Hepatocyte Growth Factor Prevents the Development of Chronic Allograft Nephropathy in Rats

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Abstract. Long-term renal isografts in humans and laboratory animals exhibit features similar to those of chronic allograft nephropathy (CAN), indicating that antigen-independent factors, such as acute renal ischemia, are likely to be involved in the development of CAN. Hepatocyte growth factor (HGF) has been demonstrated to play a renotropic role in renal regeneration and protection from acute ischemic injury. This study was thus conducted to investigate the effect of HGF on the development of CAN, using an established rat model. HGF was administered daily (100 μg/d, intravenously) for 4 wk after engraftment. Control animals received saline solution. Allografts from control animals exhibited early evidence of severe structural collapse and necrotic cell death in the proximal tubules and outer medulla, with mononuclear cell infiltration, within 1 wk after engraftment. This was followed by sequential upregulation of adhesion molecules and cytokines, accompanied by dense macrophage infiltration. Fibrogenic events, as indicated by marked increases in transforming growth factor-β1 expression and the accumulation of smooth muscle α-actin, occurred during the same period. Control animals ultimately developed features typical of CAN, with functional deterioration and severe histologic changes; a survival rate of 50.6% by 32 wk was observed. In contrast, remarkably little early injury and no late fibrogenic events were observed for the HGF-treated group. All treated animals survived, with well preserved graft function, during the 32-wk follow-up period. These results indicate that renal protection and recovery from early allograft injury with HGF treatment greatly contribute to a reduction of susceptibility to the subsequent development of CAN in a rat model. The potential application of HGF in the prevention of CAN warrants further attention.

Chronic allograft nephropathy (CAN) is characterized by the deterioration of renal allograft function and is associated with typical morphologic changes within the allograft, including glomerulosclerosis, arterial intimal and smooth muscle cell proliferation, tubular atrophy, and interstitial fibrosis (1). Although CAN is the major cause of late graft loss, the underlying mechanisms that lead to this form of injury remain poorly understood, with no practical treatments being currently available for this condition. Tilney (2) demonstrated that kidney transplants between identical twins develop changes typical of CAN in the long term and that transplanted rat renal isografts develop similar changes (3). A CAN-type condition also occurs in a rat single-kidney model subjected to ischemic injury, indicating the importance of ischemia in the process of CAN (4,5). These observations strongly suggest that, in addition to the alloimmune response, initial ischemic injury may play an important role in the development of CAN.

Hepatocyte growth factor (HGF), which was originally identified and cloned as a potent mitogen for mature hepatocytes (6), plays diverse roles in the regeneration of the kidney (7–9). It exerts mitogenic and antiapoptotic actions (7,10–15) and induces branching tubulogenesis in renal tubular cells (7,11,16). The expression of HGF and its receptor, c-Met, is rapidly upregulated in renal tissue after unilateral nephrectomy or renal injury caused by renal ischemia or nephrotoxic compounds (17–20). Furthermore, previous reports demonstrated that HGF protected and salvaged renal tubular cells from renal injuries, acting as a mitogenic renotropic factor (19,21,22). These data strongly suggest that HGF enhances renal regeneration and affords protection to kidneys subjected to acute renal injury, including renal ischemia. We therefore conducted this study to examine the potential effects and mechanisms of
action of HGF treatment in the development of CAN in a well-established rat model of CAN. In this study, we demonstrate that HGF treatment during the initial 4 wk after engraftment remarkably prevented the onset of CAN and associated death.

Materials and Methods

**Rat Model of CAN**

Inbred 200- to 250-g male Lewis rats (RT1\(^1\)) were used as graft recipients, and Fisher 344 rats (RT1\(^{1/2}\)) served as donors. The left kidney was removed from a Fisher 344 rat and transplanted orthotopically to the left renal vessels and the left ureter of the Lewis recipient, using end-to-end anastomosis with 10-0 Prolene sutures (23). The technique is well established, and the ischemic time is stable. During the procedure, the donor kidneys were subjected to 30 min of warm ischemic injury. The right kidney was removed 10 d later. All recipients received cyclosporin A at a dose of 5 mg/kg per d for 10 d, to prevent the development of acute rejection. All experimental protocols were conducted in accordance with the policies of the animal ethics committee at our institution.

