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Inhibition of monocyte chemoattractant protein-1 expression in tubular epithelium attenuates tubulointerstitial alteration in rat Goodpasture syndrome

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Inhibition of monocyte chemoattractant protein-1 expression in tubular epithelium attenuates tubulointerstitial alteration in rat Goodpasture syndrome.

Background. To examine the role of monocyte chemoattractant protein-1 (MCP-1) expressed by tubular epithelium in tubulointerstitial alterations in situ, the level of MCP-1 mRNA in tubular epithelium was lowered selectively in the rat model of Goodpasture syndrome (GPS).

Methods. Intravenously administered antisense oligodeoxynucleotide (ODN) is taken up by renal tubular epithelium and has been found to block expression of target genes in rats. MCP-1 antisense ODN was injected into GPS rats every second day from days 27 to 35 after immunization (this represents the time when renal MCP-1 mRNA level was increased and interstitial mononuclear cell infiltration was aggravated).

Results. In addition to a reduction in the level of tubular MCP-1 mRNA, antisense ODN treatment attenuated monocyte infiltration significantly and preserved renal function in GPS rats. However, ODN injection did not affect glomerular MCP-1 expression and glomerular histopathology, and there were no significant changes in the urinary protein excretion rate.

Conclusion. Our findings provide direct evidence that MCP-1, expressed by tubular epithelium, plays a pivotal role in mediating secondary tubulointerstitial alterations in the GPS model.

In human glomerular disease, several investigators have found that the secondary renal tubulointerstitial alterations correlate better with renal function and disease prognosis than the glomerular alteration itself [1–3]. However, it remains unknown as to how glomerular diseases lead to tubulointerstitial alterations such as interstitial cell infiltration, tubular atrophy, and fibrosis [4–7].

An initial event of progressive interstitial alterations in most experimental models of renal diseases is monocyte infiltration into the interstitial compartment [5, 6, 8, 9]. Monocyte/macrophages play a role as antigen-presenting cells in the immunologic process [10] and are the main source of a number of humoral mediators, including transforming growth factor-β (TGF-β), at sites of inflammation. TGF-β is one of the key cytokines contributing to renal fibrosis, resulting in a permanent loss of renal function [5, 11, 12]. Thus, the C-C chemokines, for example, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and RANTES, which preferentially recruit monocytes into the extravascular interstitium, have been examined intensively [7, 13, 14].

In this study, we have focused on the role of MCP-1 as a chemoattractant for monocyte, T lymphocyte, and natural killer (NK) cells [15]. MCP-1 appears to contribute to the inflammatory component of human diseases, including atherosclerosis, multiple sclerosis, or rheumatoid arthritis [15]. Mice with targeted deletions of the C-C chemokine receptor 2, the receptor for MCP-1, exhibited impaired monocyte migration to sites of inflammation [16]. In contrast, enforced expression of MCP-1 could induce controlled recruitment of monocytes to specific organs [17, 18]. In the kidney, a marked up-regulation of MCP-1 was demonstrated not only in glomerular cells but also in tubular epithelial cells in human and experimental nephritis. The up-regulation of MCP-1 was associated closely with monocyte accumulation [7, 19, 20]. In more direct studies, cultured proximal tubular epithelial cells were induced to synthesize MCP-1 by treatment with interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) or urinary protein [21, 22], suggesting that this chemokine might be involved in mediating interstitial

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alterations. However, there is little definitive evidence for a role of MCP-1 in mediating the accumulation of monocytes in tubulointerstitial diseases [4, 6, 7].

Several investigators have shown that anti–MCP-1 antibodies inhibit the progression of glomerular damage and proteinuria in nephrotoxic nephritis [23–26]. In addition, others have investigated the effects of anti–MCP-1 antibodies on interstitial leukocytes [25]. This study showed that in mice, MCP-1 was more important than RANTES in the induction of glomerular crescent formation and interstitial fibrogenesis in nephrotoxic nephritis [25]. However, it remains unclear whether the effects on the interstitium are due to either the direct interaction of the antibody with MCP-1 expressed by tubular epithelium or the beneficial actions of the antibody on glomerular damage with subsequent effects on the tubulointerstitium.

Recently, oligodeoxynucleotides (ODNs), administered into the systemic circulation, have been demonstrated to be selectively taken up by renal tubular epithelial cells in mice and rats [27, 28]. Proximal tubular epithelial cells are thought to take up ODNs efficiently, without detectable degradation, through the activity of ODN-binding proteins on the brush border membrane [28]. Based on these findings, Noiri et al reported that antisense ODN for inducible nitric oxide synthetase (iNOS) attenuated NO production in renal epithelium, avoiding exacerbation of postischemic acute renal failure [29]. Our preliminary studies have shown that in vivo, MCP-1 antisense ODN is selectively taken up by proximal tubular epithelial cells and results in decreased MCP-1 mRNA levels. To test whether MCP-1 expression by tubular epithelium induces accumulation of monocytes in the interstitium, we repeatedly injected MCP-1 antisense ODN into Goodpasture syndrome (GPS) rats.

METHODS

Oligodeoxynucleotide application to cultured renal tubular epithelial cells by streptolysin-O permeabilization

Phosphorothioate-capped ODNs were synthesized by an automated synthesizer. The MCP-1 sense ODN sequence comprised 5′-ACCACTATGCAGGTCTCT-3′. The antisense ODN sequence comprised 5′-AGAGACC TGCATAGTGGT-3′, and the mutated antisense ODN sequence comprised 5′-ACACAGGTCGATACTCC T-3′; these sequences were chosen from the rat MCP-1 gene [30]. A rat renal proximal tubular epithelial cell line, RPTC (kindly provided by Dr. J. Ingelfinger, Harvard Medical School), was used in this study, and the method for streptolysin-O (SL-O; Sigma, St. Louis, MO, USA) permeabilization was essentially that of Spiller et al [31]. After confirmation of attachment of ODN-treated RPTC cells to the bottom of the culture dish, stimulation with TNF-α (R&D Systems, Minneapolis, MN, USA) at 10 ng/mL in 0.5% fetal calf serum (FCS) media was carried out for four hours [21]. Untreated cells were permeabilized with SL-O without ODN treatment and stimulated with TNF-α, and the control cells were also permeabilized with SL-O without ODN treatment and without stimulation by TNF-α.

Northern blot and reverse transcription-polymerase chain reaction analysis

Total RNA was extracted from culture cells, whole kidneys, or glomeruli isolated by a sieving method, using TRIzol™ (GIBCO BRL, Grand Island, NY, USA) according to the manufacturer's instructions. Murine JE (MCP-1) cDNA (628 bp; the exon parts in the sequence +508/+2207 of murine JE gene; Genbank, accession no. M19681) was obtained by reverse transcription-polymerase chain reaction (RT-PCR) and subcloned into pGEM-7Zf(+) (Promega, Madison, WI, USA). MCP-1 cDNA and GAPDH cDNA were labeled with random primers and ^{32}PdCTP and were used as probes. Northern blotting was carried out as reported previously [32].

