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Inhibition of intercellular adhesion molecule-1 with antisense deoxynucleotides prolongs renal isograft survival in the rat

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Inhibition of intercellular adhesion molecule-1 with antisense deoxynucleotides prolongs renal isograft survival in the rat.

Background. Delayed graft function from ischemia-reperfusion injury has a negative impact on long-term renal graft survival. We tested the utility of antisense oligodeoxynucleotide (ODN) against intercellular adhesion molecule-1 (ICAM-1) in the pretransplant treatment of renal isografts in improving long-term graft survival.

Methods. Three groups of 16 inbred Lewis rats each underwent unilateral nephrectomy and were then transplanted with a kidney from a Lewis donor rat, which had received antisense ODN, reverse sense ODN, or saline vehicle six hours prior to nephrectomy. The kidneys were subjected to one hour of warm ischemia and 30 minutes of cold ischemia, which when untreated results in delayed graft function. The remaining native kidney was removed 10 days later. Serum creatinine and urinary protein excretion were measured in surviving rats at weeks 2, 4, 6, 8, 12, 16, and 20 after native nephrectomy.

Results. A Kaplan-Meier analysis revealed that by week 6 one half of the animals receiving reverse sense ODN and saline vehicle treatment had died, while all but 2 rats in the antisense ODN-treatment group survived to 20 weeks. Serum creatinine concentrations and urine protein excretion of surviving reverse sense and saline vehicle-treated rats were significantly higher than antisense treated rats at every time point. Histology at week 20 revealed marked interstitial fibrosis, focal glomerular sclerosis, vascular intimal and medial thickening and tubular atrophy in reverse sense and saline vehicle-treated kidneys, while antisense ODN-treated kidneys showed only modest changes. Immunohistochemistry showed macrophage and lymphocyte infiltration, as well as substantial up-regulation of MHC class II, in reverse sense and saline vehicle-treated kidneys compared to antisense ODN-treated kidneys.

Conclusions. These results suggest that by ameliorating acute nonimmunological renal isograft injury, the long-term chronic nonimmunologic processes are improved as well. Furthermore, the data suggest that an antisense ODN strategy directed against ICAM-1 may have utility in human kidney transplantation.

Key words: antisense, ICAM-1, adhesion molecules, transplantation, isografts.

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Renal transplantation has made substantial strides in the past few decades, with the introduction of improved immunosuppressive regimens, organ preservation, and pre- and postoperative care. Nevertheless, there is considerable room for improvement, particularly in terms of improving long-term outcome [1]. Initial ischemia/reperfusion injury occurring secondary to organ retrieval, storage, and transplantation has been associated with late renal allograft deterioration and failure [2–6]. In addition, there is an apparent synergy between the initial injuries of ischemia/reperfusion and acute graft rejection that has been reported in several clinical series. The long-term results of graft survival are significantly decreased after both events in combination as compared with either alone [7–9]. The pathogenesis of ischemia/reperfusion injury is now known to involve cytokines and particularly surface adhesion molecules, the expression of which initiates the attachment of inflammatory cells [10, 11]. Evidence from experimental animals with acute renal ischemia has shown that the intercellular adhesion molecule-1 (ICAM-1) is promptly up-regulated after injury and that neutrophil, T cell, and macrophage infiltrations subsequently occur. After an initial recovery and a period of relative quiescence, proteinuria develops and progressive morphological changes begin, including glomerulosclerosis, arterial obliteration, and interstitial fibrosis. These phenomena are accompanied by a re-expression of ICAM-1, progressive macrophage infiltration and their associated products, particularly, interleukin 1, tumor necrosis factor-α, transforming growth factor-β, and inducible nitric oxide synthase. Monocyte chemotactic protein 1 is subsequently up-regulated, with a dramatic increase in infiltrating cells [12].

We have developed a novel strategy to suppress the initial expression of ICAM-1 after ischemic injury [13] to block the subsequent cascade outlined above [12]. We employed antisense oligodeoxynucleotides (ODN) specifically directed against ICAM-1 mRNA and were able to evoke a dramatic reduction in ischemia/reperfusion injury initiated by a timed obstruction of the rat renal artery. The

antisense ODN were facilitated by means of a lipofectin vehicle and were given intravenously six hours prior to the induction of ischemia. ICAM-1 expression as measured by Western blotting and immunohistochemistry was markedly reduced, the infiltration of inflammatory cells was dramatically impeded, and the decrease in glomerular filtration rate resulting from ischemia/reperfusion injury was totally abolished [13]. We next extended these observations to an acute model of renal autotransplantation in the rat [14–16]. This study examines chronic syngeneic graft survival in the rat. We found a dramatic increase in long-term graft survival, and there was a substantial reduction in the so-called nonimmunological chronic graft deterioration.

METHODS

Phosphorothioate oligodeoxyribonucleotides (ODN) were purchased (Tib Molbiol, Berlin, FRG). We selected one of the sequences (5′-ACC GGA TAT CAC ACC TTC CT) hybridizing to the 3′-untranslated region of ICAM-1 mRNA nearly 300 bases 3′ to the translation termination site. The reverse ODN sequence (5′-TCC TTC CAC ACT ATA GGC CA) was used as control. We used a cationic lipid solution (Lipofectin; GIBCO BRL, Life Technologies, Hamburg, FRG) to enhance ODN uptake.