**Administration of HGF and Experimental Design**

Recombinant human HGF was purified from the culture medium of Chinese hamster ovary cells that had been transfected with expression vector containing HGF cDNA (6,22). In this preparation, the purity of HGF exceeds 98%, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and protein staining. One hundred microliters of HGF dissolved in 0.5 ml of saline was administered daily through the penile vein for 4 wk after engraftment (HGF group). Control animals received saline solution (control group). Animals were killed at 2, 4, 6, and 14 d after transplantation, for investigation of changes in early periods, and at 8, 16, and 32 wk after transplantation, for investigation of changes in the late phase. More than five animals/timel point were used in each group.

**Graft Function**

Alternate-day 24-h urine samples were collected for the first 2 wk after transplantation and then every 2 wk until the end of the experiment. Protein excretion was determined by measuring precipitation after the addition of 3% sulfosalicylic acid to the urine. Turbidity was assessed by measuring absorbance at a wavelength of 595 nm, using a spectrophotometer. Blood samples were obtained from recipients every 4 wk, and the serum creatinine levels were determined by using the Jaffe reaction method.

**Morphologic Assessments**

Renal tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline. Paraffin sections were stained with hematoxylin and eosin and with periodic acid-Schiff stain and were then assessed by light microscopy. The frequency of focal and segmental glomerulosclerosis and hyalinosis was determined by examining all glomeruli in the section: the number of glomeruli with sclerotic changes was expressed as a percentage of the total number of glomeruli counted. For arterial changes, the number of affected arteries demonstrating luminal narrowing and intimal and smooth muscle cell proliferation was expressed as a percentage of the total number of arteries in the section.

**Immunohistochemical Analyses**

Staining for ED1-positive macrophages was performed with the alkaline phosphatase/anti-alkaline phosphatase method, using a Dako APAAP kit (Dako Japan, Kyoto, Japan) according to the instructions provided by the manufacturer. A monoclonal antibody against ED1 was purchased from Quantum Appligene (Parc d’Innovation, Illkirch, France). Staining for intercellular adhesion molecule-1 (ICAM-1), monocyte chemotactrant protein-1 (MCP-1), and fibronectin was performed with the indirect immunoperoxidase method, using a Vectorstain Elite ABC kit (Vector Laboratories, Burlingame, CA). Monoclonal antibodies against ICAM-1, MCP-1, and fibronectin were purchased from Santa Cruz Biotecnology (Santa Cruz, CA), PeproTech (London, England), and Chemicon International (Temecula, CA), respectively. Smooth muscle α-actin (SMAα) was stained with the direct immunoperoxidase staining method, using an EPOS kit (Dako Japan). The reaction product was observed with 3,3’-diaminobenzidine.

The number of marker-positive cells was expressed as the mean ± SD of the number of cells/field of view. More than 20 fields of view were evaluated, at a magnification of ×400, for each section/specimen. For evaluation of macrophage infiltration into glomeruli, more than eight glomeruli (>160 μm in diameter) were selected and the number of infiltrating marker-positive cells was counted and expressed as the mean ± SD of the number of cells/glomerulus. The expression of adhesion molecules, cytokines, and extracellular matrix was quantified on a scale of 0 to 4+ (4+ = dense).

**Terminal Deoxynucleotidyl Transferase-Mediated dUTP-Biotin Nick-End-Labeling Staining**

Tissue sections were prepared as described previously (24) and fixed in 4% paraformaldehyde in phosphate-buffered saline. DNA fragmentation was observed using an in situ apoptosis detection kit, according to the instructions provided by the manufacturer (Takara, Tokyo, Japan).

**Transmission Electron Microscopy**

Small kidney blocks were washed and fixed overnight at 4°C with 2% glutaraldehyde in 0.2 M sodium cacodylate buffer, postfixed with 1% osmium tetroxide in 0.2 M sodium cacodylate buffer, dehydrated, and embedded in Epon 812 (NACALAI TESQUE, Kyoto, Japan). Ultrathin sections were poststained with uranyl acetate and lead citrate and viewed with a transmission electron microscope (HT100; Hitachi, Tokyo, Japan).