The GeneAmp RNA PCR Kit (Perkin-Elmer Cetus, Norwalk, CT, USA) was used for cDNA synthesis and PCR amplification, following the method of Wang et al [22]. The primers for MCP-1, sense primer, 5′-TATG CAGGTCTCTGTCACGC-3′, and antisense primer, 5′-AAGTGTTGAACCAGGATTCACA-3′, were used to amplify a 595 bp sequence in combination with primers for the housekeeping gene GAPDH yielding 520 bp. The primers for GAPDH were 5′-AATGCATCCTGCAC CACCAA-3′ as the sense primer, and 5′-GTAGCCA TATTCATTGTCATA-3′ as the antisense primer. Linearity of the amplification, in some experiments, was verified by the use of serial quantities of both template and cycles of PCR. PCR was carried out for 30 cycles in this study.

Quantitative densitometry was performed on autoradiograms of Northern blot analysis using a computer-based measurement system (Mac SCOPE, ver. 2.5; Mitani Corp., Hukui, Japan). The expression of the MCP-1 gene was corrected by dividing the signal density by that obtained for GAPDH.

Animal manipulation and experimental protocols

Male Wistar rats (160 to 200 g) were purchased from Clea Japan (Tokyo, Japan). Animal care and treatment conformed to the institutional guidelines. To generate GPS in rats, rats were immunized with 25 μg of α3(IV) NC1 domains as reported previously [33, 34]. Initially, to characterize GPS rats, daily urinary protein excretion, renal histopathology, and MCP-1 expression were monitored for two months. In addition, to visualize the distribution of ODN and the effects of antisense ODN, FITC-conjugated ODN, MCP-1 sense, and antisense ODNs

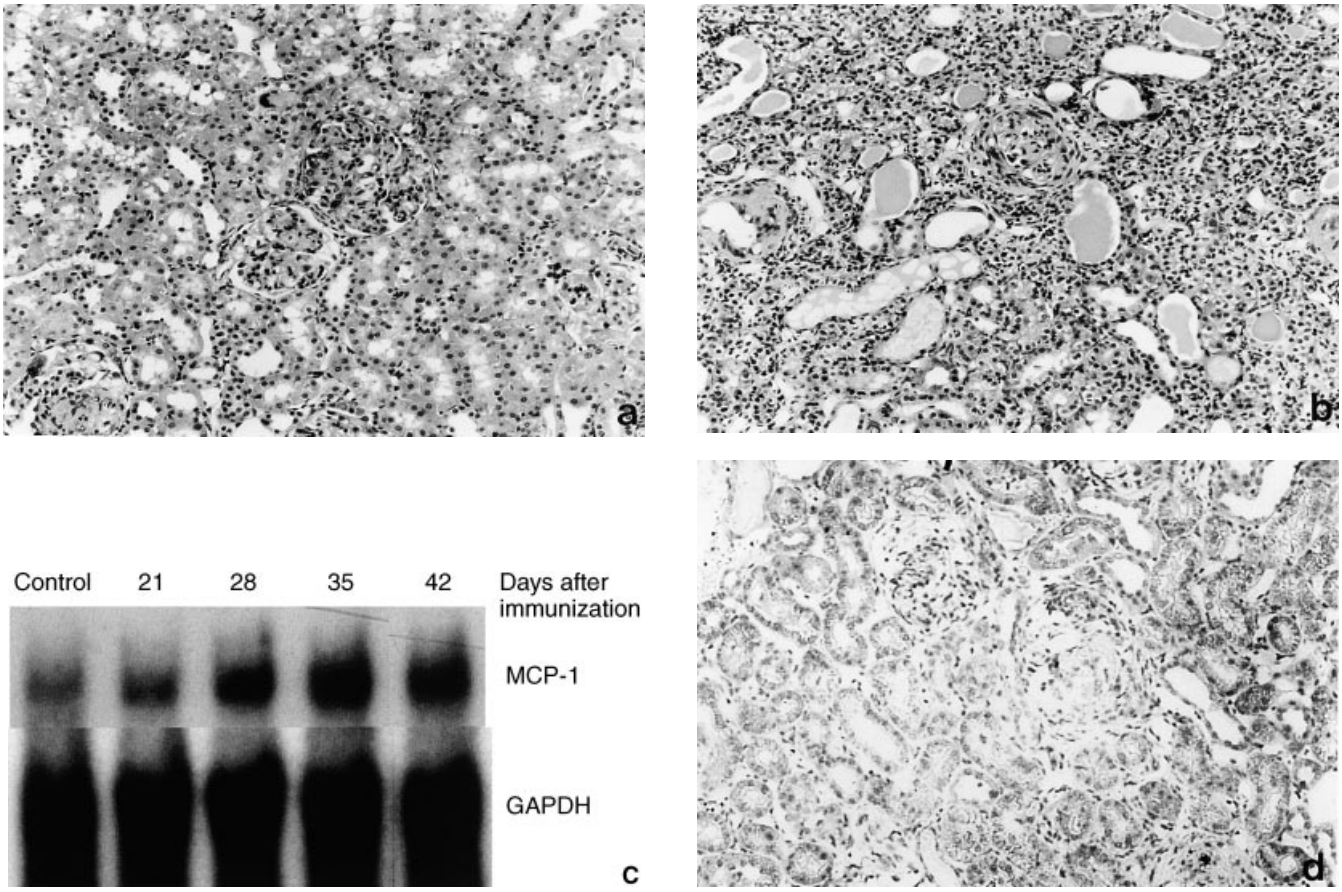


Fig. 1. Characterization of kidneys of Goodpasture syndrome (GPS) rats. (*a*) Day 28. Crescent formation was observed in some glomeruli, but no significant interstitial mononuclear cell infiltration was seen (HE stain, ×200). (*b*) Day 35. Prominent mononuclear cell infiltration in the interstitium appeared in parallel to the progression of glomerular and tubular damage (HE stain, ×200). (*c*) monocyte chemoattractant protein-1 (MCP-1) mRNA expression in the kidneys. Northern blot analysis with total RNA extracted from whole nephritic kidneys revealed that MCP-1 mRNA began to increase on day 21 and reached a peak level on day 35. A representative blot selected from three separate experiments is shown. (*d*) MCP-1 protein expression on day 28. MCP-1 is located in the glomeruli and in the tubular epithelium (×200).

were injected intravenously at a concentration of 1.0 mg/kg into day 27 GPS rats. Day 27 was selected because a significant increase in the MCP-1 expression was found in GPS rat kidney on day 28. The dose of ODN (1.0 mg/kg) was determined based on the report by Noiri et al [29], and its efficacy was reconfirmed by the observation that FITC-conjugated ODN injected intravenously at the relevant dose was accumulated in the tubular epithelium of normal rats and day 28 GPS rats (described later in this article). The experimental groups of the present study include MCP-1 antisense ODN-treated GPS rats (MCP-1-AS, $N = 6$), MCP-1 sense ODN-treated GPS rats (MCP-1-S, $N = 6$), untreated GPS rats (untreated, $N = 6$), and control normal rats ($N = 4$). On days 27, 29, 31, 33, and 35, either sense or antisense ODNs were injected intravenously at a concentration of 1 mg/kg. The interval between each injection was determined based on our finding that FITC-conjugated ODN remained in the kidney 24 hours after injection. On day 36, rats from each group were placed in metabolic cages for 24 hours to collect urine. Kidney tissues and blood samples were collected from each rat at the time of sacrifice on day 37. One kidney was used for RNA and protein extraction. Tissues from the other kidney were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight. One half of each fixed tissue was processed into paraffin blocks for histopathology and immunohistochemistry. The other half was rinsed in serial concentrations of sucrose solutions and then snap frozen for immunolocalization and in situ hybridization.

