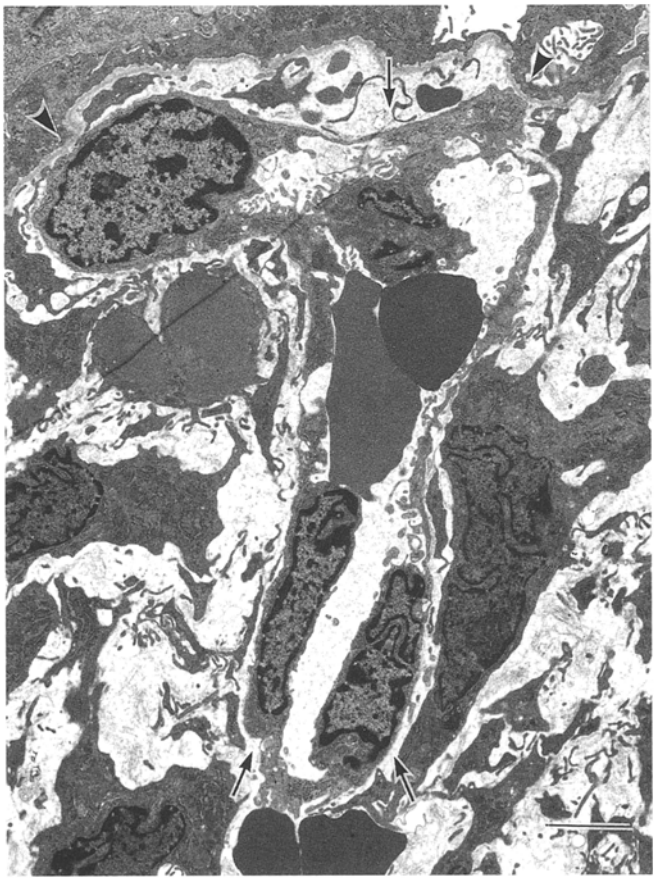


Fig. 10 The hypercellular region is mainly composed of young mesangial cells (*arrow*), with a mixture of endothelial cells which form frequent premature capillaries (*arrowhead*) without definite formation of the GBM at day 6. Some mesangial cells (*double arrow*) contact with adjacent endothelial cells. ×3,570; calibration bar=3 μm



strated that TM positive cells, which were connected with the afferent arteriole, also expressed PCNA. (Fig. 5a, b).

Electron microscopy

At day 1 most of the mesangial matrix had dissolved or disappeared, and many mesangial cells had degenerated and been removed from the mesangial area. Although a few endothelial cells remained in the peripheral cystic lesion with preserved attachment to GBM, most of the endothelial cells were detached from the mesangial matrix and GBM and could not be detected in the cystic lesion (Fig. 8). At day 2, endothelial cells now reappeared in the cystic lesion. Some endothelial cells were connected by junctional complex with adjacent endothelial cells which preserved attachment to the GBM, and elongated their cytoplasm (Fig. 9). At day 6, prominent proliferative lesions occupied the site of prior mesangiolytic lesions, the matrix was increased. The main contributions to hypercellularity were young mesangial cells as judged by cell position and ultrastructual features. These cells had cytoplasmic processes which rarely formed junctions with adjacent cells, dense cytoplasm, prominent Golgi

Fig. 11 In the proliferative lesion, the premature capillary (*arrow*) branches out and attaches to GBM (*arrowhead*) at day 7. ×3,450; calibration bar=3 μm

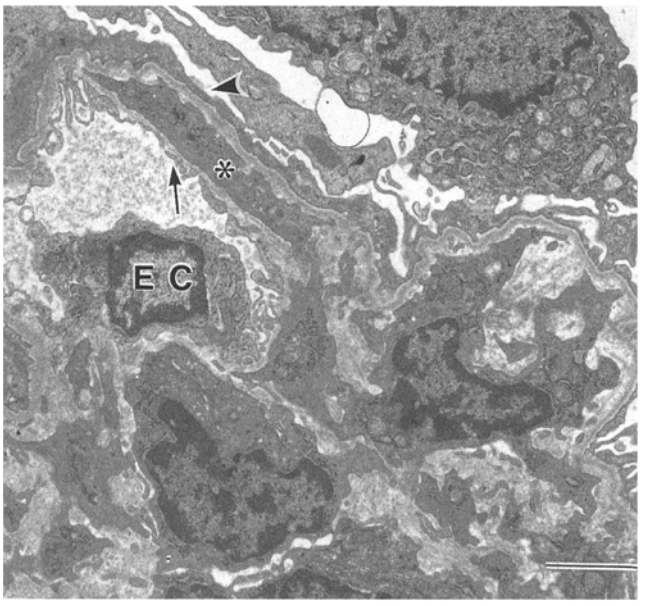


Fig. 12 Mild segmental proliferative lesion still seen 14 days after venom injection. Mesangial cells (*) are interposed between newly formed (*arrow*) and original GBMs (*arrowhead*) (*EC*, Endothelial cell) ×5,200; calibration bar=2 μm