**Analysis of DNA Fragmentation by Agarose Gel Electrophoresis**

Collected frozen kidney tissues were homogenized and lysed on ice for 20 min in a buffer containing 0.5% Triton X-100, 20 mM ethylene-diaminetetraacetate, and 10 mM Tris (pH 8.0). DNA fragments were separated from intact chromatin by centrifugation for 15 min at 15,000 rpm. DNA in the supernatants (fragmented DNA) was precipitated in isopropanol containing 0.5 M NaCl, digested for 60 min at 37°C with 1 mg/ml RNase A and 200 µg/ml proteinase K, and subjected to electrophoresis in a 1.5% agarose gel. DNA was subsequently observed by ethidium bromide staining.

**Reverse Transcription-PCR Assays**

RNA was extracted from tissues using RNeasy total RNA kits (Qiagen, Hilden, Germany), according to the instructions provided by the manufacturer. The quality of the RNA was confirmed with formaldehyde-agarose gel electrophoresis, and the cDNA were prepared as described previously (25). PCR was performed with a GeneAmp 9600 PCR system (Perkin-Elmer, Norwalk, CT), using
primers for MCP-1, transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH). [32P]dCTP was included for quantitative PCR. The primer sequences and numbers of cycles were as follows: MCP-1, 5’ primer, ATGCAGGTCTCTGTCACG; 3’ primer, CTAGTTCTCTGT-

Figure 1. Histologic appearance of renal allografts 4 d after transplantation. Many proximal tubules exhibited severe structural deformity and collapse, with mononuclear cell infiltration (a and b), and remarkable protein deposition, with structural disruption, was observed in the outer medulla (c and d) in the allografts from control recipients. In contrast, allografts from hepatocyte growth factor (HGF)-treated recipients were remarkably well preserved, with only minor mononuclear cell infiltration. The tubular structure remained normal, with no protein deposition, in both the proximal tubules (e and f) and the outer medulla (g and h). Original magnification, ×100 in a, c, e, and g; ×400 in b, d, f, and h.
CATACT (28 cycles); TGF-β1, 5' primer, TGAACCAAGGAGACG-GAATAACAG; 3' primer, TACTGTGTGTCCAGGCTCCAAATG (26 cycles); TNF-α, 5' primer, TACTGAACCTCGGGGTTGGTGC; 3' primer, CAGCCTTGCCCTTGAAGAGAACC (30 cycles); IL-6, 5' primer, CAAGAGACTTCCAGCCAGACC (30 cycles); iNOS, 5' primer, TTGCCGAGTAGACCTCATAGTGACC (30 cycles); G3PDH (35 cycles). The annealing temperature for PCR was optimized for each reaction. PCR products were subjected to electrophoresis on 1.5% agarose gels, and [32P]dCTP incorporated into specific bands of PCR products was measured on dried gels using a PhosphorImager system (Molecular Dynamics, Sunnyvale, CA), as described elsewhere (26). PCR amplification of G3PDH was performed to assess variations in total RNA or cDNA loading among samples. Corrected values were derived by dividing the measured 32P value for the transcript of interest by the mean.

Figure 2. Distribution and appearance of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end-labeling (TUNEL)-positive cells in the allografts at day 4 (a to d) and the time course in the early period (e). DNA fragmentation in the nuclei was stained using the TUNEL method. Many TUNEL-positive cells were demonstrated in the kidneys from the control animals (a and b), whereas the number of TUNEL-positive cells was remarkably less in the kidneys from HGF-treated animals (c and d). The number of TUNEL-positive cells increased remarkably in the kidneys from the control animals, peaking at day 4 after transplantation, whereas the number of positive cells was significantly less in the kidneys from HGF-treated animals (e). FV, field of view. Original magnification, ×100 in a and c; ×400 in b and d.
G3PDH value for that sample. PCR analysis was performed for all samples, and each analysis was performed at least four times. The mean value for the corrected levels was obtained by pooling measurements for all animals in each experimental group.