Histopathology, immunohistochemistry and immunolocalization

Four micrometer paraffin sections were stained with hematoxylin and eosin (HE). Glomerular hypercellularity and interstitial mononuclear cell infiltration were quantitatively evaluated with a computer-assisted image

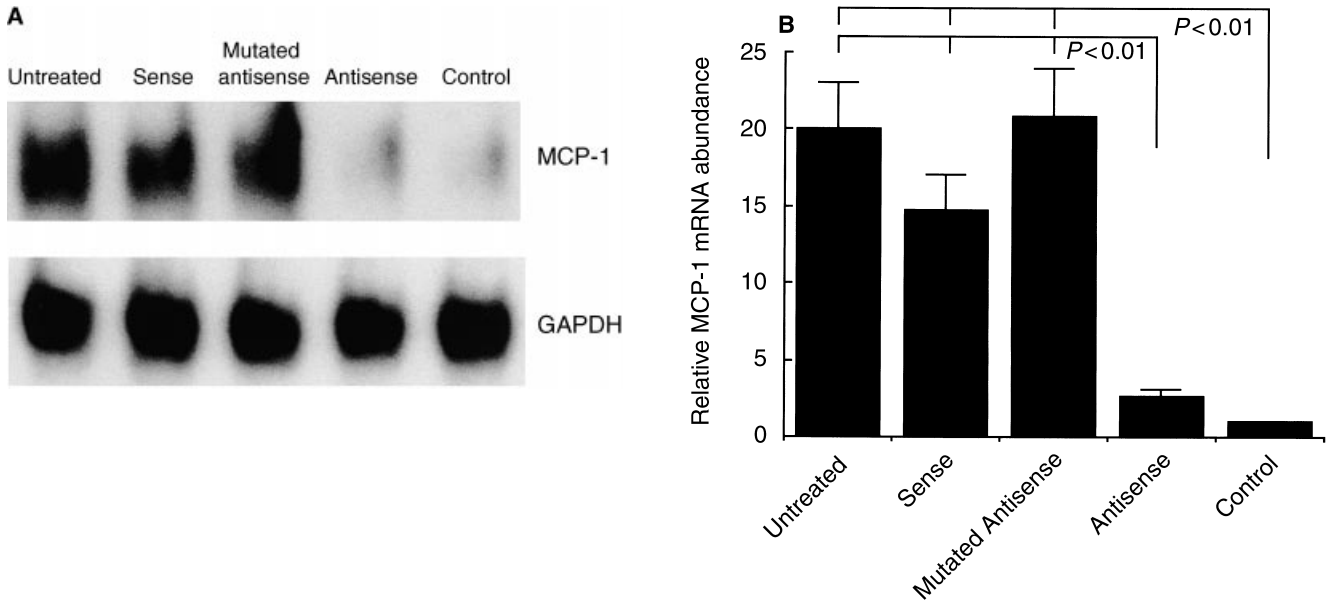


Fig. 2. Monocyte chemoattractant protein-1 (MCP-1) antisense oligodeoxynucleotide (ODN) application in vitro. (*A*) Northern blot analysis of MCP-1 mRNA in RPTC cells treated with antisense ODN. (*B*) Quantitative densitometric analysis of (A). Antisense ODN significantly lowered the MCP-1 mRNA level (lane 4 in A, column 4 in B) in relationship to the control level (lane 5 in A, column 5 in B). However, sense ODN-treated, mutated antisense ODN-treated and untreated RPTC cells strongly express MCP-1 mRNA (lanes 1 through 3, columns 1 through 3, respectively). A representative blot from three separate experiments is shown; densitometric data were obtained from all three blots.

analysis system (Mac SCOPE) in 30 glomeruli and 30 high-power (×200) cortical fields, respectively. Each histopathological index was calculated as the mean cell number per 10 glomeruli or one high-power cortical field. Immunohistochemistry was carried out according to the method reported previously [32] with a slight modification. In this study, the deparaffin sections were boiled in citrate buffer under microwave in addition to the treatment with proteinase K for unmasking antigenicity. Rabbit antiserum to rat MCP-1 (obtained from Pepro Tech, London, UK) was used as the primary antibodies to detect MCP-1. Biotinylated goat antirabbit IgG (American Qualex, LaMirada, CA, USA) was then applied as the secondary antibodies. For immunolocalization, 4 μm frozen sections were reacted with a murine monoclonal antirat monocyte/macrophage antibody, ED-1 (Serotec, Oxford, UK), to detect monocytes/macrophages. Then the sections were reacted with FITC-conjugated goat antimouse IgG (American Qualex) and analyzed by fluorescent microscopy. ED-1–positive monocyte/macrophages in the glomeruli and the interstitium were counted as described earlier in this article. Sections of renal tissues from rats injected with FITC-conjugated ODN were observed directly with fluorescent microscopy. Additionally, to localize the distribution of ODN, the same sections were stained with TRITC-conjugated Tetra-gon-olobus purpureas (Sigma) as the proximal tubular epithelium marker or with TRITC-conjugated Arachis hypogaea (Sigma) as the distal tubular epithelial marker [35] and were observed by confocal microscopy (MRC600; Bio-Rad, Hercules, CA, USA). Negative controls for immunostaining consisted of replacing each of the primary antibodies with equivalent concentrations of rabbit or murine IgG.

Western blot analysis

Kidney tissues were sonicated in cold RIPA lysis buffer [1% NP40, 0.1% sodium dodecyl sulfate (SDS), 100 μg/mL phenylmethylsulfonylfluoride, 0.5% sodium deoxycholate, 1 mmol/L sodium orthovanadate, 2 μg/mL aprotinin, 2 μg/mL antipain, and 2 μg/mL leupeptin in PBS], and the homogenates were centrifuged for five minutes at 4°C. Protein concentration of the supernatant was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA), and then 30 μg of proteins were examined as described previously with rabbit anti–MCP-1 [32].

In situ hybridization

Monocyte chemoattractant protein-1 mRNA transcripts were detected in paraformaldehyde-fixed, frozen tissues by in situ hybridization with MCP-1 cRNA probes generated from the mouse MCP-1 cDNA according to the method of Hirota et al [36]. Hybridization was carried out at 45°C for 16 hours, and the final washing was in 0.2 × standard saline citrate for 15 minutes at 60°C.