complexes and rough endoplasmic reticulum. However, endothelial cells which formed premature capillaries without definite basement membrane were observed in these proliferative lesions. Around these premature capillaries, mesangial cells were present and some were in contact with adjacent endothelial cells (Fig. 10). Thereafter the premature capillaries branched out into the proliferative lesion and some capillaries attached to the original GBM (Fig. 11). At day 14, we observed mature capillary loops with endothelial cells and newly formed definite GBM, which was integrated into the original GBM (Fig. 12). The mesangial cells were interposed between the newly formed and the original GBM, mimicking mesangial interposition (Fig. 12). The number of interposed mesangial cells fell with time and at day 42 most of the glomeruli appeared to be almost normal, except for some with segmental sclerosis.

Discussion

Miura and Sumikawa first reported in 1902 [25] that glomerular injury could be induced by injection of HSV into rabbits. Since then, many reports have demonstrated mesangiolysis and mesangial proliferative glomerular lesions in experimental animals after administration of HSV [3, 4, 5, 8, 30]. A feature of this model is that damaged glomeruli eventually recover their original structure with capillary tuft reconstruction [26]. These morphological changes, which are characterized by rapid repair of the original structure followed by marked mesangial cell proliferation, are similar to those described in anti-thymocyte antibody induced mesangiolytic nephritis [34].

When considering the pathophysiology of glomerulonephritis, many investigations have focused on the mesangial cells and mesangial matrix. However the behaviour of endothelial cells, which are assumed to be important for vascular construction, has not yet been thoroughly analysed in human and experimental glomerulonephritis. In this study, we investigated the endothelial cell during the recovery process following HSV induced glomerular injury.

The kinetics of the endothelial cells was analysed by electron microscopy and by immunohistochemical studies of TM. TM is a cell surface protein of the vascular endothelium [12, 27]; it is found on cells cultured from vascular beds [10], on the endothelium throughout the rabbit vasculature [9] and on the endothelial cells of arteries, veins, capillaries and lymphatics in all human tissues and organs tested, except for the brain [24]. De Bault has proposed that TM is an excellent specific marker for the endothelial cell [9]. Furthermore in glomeruli, Horvat and Palade [16] showed, by indirect immunofluorescence and immunogold labelling procedures in the rat, that TM is located in the endothelial cell of the glomerular capillary loop and absent from the mesangial cell and the podocyte. In agreement with the above data, our study showed that TM was present along the exposed surface of the endothelial cell in both control and experimental rats.

Morphometric analysis demonstrated that the number of endothelial cells decreased significantly at day 1, compared with the control group, due to mesangiolysis. This was followed by a rapid increase to a plateau at day 7 that did not significantly differ from control values, as shown in Fig. 2c. Morphologically, at day 1, when the glomeruli showed a cystic lesion, we could not find endothelial cells inside these lesions except for those endothelial cells where attachment to the original GBM was preserved in the peripheral cystic lesion. However in the early proliferative phase, endothelial cells could already be detected inside the cystic lesion. A rapid proliferation of endothelial cells in the early phase of repair processes has been observed in chemically cauterized cornea [6] and in the endothelial removed aorta [31]. In agreement with above data, our results indicated that rapid endothelial cell proliferation occurred in the early phases after glomerular injury induced by HSV. However, α-SMA could not be detected in the cystic lesion on these newly appearing endothelial cells. However, α-SMA was prominently expressed in the mesangial region, in accordance with mesangial proliferation seen in the later phases. Alpha-SMA is considered to be an inducible marker of activated mesangial cells in disease states in human and rat glomeruli [1, 19]. These findings suggest that endothelial cells grow rapidly prior to the onset of mesangial cell activation in injured glomeruli.

Previously studies have demonstrated that segmental glomerular hypercellularity is mainly due to the proliferation of mesangial cells [3, 4, 5]. Barnes et al. [4] showed that the cell type involved in the proliferative lesion induced by HSV in the unilaterally nephrectomized

rat was almost exclusively the mesangial cell; this would exclude a significant contribution by endothelial and epithelial cells to the proliferating population. However in the current study, immunohistochemical and electron microscopic examination showed that the proliferative lesions contained not only mesangial but also endothelial cells. This contradictory result may be due to the methods employed (unilateral nephrectomy) which might accelerate glomerular injury and mesangial activation after HSV injection. We were unable to resolve one important question, namely the origin of the newly appearing endothelial cells. The finding that TM positive cells, which were connected to the afferent arterioles, also expressed PCNA, indicates that the endothelial cells may extend from the vascular pole into the cystic lesion. This is supported by the findings from serial sections, showing that TM positive cells were continuous with the vascular pole in the severe proliferative lesion at day 5. Furthermore, these cells formed premature capillaries with outgrowth and connected with the endothelial cells in the peripheral region of the capillary loop. These observations may be similar to those seen in angiogenesis, in which new vessels grow from the parent capillary [14, 28, 36].