### Statistical Analyses

One-way ANOVA was performed for the proteinuria and serum creatinine concentration values. Graft survival was evaluated with the Wilcoxon test. The unpaired *t* test was used for cellular infiltration in immunohistochemical analyses. The Mann-Whitney *U* test was used for histologic analyses and immunohistochemical data on the expression of adhesion molecules, cytokines, and extracellular matrix. Results from reverse transcription-PCR assays were subjected to ANOVA without replication. If the ANOVA results indicated significance, then individual comparisons were performed using the *t* test. *P* values of <0.05 were considered to be statistically significant.

### Results

**Reduction of the Initial Histologic Damage in Allografts with HGF Treatment**

In control recipients, marked destruction of renal tissue occurred within 1 wk after engraftment, especially at days 4 to 5 after engraftment. Samples taken from control allografts collected 4 d after transplantation demonstrated widespread changes, with mononuclear cell infiltration and disruption of normal renal architecture. Many proximal tubules demonstrated severe structural deformity and collapse, with infiltration of mononuclear cells (Figure 1, a and b). Remarkable protein deposition, with structural disruption, was observed in the outer medulla (Figure 1, c and d). The histologic changes in glomeruli were generally unremarkable; however, there was some engorgement of glomerular capillaries, which is often observed in ischemic glomerulopathy. In contrast, allografts from HGF-treated recipients were remarkably well preserved, and mononuclear cell infiltration was strongly reduced (Figure 1, e to h). Tubular structure remained mostly normal, with no

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*Figure 3.* Electron microscopic appearance of allografts (a and b) and degradation of genomic DNA from allografts (c). (a and b) Allografts were obtained 4 d after transplantation. Characteristics of cell necrosis, including flattened nuclei (NN) with severe heterochromatin and injured subcellular organelles, especially enlarged mitochondria (black arrows) and vacuoles (white arrows) in the cytoplasm, were demonstrated in the kidneys from control recipients (a). However, most cells remained normal, with intact nuclei (N) and mitochondrial architecture (black arrows) and a fine chromatic pattern in the kidneys from HGF-treated animals (b). Original magnification, ×4000 in a and ×2600 in b. (c) Genomic DNA from allografts was analyzed by agarose gel electrophoresis. The extracted DNA from the control animals (C3 and C4) demonstrated a predominant smear pattern resulting from random DNA degradation, indicating necrotic cell death by day 4. In contrast, no smear pattern was noted in the kidneys from HGF-treated animals (H3 and H4).

*Figure 4.* Survival times for rats from both groups. More than five animals/time point were used in each group. Almost 50% of animals in the control group died by 32 wk, whereas no animals treated with HGF died during the follow-up period.
apparent protein deposition throughout the graft tissue. Most glomeruli exhibited no remarkable changes.

**Remarkable Decreases in Cell Necrosis with HGF Treatment**

These initial changes in the allografts were further investigated by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end-labeling (TUNEL) staining, transmission electron microscopy, and DNA gel electrophoresis. TUNEL staining demonstrated many positive cells in the kidneys from the control group, particularly in the proximal tubules in the cortex and outer medulla on day 4 after engraftment (Figure 2, a, b, and e). In contrast, the number of TUNEL-positive cells was significantly reduced in the kidneys from HGF-treated animals (Figure 2, c, d, and e).

Transmission electron microscopy demonstrated that many tubular cells exhibited characteristics of cell necrosis, i.e., deformed nuclei, cytoplasmic vacuoles, and degenerative organelles, with particularly swollen and enlarged mitochondria (Figure 3a). In contrast, in the kidneys from HGF-treated animals, most tubular cells were morphologically well preserved, with intact nuclei and mitochondrial architecture and a fine chromatic pattern (Figure 3b). The DNA fragmentation pattern for the samples from the control group on day 4 demonstrated a predominant smear resulting from random DNA degradation, which is compatible with necrotic cell death (Figure 3c), whereas no smear pattern was noted during the initial 1 wk for the kidneys from HGF-treated animals.