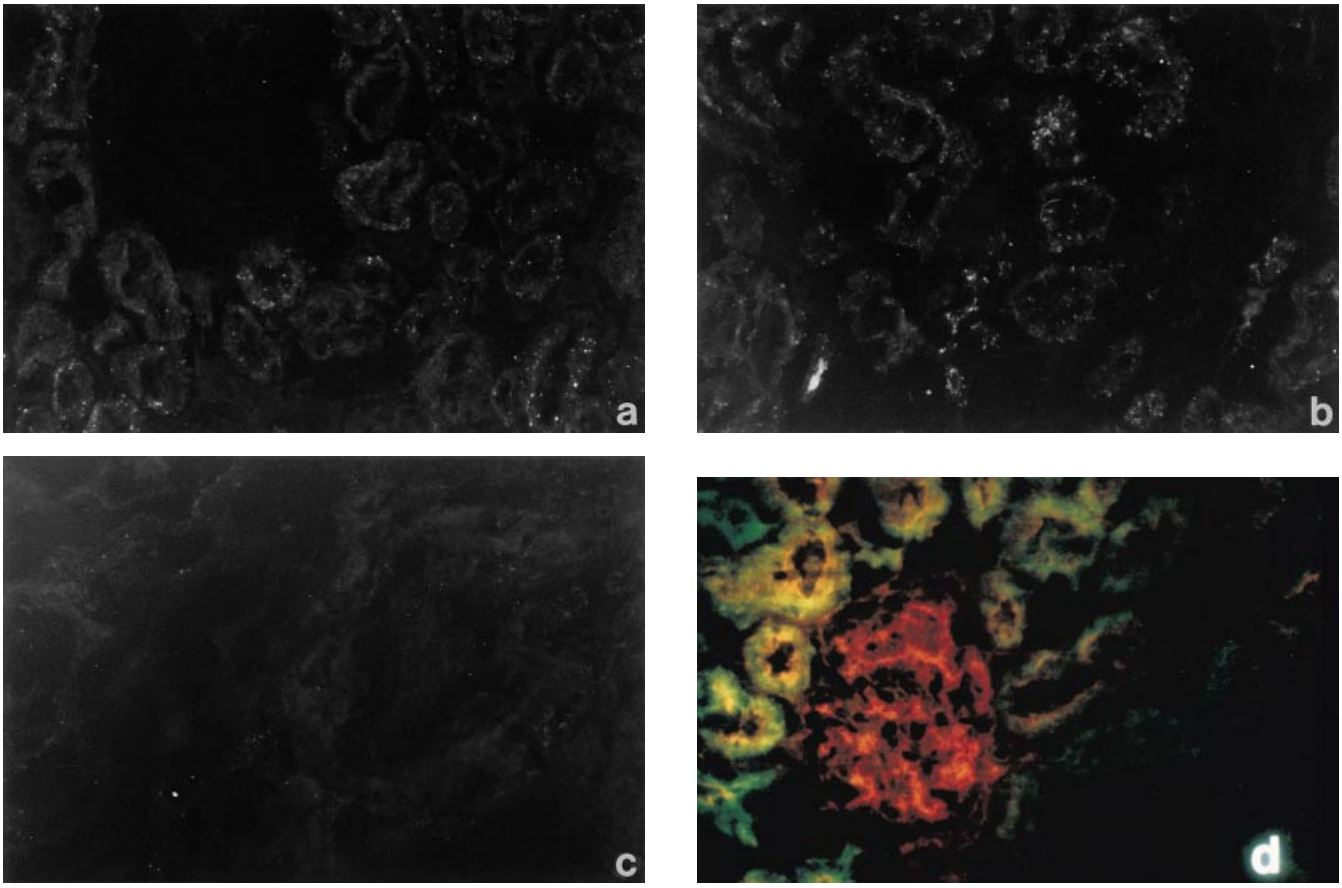


Fig. 3. Distribution of FITC-conjugated ODN in the kidney at 12 hours after intravenous injection. (*a*) Normal kidney. FITC-conjugated ODN was observed in the tubular epithelium (×300). (*b*) Nephritic kidney of GPS rats on day 28. FITC-conjugated ODN was taken up by the tubular epithelium, and its distribution was substantially the same as in the normal kidney (×300). (*c*) The autofluorescence of nephritic kidney was negligible (×300). (*d*) Dual staining with TRITC-conjugated Tetragonolobus purpureas (TP) as a proximal tubular epithelium marker. FITC-conjugated ODN (in green) colocalized with TP (in orange) in the proximal tubular epithelium, yielding a yellow color and indicating that ODN was taken up by proximal tubular epithelial cells exclusively. TP, only, localized to the glomeruli, yielding an orange color (×300).

Urinary protein and renal function

Blood and 24-hour urine collections were taken on day 37 prior to sacrifice. Urinary protein was measured using a BCA protein assay kit (Pierce). Concentrations of serum and urine creatinine were determined using the standard Jaffe rate reaction (alkaline picrate; Sigma). Urine volume was measured gravimetrically, and urine flow rate was factored per gram body weight. Creatinine clearance (C_{Cr}) was calculated as the ratio of the urine/plasma concentration of creatinine multiplied by the urine flow rate. Urinary protein excretion rate was calculated as the ratio of urinary protein per min/C_{Cr} to exclude the influence of C_{Cr} on the glomerular protein trafficking, resulting in an indicator of glomerular damage.

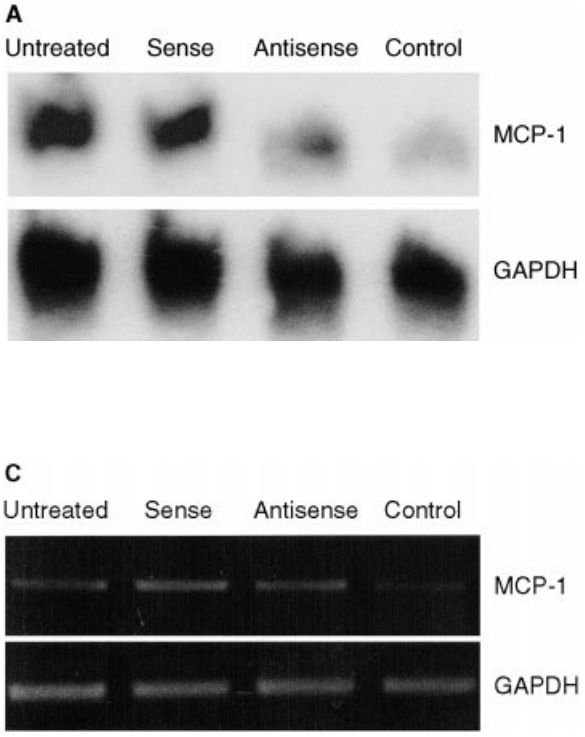
Data analysis

Values were presented as mean ± SE. Statistical differences between groups were evaluated by analysis of variance, followed by Duncan's multiple range test [37], with $P < 0.05$ used as the requirement for significance.