Capillary tuft reconstruction was not over at day 7, although endothelial cell proliferation was complete as shown in Fig. 2c. These observations suggest that the endothelial cell may elongate its cytoplasm without proliferation after day 7. In fact, Sholley et al. [33] reported that endothelial sprouting in the cauterized rat cornea was observed although endothelial proliferation had been inhibited by irradiation. The factors that participate in these capillary tuft reconstructions and endothelial regeneration have not been fully investigated in glomeruli. However, basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), which stimulate endothelial cell growth and angiogenesis [20], are produced by glomerular resident cells [13, 18]. These factors may enhance endothelial regeneration and capillary reconstruction after glomerular injury in this model.

We observed a close correlation between the completion of endothelial cell proliferation and the decrease in the number of total cells after day 7; this decrease was more dependent on the reduction in number of mesangial cells. It has been reported that mesangial cell proliferation is regulated by intercellular contact with endothelial cells in the co-culture system [29] and inhibited by a peptide with heparin-like activity, produced by glomerular endothelial cells [7]. By analogy with the above data in vitro, mesangial cell growth, including that of interposed mesangial cells with both newly formed and original GBM, may be ceased by mesangial-endothelial cell interactions and after this, capillary lumina grow in size.

It has been reported that the endothelial cell cannot be detected in the sclerotic lesions of human and mice glomeruli [35] and that endothelial injury or dysfunction is an initial event in atherosclerotic lesions, which seem to be analogous to focal and segmental glomerulosclerosis [11]. Therefore, the breakdown of endothelial cell growth may cause sclerotic lesions in glomeruli. In this context, endothelial cell growth seems to be indispensable in maintaining the original structure of the glomeruli.

In conclusion, following HSV induced glomerular injury reconstitution of the original structures is the natural course, and migration and proliferation of endothelial cells with capillary formation along with mesangial cell proliferation are essential for the repair process.

Acknowledgements The authors are grateful to Professors Stephen Bastford and Akiroh Terashi for their critical reviews. We also wish to thank Doctors David Stern and Yukio Yuzawa for providing the anti-TM antibody. Technical assistance provided by Doctors Yukinari Masuda and Akira Shimizu and Mr. Takakuni Matsunami is also acknowledged.

References

1. Alpers CE, Hudkins KL, Gown AM, Johnson RJ (1991) Enhanced expression of muscle specific actin in glomerulonephritis. Kidney Int 41:1134–1142
2. Baker AJ, Mooney A, Hughes J, Lombrdi D, Johnson RJ, Savill J (1994) Mesangial cell apoptosis: the major mechanism for resolution of glomerular hypercellularity in experimental mesangial proliferative nephritis. J Clin Invest 94:2105–2116
3. Barnes JL, Abboud HE (1993) Temporal expression of autocrine growth factors corresponds to morphological features of mesangial proliferation in Habu snake venom-induced glomerulonephritis. Am J Pathol 143:1366–1376
4. Barnes JL, Hevey KA, Hastings RR, Bocanegra RA (1994) Mesangial cell migration precedes proliferation in Habu snake venom-induced glomerular injury. Lab Invest 70:460–467
5. Bradfield JWB, Cattel V, Smith J (1977) The mesangial cell in glomerulonephritis II; mesangial proliferation caused by Habu snake venom in the rat. Lab Invest 36:487–492
6. Burger PC, Klintworth KG (1981) Autoradiographic study of corneal neovascularization induced by chemical cautery. Lab Invest 45:328–334
7. Castellot JJ, Hoover RL, Karnovsky MJ (1986) Glomerular endothelial cells secrete a heparin-like inhibitor and a peptide stimulator of mesangial cell proliferation. Am J Pathol 125: 493–500
8. Cattel V, Bradfield JWB (1977) Focal mesangial proliferative glomerulonephritis in the rabbit caused by Habu snake venom. Am J Pathol 87:511–524
9. De Bault LE, Esmon N, Olson JR, Esmon CT (1986a) Distribution of the thrombomodulin antigen in the rabbit vasculature. Lab Invest 54:172–178
10. De Bault LE, Esmon N, Smith GP, Esmon CT (1986b) Localization of thrombomodulin antigen in rabbit endothelial cells culture. Lab Invest 54:179–187
11. Diamond JR, Karnovsky MJ (1988) Focal and segmental glomerulosclerosis: analogies to atherosclerosis. Kidney Int 33: 917–924
12. Esmon CT, Owen WG (1981) Identification of an endothelial cell co factor for thrombin-catalyzed activation of protein C. Proc Natl Acad Sci USA 78:2249–2252
13. Floge J, Eng E, Lindner V, Alpers CE, Young BA, Reidy MA, Johnson RJ (1992) Rat glomerular mesangial cells synthesize basic fibroblast growth factor. Release, upregulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. J Clin Invest 90(6):2362–2369
14. Folkmann J (1985) Tumor angiogenesis. Adv Cancer Res 43: 175–203
15. Garg UC, Hassid A (1989) Inhibition of rat mesangial cell mitogenesis by nitric oxide – generating vasodilators. Am J Physiol 257(1 pt 2):F60–66
16. Horvat R, Palade GE (1993) Thrombomodulin and thrombin localization on the vascularture endothelium; their internaliza-