**Prevention of Animal Death and Significant Preservation of Graft Function for Long Periods with HGF Treatment**

Almost one-half (49.4%) of the animals in the control group died by 32 wk, compared with no deaths in the HGF-treated group. Figure 4 presents animal survival times. Renal function, as indicated by serum creatinine levels and proteinuria, was also markedly different between the two groups. The serum creatinine levels for control recipients began to increase 16 wk after transplantation and continued to increase thereafter. In contrast, the serum creatinine levels for HGF-treated rats remained stable, within the normal range, throughout the follow-up period (Figure 5A). The mean serum creatinine concentration for surviving recipients in the control group was more than threefold higher than that for HGF-treated rats (2.73 ± 1.63 versus 0.77 ± 0.10 mg/dl, *P < 0.05*) at 32 wk after transplantation. Likewise, a variable degree of proteinuria was observed for the control recipients starting 16 wk after transplantation. Urinary protein levels progressively increased until 32 wk after transplantation, whereas no excessive proteinuria was observed in the treated group during the follow-up period (62.0 ± 41.8 versus 11.4 ± 0.8 mg/dl at 32 wk, *P < 0.05*) (Figure 5B). These results indicate that HGF treatment also significantly prevented progressive late graft dysfunction.

**Remarkable Preservation of Graft Morphologic Features in Allografts from HGF-Treated Recipients throughout the Follow-up Period**

Consistent with the results of the renal function studies, HGF treatment remarkably prevented the histologic changes of graft destruction that were observed for the control group. In grafts collected from the control group 8 wk after transplantation, there was evidence of scant mononuclear cell infiltration in the periglomerular and perivascular areas, with a small number of atrophic tubules. By 16 wk, this cellular infiltration had become more florid in the glomeruli and perivascular areas, and the tubules associated with damaged glomeruli exhibited striking atrophy. By 32 wk, interstitial fibrosis had become widespread and was accompanied by severe tubular atrophy; some tubules exhibited remarkable dilation, with intratubular casts (Figure 6a). Almost 40% of glomeruli developed various degrees of...
Figure 6. Histologic changes in renal allografts 32 wk after transplantation in rats with or without HGF treatment. (a through f) Periodic acid-Schiff staining of histologic sections. The kidneys from control recipients exhibited widespread interstitial fibrosis, with atrophic tubules (a), severe glomerulosclerosis (b), and arterial obliteration (c). In contrast, no remarkable fibrosis was noted, with normal-appearing tubules, in the kidneys from HGF-treated animals (d). Most glomeruli remained intact (e), and no remarkable arterial obliteration was noted (f). Original magnification, ×100 in a and d; ×400 in b, c, e, and f. (g) Development of glomerulosclerosis and arterial obliteration. At week 32, approximately 40% of glomeruli and graft arteries were affected in the kidneys from control recipients, compared with only 10% in allografts from HGF-treated recipients.
glomerulosclerosis, with wrinkling and collapse of glomerular capillaries, mesangial cell proliferation, and peripheral mesangial interposition (Figure 6, b and g). Some glomeruli developed remarkable cellular crescents. Arterial changes were also manifest, with luminal narrowing and intimal and smooth muscle cell proliferation (Figure 6, c and g). In contrast, in the HGF-treated recipients, most glomeruli remained normal, with significantly decreased interstitial fibrosis and tubular changes and only minimal vascular abnormalities (Figure 6, d through g).

**Figure 7.** Expression of smooth muscle α-actin (SMαA) and intercellular adhesion molecule-1 (ICAM-1) in allografts at 8 wk. In control recipients, SMαA was densely expressed in the interstitial areas, especially in the outer medulla (a and b), whereas it was remarkably suppressed in the kidneys from HGF-treated recipients (c and d). Expression of ICAM-1 was markedly upregulated in the control recipients, especially in the glomeruli (e) and arterial intima (f). In contrast, it was significantly reduced in the kidneys from rats treated with HGF (g and h). Original magnification, ×100 in a and c; ×400 in b and d through h.