RESULTS AND DISCUSSION

Characterization of Goodpasture syndrome rats and expression of monocyte chemoattractant protein-1 in the nephritic kidneys

As others have reported [33], we found that when rats were immunized with α3(IV) NC1 domains, they developed significant proteinuria on day 21 that increased to a peak level at approximately day 35 (data not shown). Rat IgG was found distributed linearly along glomerular basement membranes by day 14, and focal crescentic glomerulonephritis with marked glomerular hypercellularity was observed on day 28 (Fig. 1a). Crescent formation in the glomeruli became more apparent, and tubulointerstitial alterations with peritubular mononuclear cell infiltration became detectable on day 35 (Fig. 1b). In parallel to peritubular mononuclear cell infiltration, MCP-1 mRNA expression across the entire nephritic kidney was significantly up-regulated from day 21 and increased to a peak level on day 35 (Fig. 1 c, d). MCP-1 protein was found



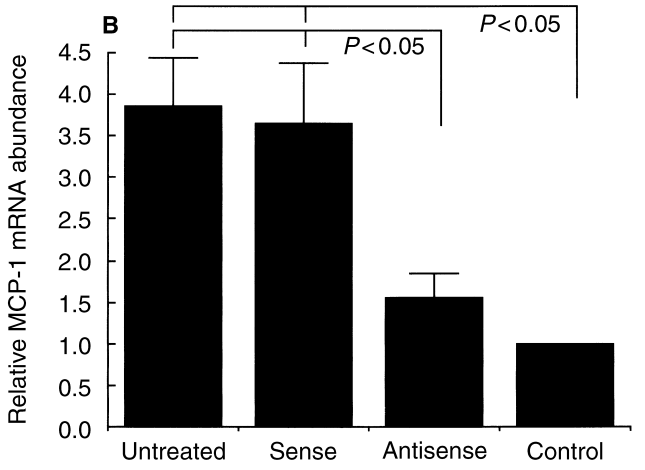


Fig. 4. MCP-1 antisense ODN application in vivo. (*A*) Northern analysis of total RNA extracted from whole nephritic kidneys at 12 hours after the administration of ODN on day 28. (*B*) Quantitative densitometric analysis of (A). MCP-1 antisense ODN reduced the MCP-1 mRNA to control levels (lanes 3 and 4 in A, columns 3 and 4 in B). MCP-1 mRNA levels in the kidneys of sense ODN-treated GPS rats (lane 2 in A, column 2 in B) were similar to the untreated GPS rats (lane 1 in A, column 1 in B). A representative blot selected from four separate experiments is shown, and the densitometric data were obtained from these four blots. (*C*) RT-PCR detection of MCP-1 mRNA in the glomeruli isolated by serial sieving. The glomerular MCP-1 mRNA level was increased in the untreated GPS rats on day 28 when compared with the control rats (lanes 4 and 1). MCP-1 antisense or sense ODN did not affect the increased level of MCP-1 mRNA in the glomeruli (lanes 2–4). These are the representative data selected from three separate experiments.

not only in the glomeruli but also in the tubular epithelium on day 28 (Fig. 1e), which is consistent with findings in other experimental models and in human nephritis [7, 19, 20]. Some humoral factors, including IL-1β, TNF-α [21], and urinary protein [22], directly or indirectly enhance MCP-1 mRNA expression in tubular epithelium. Although the exact regulation of tubular MCP-1 expression in GPS remains unclear, most of these factors prevail ubiquitously and are likely to induce MCP-1 and other downstream members of proinflammatory cascade in this model [4–9, 14].

Application of MCP-1 antisense ODN in vivo and its effects on Goodpasture syndrome rats

Consistent with other studies [21], we found that TNF-α (10 ng/mL) stimulates RPTC cells to express MCP-1 mRNA. TNF-α–induced MCP-1 mRNA expression reached a peak level within four hours. Treatment of SL-O permeabilized RPTC cells with MCP-1 antisense ODN after four hours of incubation with TNF-α prevented the increase in MCP-1 mRNA expression (Fig. 2). Sense ODN and mutated antisense ODN treatments produced no significant effects (Fig. 2). This result demonstrated that the sequence employed as MCP-1 antisense was effective. To demonstrate the delivery of ODN into renal tubular epithelium, FITC-conjugated ODN was injected intravenously into normal rats and day 27 GPS rats at a concentration of 1 mg/kg. Kidneys were harvested and evaluated at 12, 24, and 48 hours after injection. The kidneys showed remarkable accumulation of ODN in the tubular epithelium at 12 hours after injection (Fig. 3 a, b). Detectable ODN remained there for as long as 24 hours and subsequently disappeared 48 hours later (data not shown). FITC-conjugated ODN colocalized exclusively with the proximal tubular epithelial marker, Tetragonolobus purpureas (Fig. 3d), but not with the distal tubular epithelial marker, Arachis hypopaea (data not shown). Administration of MCP-1 sense ODN had no effect, but MCP-1 antisense ODN lowered the MCP-1 mRNA level in whole nephritic kidney from day 28 GPS rats at 12 hours after injection (Fig. 4 A, B). RT-PCR revealed that the level of MCP-1 mRNA in the glomeruli of day 28 GPS rats was increased when compared with those of control rats. In addition, neither antisense nor sense ODNs affected the glomerular MCP-1 mRNA level (Fig. 4C). These results show that ODNs were selectively taken up by tubular epithelial cells in the nephritic kidney and suggest that MCP-1 antisense ODN lowered MCP-1 mRNA expression in tubular epithelium in vivo.