tion and transcytosis by plasmalemmal vesicles. Eur J Cell Biol 61:299–313
17. Hsu SM, Raine L, Fanger H (1981) Use of avitin – biotin – peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580
18. Iijima K, Yoshikawa N, Connolly DT, Nakamura H (1993) Human mesangial cells and peripheral blood mononuclear cells produce vascular permeability factor. Kidney Int 44(5): 959–966
19. Johnson RJ, Iida H, Alpers CE, Majesky MW, Schwartz SM, Pritzl P, Gordon K, Gown AM (1991) Expression of smooth muscle cell phenotype by rat mesangial cells in immune complex nephritis. J Clin Invest 87:847–858
20. Klagsburn M, D'Amore PA (1991) Regulators of angiogenesis. Annu Rev Physiol 53:217–239
21. Klahr S, Schreiner G, Ichikawa I (1988) The progression of renal disease. N Engl J Med 318:1657–1666
22. Kurki P, Vanderlaan M, Dolbeaare F, Gray J, Tan EM (1986) Expression of proliferating cell nuclear antigen (PCNA) / cyclin during the cell cycle. Exp Cell Res 166:209–219
23. Marti HP, Lee L, Kashgarian M, Lovett DH (1994) Transforming growth factor – β1 stimulates glomerular mesangial cell synthesis of the 72-kD type IV collagenase. Am J Pathol 144: 82–94
24. Maruyama I, Bell CE, Majerus PW (1985) Thrombomodulin is found on endothelium of arteries, veins, capillaries, and lymphatics and on syncytiotrophoblast of human placenta. J Cell Biol 101:363–371
25. Miura M, Sumikawa T (1902) Beitrag zur Untersuchung des Schalan gengifter. Zentrabl Allg Pathol 13:980–986
26. Morita T, Kihara I, Oite T, Yamamoto T, Suzuki Y (1978) Mesangiolysis; sequential ultrastructural study of Habu venom induced glomerular lesion. Lab Invest 38:94–102
27. Owen WG, Esmon CT (1981) Functional properties of an endothelial cell cofactor for thrombin – catalyzed activation of protein C. J Biol Chem 256:5532–5535
28. Rhodin JGA, Fujita H (1989) Capillary growth in the mesentery of normal young rat. Intra vital video and electron microscope. J Submicrosc Cytol 21(1):1–34
29. Saeki T, Morioka T, Arakawa M, Shimizu F, Oite T (1991) Modulation of mesangial cell proliferation by endothelial cells in coculture. Am J Pathol 139:949–957
30. Sakaguchi H, Kawamura S (1963) Electronmicroscopic observation of mesangial lesion. Keio J Med 12:99–105
31. Schwartz ST, Haudenschild CC, Eddy EM (1978) Endothelial regeneration. I. Quantitative analysis of intimal stages of endothelial regeneration in rat aortic intima. Lab Invest 38:568–580
32. Shimizu A, Kitamura H, Masuda Y, Ishizaki M, Sugisaki Y, Yamanaka N (1995) Apoptosis in repair process of experimental mesangial proliferative glomerulonephritis. Kidney Int 47: 114–121
33. Sholley MM, Ferguson GP, Seibel HR, Montour JL, Wilson JP (1984) Mechanism of neovascularization. Vascular sprouting can occur without proliferation of endothelial cells. Lab Invest 51:624–663
34. Yamanaka N, Ishizaki M (1991) Anti-thymocyte antibody induced mesangiolytic nephritis. In: Hatano M (ed) Proceedings of the XIth international congress of Nephrology. Springer Tokyo, pp. 1004–1014
35. Yonezawa S, Irisa S, Nakamura T, Uemura S, Otsuji Y, Ohi Y, Sato E (1983) Deposition of α-1-antitrypsin and loss of glycoconjugate carrying *Ulex europaeus* agglutinin-1 binding site in the glomerular sclerotic process. Nephron 33:38–43
36. Wakui S (1988) Two- and three-dimensional ultrastructural observations of two cell angiogenesis in human granulation tissue. Virchows Arch [B] 56:127–139