Reduced Macrophage Infiltration, Inflammatory Responses, and Fibrogenic Events at Late Times with HGF Treatment

In the control recipients, dense expression of SMαA (a marker of myofibroblasts involved in the development of renal fibrosis) appeared in the interstitial areas, especially in the outer medulla, by 8 wk after transplantation (Figure 7, a and b). Upregulation of ICAM-1, which is involved in the inflammatory reaction in grafted kidneys, was observed in the glomeruli and arterial intima during the same time period (Figure 7, e and h).
and TGF-β grafts from control recipients, the mRNA production of MCP-1 was evaluated by semiquantitative reverse transcription-PCR. In the early period after engraftment, sequential inflammatory responses, including upregulation of ICAM-1 and MCP-1, occurred and were accompanied by a dramatic influx of macrophages by 16 wk after transplantation (35,36). Infiltrating macrophages are thought to be responsible for increased production of cytokines such as TGF-β, TNF-α, and IL-6, which are known to cause sclerotic changes in glomeruli by promoting mesangial cell proliferation and production of extracellular matrix (37–41). Concomitant with these changes, molecular mechanisms for the prevention of chronic injury in this model.

The mechanisms that cause CAN are currently poorly understood. Although the process of CAN is primarily related to MHC disparities, Tilney (2) revealed that changes similar to CAN developed in human kidney transplants between identical twins, as well as in rat kidney isografts (3). Such changes cannot be explained by antigen-dependent factors; antigen-independent factors are likely to be involved in the onset of CAN (27,28). Several reports have indicated that initial ischemic injury is one of the major risk factors for the development of CAN (1,29,30). Our previous report that demonstrated that nontransplanted single-kidney rats subjected to renal ischemia developed long-term changes similar to CAN emphasized the importance of ischemic injury as a precipitating factor in the development of CAN (31). In this study, severe injury, producing structural deformity and collapse, was observed in the proximal tubules and outer medulla of allografts from non-HGF-treated animals in the first 1 wk after engraftment. A large number of necrotic tubular cells were observed in these regions. By comparison, graft tissue from HGF-treated animals was well preserved, with no evidence of cell necrosis, during this period. Morphologic changes resulting from ischemic injury occur predominantly in proximal tubules and the medullary thick ascending limb of Henle (32). The changes are potentially reversible, and recovery has been demonstrated to occur through the regeneration or replacement of injured cells (33). Previous studies indicated that HGF promotes DNA synthesis in renal tubular cells in vitro (10,34) and that rats given HGF after acute renal injury caused by renal ischemia or nephrotoxic compounds exhibited more satisfactory renal function and much less histologic damage, compared with control rats (19,21,22). The results presented here indicate that ischemic injury is a critical event in the early period after engraftment and that HGF salvaged and protected allografts from such early injury after engraftment.

After a period of quiescence, sequential inflammatory responses, including upregulation of ICAM-1 and MCP-1, occurred and were accompanied by a dramatic influx of macrophages by 16 wk after transplantation (35,36). Infiltrating macrophages are thought to be responsible for increased production of cytokines such as TGF-β, TNF-α, and IL-6, which

Discussion

In this study, control recipients without HGF treatment developed progressive renal failure and histologic changes characteristic of CAN. Almost one-half of the animals died, as a result of renal failure, by 32 wk. In contrast, there was remarkably little early injury and no subsequent fibrogenic events in the allografts from the HGF-treated group. All animals survived, with well preserved graft function, to the end of the study period. These results indicate the importance of initial events after engraftment in the development of CAN. The protection and recovery of grafted kidneys from initial injury with HGF treatment seem to be the underlying mechanisms for the prevention of chronic injury in this model.

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The mRNA production of macrophage-associated proteins, such as MCP-1, TGF-β1, TNF-α, IL-6, and iNOS, was evaluated by semiquantitative reverse transcription-PCR. In the grafts from control recipients, the mRNA production of MCP-1 and TGF-β1 was significantly increased after 8 wk (Figure 10). The production of TNF-α, IL-6, and iNOS was progressively augmented by 32 wk. In contrast, those products exhibited different patterns of expression in the HGF-treated group, with much smaller peaks of measurable mRNA, compared with control values, through the 32-wk follow-up period.