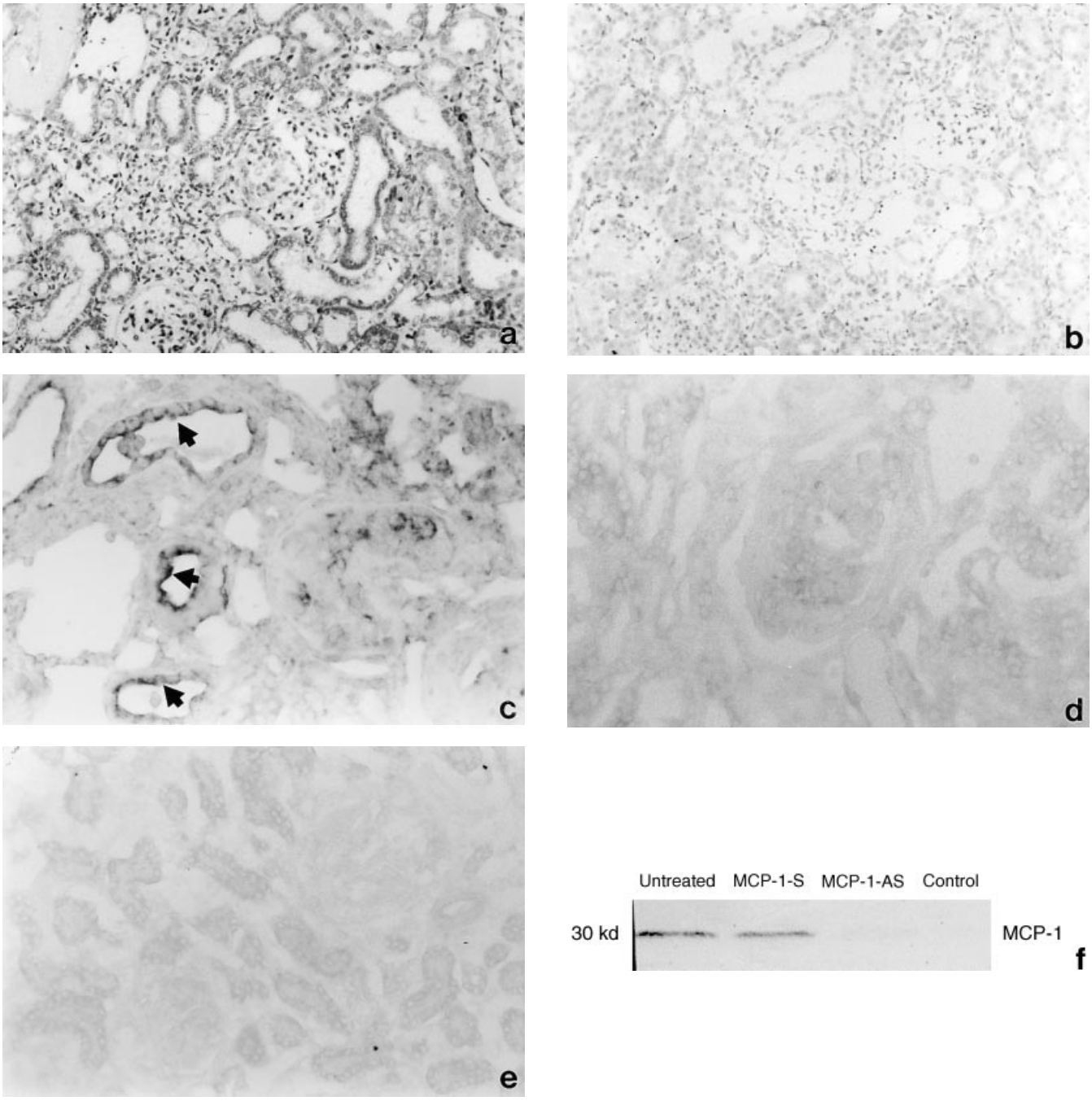


Fig. 5. MCP-1 protein and mRNA expression in the kidney of GPS rats on day 37 after multiple injections of MCP-1 antisense ODN. (*a*) MCP-1 protein expression in the kidney of MCP-1-S rats. MCP-1 protein persisted in the tubular epithelium of the MCP-1-S rats (×200). (*b*) MCP-1 protein expression in the kidney of the MCP-1-AS group. The MCP-1 protein level was reduced in the tubular epithelium (×200). (*c*) In situ hybridization analysis of MCP-1 mRNA expression in the kidney of sense ODN-treated GPS rats on day 37. Using MCP-1 antisense probe, MCP-1 mRNA was detected in the tubular epithelium (arrows) and faintly in the glomeruli (×300). (*d*) The MCP-1 mRNA level in the kidney of antisense ODN-treated GPS rats on day 37. MCP-1 mRNA was not found in the tubular epithelium of these rats (×300). (*e*) In situ hybridization using the MCP-1 sense probe did not label the kidney of sense ODN-treated GPS rats on day 37 (×300). (*f*) Western blot analysis of protein extracts from the kidneys of ODN-treated GPS rats on day 37. The MCP-1 protein level was increased in the kidney of untreated animals, and the increased levels were not affected by treatment with sense ODN in the MCP-1-S rats. However, MCP-1 protein was not detected in the sample of the MCP-1-AS group and the control rats, suggesting that antisense ODN lowered MCP-1 protein expression in the nephritic kidneys.

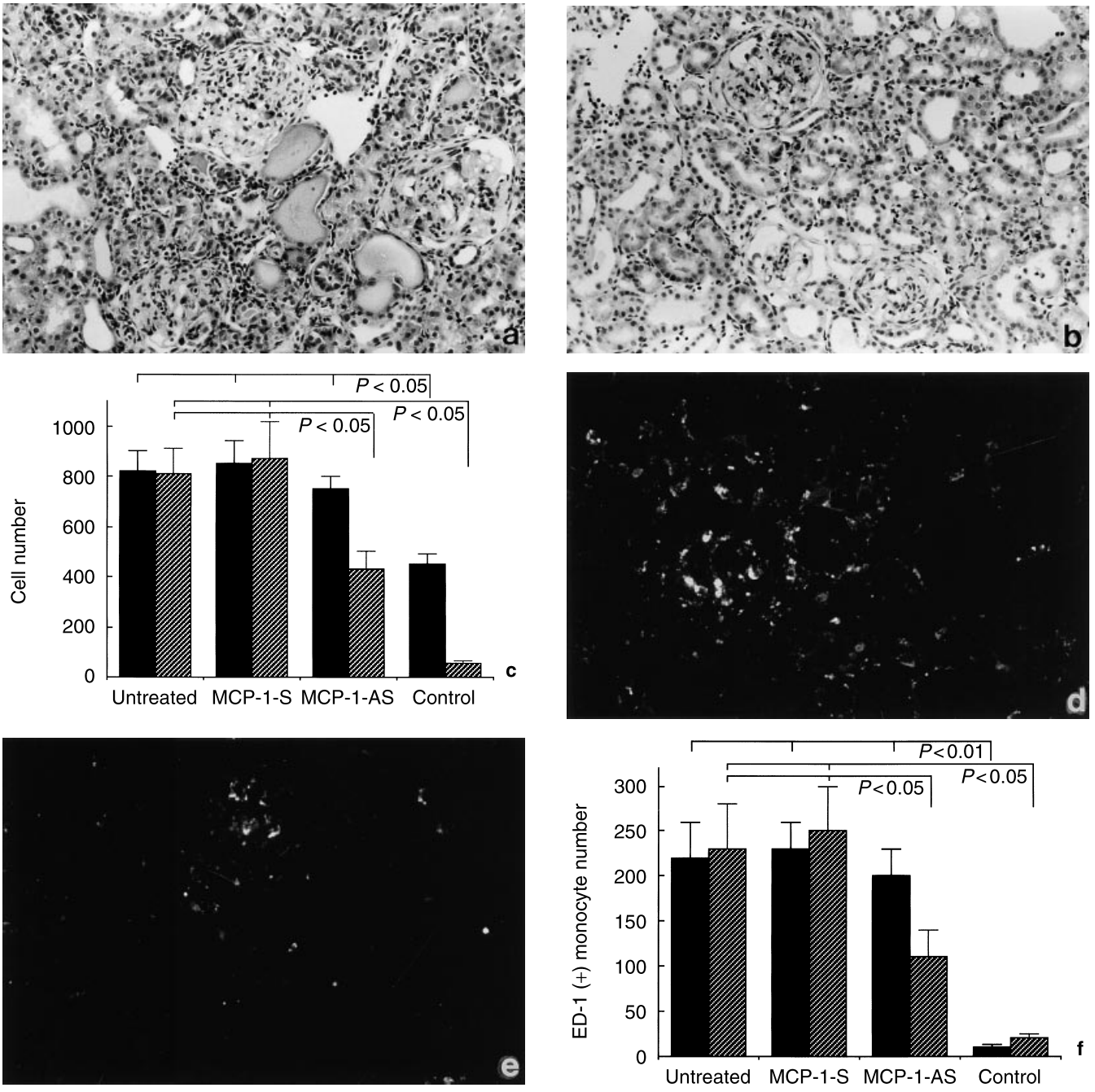


Fig. 6. Renal histopathology of GPS rats on day 37 after repeated injections of MCP-1 antisense ODN. (*a*) Renal histology of the MCP-1-S rats. Remarkable glomerular and interstitial alterations were observed (HE stain, ×300). (*b*) Renal histology of the MCP-1-AS rats. Despite extensive glomerular damage, interstitial mononuclear cell infiltration was significantly attenuated (HE stain, ×300). (*c*) Glomerular hypercellularity and interstitial mononuclear cell infiltration index [mean cell number/10 glomeruli, and mean cell number/high-power (×200) cortical field, respectively]. Symbols are: (■) glomeruli; (▨) interstitium. No significant effects on glomerular hypercellularity were seen in either the MCP-1-AS or MCP-1-S rats when compared with the untreated rats. However, the number of mononuclear cells in the interstitium was significantly lower in the MCP-1-AS group than in either the MCP-1-S or untreated groups. (*d*) ED-1–positive monocyte/macrophages in the kidney of the MCP-1-S group. A number of ED-1–positive monocyte/macrophages were seen in the glomeruli and the interstitium (×300). (*e*) ED-1–positive monocyte/macrophages in the kidney of the MCP-1-AS group. The number of ED-1–positive monocyte/macrophages in the interstitium was significantly reduced (×300). (*f*) ED-1–positive monocyte/macrophage counts in the glomeruli (■) and the interstitium (▨) [mean ED-1 (+) cell number/10 glomeruli, and mean ED-1 (+) cell number/high-power (×200) cortical field, respectively]. Although the numbers of ED-1–positive monocyte/macrophages in the glomeruli were not different between the MCP-1-S and MCP-1-AS rats, the numbers of ED-1–positive monocyte/macrophages in the interstitium of the MCP-1-AS group were significantly lower than those of the MCP-1-S and untreated groups.

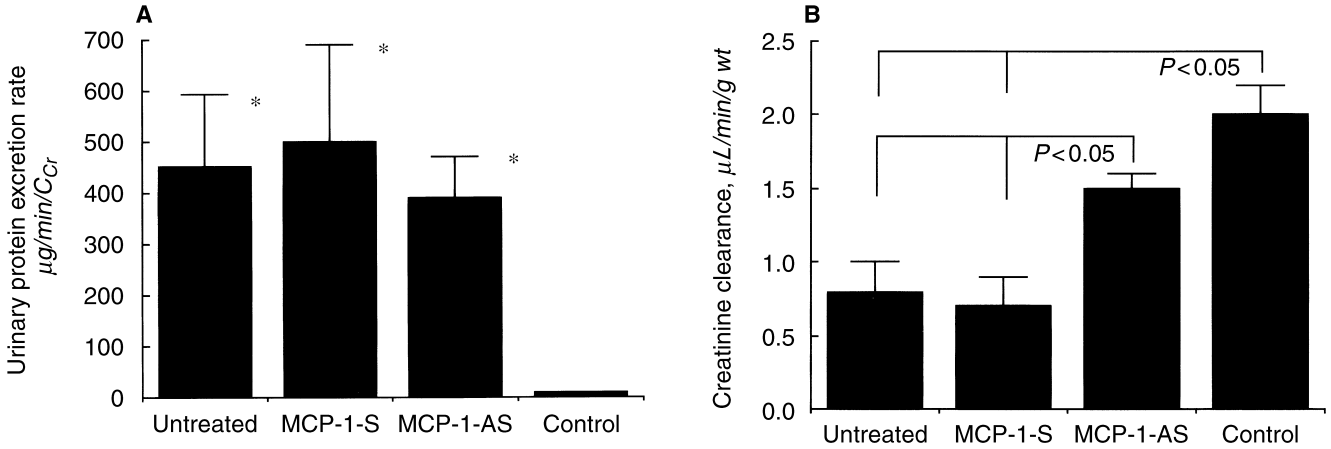


Fig. 7. Renal functional parameters of GPS rats on day 37 after multiple injections of MCP-1 antisense ODN. (*a*) Urinary protein excretion rate. Urinary protein excretion rate was not affected in either the MCP-1-AS or MCP-1-S groups when compared with the untreated group. (*b*) Creatinine clearance (C_{Cr}). The decrease in C_{Cr} of the MCP-1-AS group was significantly suppressed compared with the untreated and MCP-1-S groups.

Modification of renal deterioration in Goodpasture syndrome rats

After multiple injection of ODNs between days 27 and 35, examination of MCP-1-S rats on day 37 revealed strong expression of MCP-1 protein and mRNA in the tubular epithelium (Fig. 5 a, c, f). Expression levels were similar to those found in the control group. However, MCP-1 protein and mRNA expression in MCP-1-AS rats were significantly attenuated (Fig. 5 b, d, f). Although the glomerular hypercellularity in all three experimental groups progressed to a similar degree (Fig. 6 a–c), aggravation of the interstitial mononuclear cell infiltration in the MCP-1-AS group was significantly attenuated compared with that observed in the MCP-1-S and untreated groups on day 37 (Fig. 6 a–c). The number of ED-1–positive monocyte/macrophages in the interstitium was also significantly lower in the MCP-1-AS group than in the MCP-1-S group, regardless of the presence of a number of ED-1–positive monocyte/macrophages in the glomeruli of both groups (Fig. 6 d–f). These results suggest that MCP-1, expressed by tubular epithelium, plays an essential role in mediating interstitial alterations, especially monocyte infiltration.

No significant difference was found in the urinary protein excretion rate between the MCP-1-AS and MCP-1-S groups on day 37 (Fig. 7A). Interestingly, glomerular histopathology was not altered in the MCP-1-AS group, which may explain why MCP-1 antisense ODN failed to lower the urinary protein excretion rate. However, the decrease in C_{Cr} was significantly lower in the MCP-1-AS group than in the MCP-1-S group (Fig. 7B). This result is consistent with the observation that the degree of tubulointerstitial alteration is a better indicator of renal function correlated than is glomerular alteration [1–3].

Recently, using MCP-1–deficient mice with the nephrotoxic serum (NTS) nephritis, Tesch et al demonstrated that an absence of MCP-1 expression in the kidney results in a reduction of tubular injury and macrophage-induced interstitial inflammation in spite of obvious glomerular injury and proteinuria [38]. Although there are two significant differences between their study and ours, the means to accomplish a reduction of MCP-1 expression and the model used, both studies provide direct evidence that MCP-1, expressed by tubular epithelium, has a pivotal role in mediating monocyte infiltration into the interstitium when secondary to glomerular diseases. Therefore, blocking MCP-1 expression in tubular epithelium may be appropriate to treat some forms of human kidney disease.

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REFERENCES

1. Striker G, Schainuck L, Cutler R, Beneditt E: Structural-functional correlations in renal disease. 1. The correlations. *Hum Pathol* 1:615–630, 1970
2. Risdon R, Sloper J, De Vardener H: Relationship between renal function and histologic changes found in renal-biopsy specimens from patients with persistent glomerulonephritis. *Lancet* 2:363–366, 1968
3. Bohle A, Mackensen-Haen S, Von Gise H: Significance of tubulointerstitial changes in the renal cortex for the excretory function and concentration ability of the kidney: A morphometric contribution. *Am J Nephrol* 7:421–433, 1989
4. Pichler R, Giachelli C, Young B, Alpers C, Couser W, Johnson R: The pathogenesis of tubulointerstitial disease associated with glomerulonephritis: The glomerular cytokine theory. *Miner Electrolyte Metab* 21:317–327, 1995
5. Okada H, Strutz F, Danoff T, Kalluri R, Neilson E: Possible mechanisms of renal fibrosis. *Contrib Nephrol* 118:147–154, 1996