![Figure 8. Time course of macrophage infiltration into allografts.](Image)
and cellular events leading to tissue fibrosis became more evident. Dense expression of SMαA, which is responsible for the accumulation of extracellular matrix proteins, appeared with augmented production of TGF-β1, which plays a critical role in the onset of renal fibrosis (42,43). Dense expression of the extracellular matrix protein fibronectin appeared, and allo-

Figure 9. Immunohistologic analysis of ED1-positive macrophages, monocyte chemoattractant protein-1 (MCP-1), and fibronectin in the grafted kidneys at 16 wk. In the kidneys from control animals, infiltration of ED1-positive macrophages was remarkably increased in the glomeruli (a) and perivascular areas (b), with marked expression of MCP-1 in the glomeruli (c) and of fibronectin in the perivascular areas (d). Macrophage infiltration was remarkably deceased in the kidneys from HGF-treated hosts (e and f), accompanied by remarkably reduced expression of MCP-1 (g) and fibronectin (h). Magnifications: ×200 in a, b, e, and f; ×400 in c and g; ×100 in d and h.
grafts ultimately developed severe widespread renal fibrosis. In contrast to the findings for control animals, these sequential inflammatory and fibrogenic events were remarkably suppressed in the allografts from HGF-treated animals, with prevention of progression to CAN. This indicates that such inflammatory and fibrogenic events are critical in the development of CAN. Prevention of early injury through the restoration of damaged cells may be the underlying mechanism for the beneficial effects of HGF on the long-term functioning of these allografts.

Although the exact mechanisms of such inflammatory and fibrogenic changes in allografted kidneys remain unclear, insufficient nephron numbers are possibly involved in the processes. As we previously observed in a rat kidney transplant model, renal allograft ablation accelerated CAN changes, which included parallel changes in the sequential patterns of cytokine production and adhesion molecule expression (4,44). Conversely, recipients with increased kidney mass, bearing two allografts, did not exhibit any signs of CAN (4,45). Several clinical reports support the concept that insufficient nephron mass is an important factor in the development of CAN (46,47). Therefore, it can be hypothesized that the presence of insufficient nephron numbers for long periods, resulting from early ischemic injury, is a critical factor in subsequent inflammatory and fibrogenic changes in allografted kidneys, although this issue remains to be further explored. Recovery from initial insults with HGF treatment may preserve more functioning nephrons in the long term, which may prevent the development of CAN.

We recently reported that the neutralization of endogenous HGF with anti-HGF IgG treatment accelerated the progression of tubular destruction, resulting in renal fibrosis, whereas the administration of exogenous HGF for a period of 4 wk suppressed fibrogenic events in a mouse nephrotic model (14). This study may support this hypothesis, but it remains to be determined whether HGF treatment at later times, after the onset of ischemic injury, can prevent the progression of CAN.

Recent improvements in immunosuppressive therapy have led to dramatic increases in renal cadaveric graft survival rates (especially the short-term rate of 87.7%); however, >20% of

Table 1. Expression of MCP-1 and fibronectin at 16 wk

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<tr>
<th>Parameter</th>
<th>MCP-1</th>
<th>Fibronectin</th>
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<tr>
<td>HGF</td>
<td>1.1 ± 0.3 b</td>
<td>0.8 ± 0.3 b</td>
</tr>
<tr>
<td>Control</td>
<td>2.8 ± 0.7</td>
<td>2.9 ± 0.6</td>
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a Staining for monocyte chemoattractant protein-1 (MCP-1) and fibronectin was performed using the indirect immunoperoxidase method; the intensities of those proteins were quantified on a scale of 0 to 4+. Dense expression of MCP-1 and fibronectin was observed by 16 wk, whereas such changes were markedly suppressed in the allografts from hepatocyte growth factor (HGF)-treated animals. b P < 0.05, HGF versus control.

Figure 10. Changes in MCP-1, transforming growth factor-β1 (TGF-β1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS) mRNA expression in the grafted kidneys, as determined by semiquantitative reverse transcription-PCR. In the control recipients, the production of MCP-1 and TGF-β1 was significantly increased after 8 wk. The production of TNF-α, IL-6, and iNOS was progressively augmented by 32 wk. In the kidneys from HGF-treated animals, mRNA expression of MCP-1, TGF-β1, and other products was remarkably reduced, compared with control values, through 32 wk of follow-up monitoring.
patients resume hemodialysis within 5 yr because of late graft loss (48). Although CAN is one of the major causes of such late graft loss, no practical treatment has been established. The results presented here suggest that HGF is a promising candidate for the prevention of CAN in the clinical setting. The potential therapeutic value of HGF in preventing the onset of CAN warrants further attention and preclinical studies.

References


Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/