6. Remuzzi G, Ruggenenti P, Benigni A: Understanding the nature of renal disease progression. *Kidney Int* 51:2–15, 1997
7. Rovin B, Phan L: Chemotactic factors and renal inflammation. *Am J Kidney Dis* 6:1065–1084, 1998
8. Klahr S: Interstitial macrophages. *Semin Nephrol* 13:488–495, 1993
9. Van Goor H, Ding G, Kees-Folts D, Schreiner G, Grond J, Diamond J: Macrophages and progressive renal disease. *Lab Invest* 71:456–464, 1994
10. Reimann J, Kaufmann S: Alternative antigen processing pathways in anti-infective immunity. *Curr Opin Immunol* 9:462–469, 1997
11. Sharma K, Ziyadeh F: The emerging role of transforming growth factor-beta in kidney diseases. *Am J Physiol* 266:F829–F842, 1994
12. Okada H, Danoff T, Kalluri R, Neilson E: Early role of Fsp1 in epithelial-mesenchymal transformation. *Am J Physiol* 273:F563–F574, 1997
13. Proost P, Wuyts A, Van Damme J: The role of chemokines in inflammation. *Int J Clin Lab Res* 26:211–223, 1996
14. Wenzel U, Abboud H: Chemokines and renal disease. *Am J Kidney Dis* 26:982–994, 1995
15. Gu L, Rutledge B, Fiorillo J, Ernst C, Grewal I, Flavell R, Gladue R, Rollins B: In vivo properties of monocyte chemoattractant protein-1. *J Leukoc Biol* 62:577–580, 1997
16. Kurihara T, Warr G, Loy J, Bravo R: Defects in macrophage recruitment and host defense in mice lacking the CCR2 chemokine receptor. *J Exp Med* 186:1757–1762, 1997
17. Grewal I, Rutledge B, Fiorillo J, Gu L, Glandue R, Flavell R, Rollins B: Transgenic monocyte chemoattractant protein-1 (MCP-1) in pancreatic islets produces monocyte-rich insulitis without diabetes: Abrogation by a second transgene expressing systemic MCP-1. *J Immunol* 159:401–408, 1997
18. Kolattukudy P, Quach T, Bergese S, Breckenridge S, Hensley J, Altschuld R, Gordillo G, Klenotic S, Orosz C, Parker-Thronburg J: Myocarditis induced by targeted expression of the MCP-1 gene in murine cardiac muscle. *Am J Pathol* 152:101–111, 1998
19. Zoja C, Liu X, Donadelli R, Abbate M, Testa D, Corna D, Taraboletti G, Vecchi A, Dong Q, Rollins B, Bertani T, Remuzzi G: Renal expression of monocyte chemoattractant protein-1 in lupus autoimmune mice. *J Am Soc Nephrol* 8:720–729, 1997
20. Grandaliano G, Gesualdeo L, Ranieri E, Monno R, Stallone G, Schena F: Monocyte chemotactic peptide-1 expression and monocyte infiltration in acute renal transplant rejection. *Transplantation* 63:414–420, 1996
21. Prodjosudjadi W, Gerritsma J, Klar-Mohamad N, Gerritsen A, Bruijn J, Daha M, Van Es L: Production and cytokine-mediated regulation of monocyte chemoattractant protein-1 by human proximal tubular epithelial cells. *Kidney Int* 48:1477–1486, 1995
22. Wang Y, Chen J, Chen L, Tay Y, Rangan G, Harris D: Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein. *J Am Soc Nephrol* 8:1537–1545, 1997
23. Fujinaka H, Yamamoto T, Takeya M, Feng L, Kawasaki K, Yaoita E, Kondo D, Wilson C, Uchiyama M, Kihara I: Suppression of anti-glomerular basement membrane nephritis by administration of anti-monocyte chemoattractant protein-1 antibody in WKY rats. *J Am Soc Nephrol* 8:1174–1178, 1997
24. Wada T, Yokoyama H, Furuichi K, Kobayashi K, Harada K, Naruto M, Su S, Akiyama M, Mukaida N, Matsushima K: Intervention of crescentic glomerulonephritis by antibodies to monocyte chemotactic and activating factor (MCAF/MCP-1). *FASEB J* 10:1418–1425, 1996
25. Lloyd C, Minto A, Dorf M, Proudfoot A, Wells T, Salant D, Gutierrez-Ramos J: RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. *J Exp Med* 185:1371–1380, 1997
26. Tang W, Qi M, Warren J: Monocyte chemoattractant protein 1 mediates glomerular macrophage infiltration in anti-GBM Ab GN. *Kidney Int* 50:665–671, 1996
27. Carome M, Kang Y, Bohen E, Nicholson D, Carr F, Kiandoli L, Brummel S, Yuan C: Distribution of the cellular uptake of phosphorothioate oligodeoxynucleotides in the rat kidney in vivo. *Nephron* 75:82–87, 1997
28. Rappaport J, Hanss B, Kopp J, Copeland T, Bruggeman L, Coffman T, Klotman P: Transport of phophorothioate oligonucleotides in kidney: Implications for molecular therapy. *Kidney Int* 47:1462–1469, 1995
29. Noiri E, Peresleni T, Miller F, Goligorsky M: In vivo targeting of inducible NO synthase with oligodeoxynucleotides protects rat kidney against ischemia. *J Clin Invest* 97:2377–2383, 1996
30. Poon M, Hsu W, Bogdanow V, Taubman M: Secretion of monocyte chemotactic activity of cultured rat aortic smooth muscle cells in response to PDGF is due predominantly to the induction of JE/MCP-1. *Am J Pathol* 149:307–317, 1996
31. Spiller D, Giles R, Grzybowski J, Tidd D, Clark R: Improving the intracellular delivery and molecular efficacy of antisense oligonucleotides in chronic myeloid leukemia cells: A comparison of streptolysin-O permeabilization, electroporation, and lipophilic conjugation. *Blood* 91:4738–4746, 1998
32. Strutz F, Okada H, Lo C, Danoff T, Carone R, Tomaszewski J, Neilson E: Identification and characterization of a fibroblast marker: Fsp1 *J Cell Biol* 130:393–405, 1995
33. Abbate M, Kalluri R, Corna D, Yamaguchi N, McCluskey R, Hudson B, Andres G, Zoja C, Remuzzi G: Experimental Goodpasture's syndrome in Wistar-Kyoto rats immunized with a3 chain of type IV collagen. *Kidney Int* 54:1550–1561, 1998
34. Kalluri R, Danoff T, Okada H, Neilson E: Susceptibility to antiglomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II gene and the emergence of T cell-mediated immunity in mice. *J Clin Invest* 100:2263–2275, 1997
35. Nadasdy T, Laszik Z, Kenneth E, Johnson D, Silva F: Tubular atrophy in the end-stage kidney: A lectin and immunohistochemical study. *Hum Pathol* 25:22–28, 1994
36. Hirota S, Ito A, Morii E, Wanaka A, Tohyama M, Kitamura Y, Nomura S: Localization of mRNA for c-kit receptor and its ligand in the brain of adult rats: An analysis using in situ hybridization. *Mol Brain Res* 15:47–54, 1992
37. Takenaka T, Mitchell K, Navar L: Contribution of angiotensin II to renal hemodynamic and excretory responses to nitric oxide synthesis inhibition in the rats. *J Am Soc Nephrol* 4:1046–1053, 1993
38. Tesch G, Schwarting A, Kinoshita K, Lan H, Rollins B, Kelley V: Monocyte chemoattractant protein-1 promotes macrophage-mediated tubular injury, but not glomerular injury, in nephrotoxic serum nephritis. *J Clin Invest* 103:73–80, 1999