To investigate the long-term influence of antisense ODN for ICAM-1 on reperfusion injury during transplantation, we used a Lewis rat isotransplantation model. Left kidneys of normal Lewis rats were removed and the left kidney from the Lewis donor rat was transplanted after 30 minutes of cold ischemia time. Donor animals were treated with antisense ODN for ICAM-1, reverse sense ODN, or saline vehicle six hours prior to transplantation. Warm ischemia was maintained at 60 minutes. After 30 minutes of cold ischemia, the left kidneys were transplanted into the Lewis recipient rats. Isotransplanted animals receiving kidneys with antisense ODN served as the "active treatment" group, those receiving kidneys with reverse ODN-treatment served as the "control treatment" group, and rats receiving kidneys from donors treated with saline vehicle served as the "no treatment" control group. Finally, we used native kidneys from nephrectomized recipients to obtain normal rat renal tissue for comparison.

Renal transplantation was carried out according to a modified protocol developed by Tullius et al [7, 17]. Male Lewis (LEW, RT^1) rats (150 to 200 g) purchased from Moellegaard Breeding & Research Center Ltd. (Ejby, Skensved, Denmark) were used in the experiments. The animals had free access to tap water and standard rat diet (No. C-1000; Altromin, Lage, FRG) and were kept under regular lighting conditions (lights on at 06:00 and off at 18:00) at a constant temperature of 24°C. All procedures were approved by local authorities (Permit AZ IV.A4/5-G 0406/95) according to guidelines corresponding to the American Physiological Society. The rats were fasted overnight before surgery. Donor animals were primarily anesthetized intraperitoneally (i.p.) with thiohexital (1.5 ml/kg of body wt of Brevimytal; Bayer, Leverkusen, FRG) and a catheter was placed in the left jugular vein. ODN (10 mg/kg body wt) were administered i.v. in a 1 ml solution containing either a lipofectin-ODN mix or saline vehicle.

Donors were allowed to recover for six hours and then were re-anesthetized with a 4% chloral hydrate solution (250 mg/kg i.p.). They were placed on a heated surgical table to maintain rectal temperature at 37°C. A long midline abdominal incision was made, the intestine was wrapped in isotonic saline moistened gauze, and placed on the thorax in order to expose the aorta and inferior vena cava together with the renal vessels. The left renal artery and vein were separated from each other and their collateral branches, adrenal artery and vein and spermatic artery and vein, were double ligated with 7-0 silk and divided with an electrocautery device. The ureter was freed from the surrounding fibrotic tissue and cut in the proximity to the bladder. Afterwards, the kidney was freed from perirenal fat. Microaneurysm clips were placed on the renal artery and the renal vein and vessels were cut with an iris microscissors. Perfusion *ex vivo* with 5 ml cold University of Wisconsin (UW) solution followed. Finally, the kidney was placed in cold UW solution (0 to 4°C) for 30 minutes. Recipient Lewis rats were anesthetized in the same fashion. A left nephrectomy was performed and then the kidney from the Lewis donor rat was removed from the cold UW solution and transplanted to the recipient. The native kidneys were preserved to be later used in histological comparisons. The anastomoses were completed end-to-end, using 10-0 prolene suture material. The anastomosis time averaged 30 minutes; clips were left over the vessels an additional 30 minutes to produce the extended warm ischemia time. The rats received cyclosporine 1.5 mg/kg for 10 days, after which the remaining native kidney was removed and the drug was discontinued.

We prepared a total of 16 rats for each of the three groups receiving kidneys from antisense, reverse and saline vehicle-treated donors. The rats were followed for 20 weeks. The rats were inspected every day. Most animals that died, did so without our being able to obtain blood or tissue. Surviving rats were placed in metabolic cages at weeks 2, 4, 8, 12, 16, and 20. Twenty-four-hour urine samples were measured for protein excretion. Blood was obtained from the jugular vein for serum creatinine concentrations. These chemistries were determined with automated methods.

To examine the status of transplanted kidneys in terms of reversible or irreversible acute tubular necrosis at the time of native nephrectomy, we prepared six additional control rats as described above. Three rats received reverse sense ODN and three were treated with vehicle. These rats were sacrificed at 10 days after transplantation and a histological analysis was performed.

Immunohistochemistry was carried out as previously

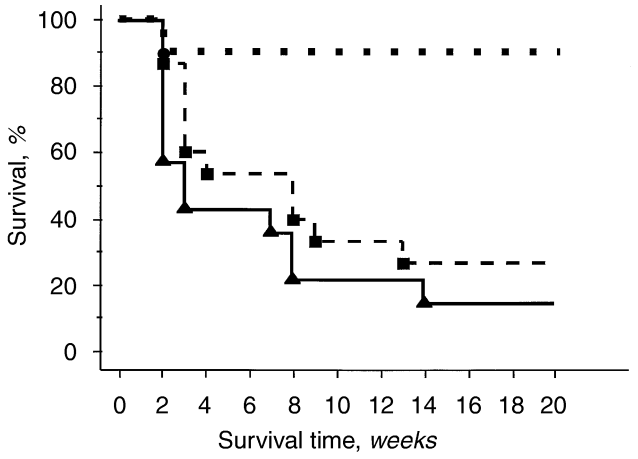

Fig. 1. Kaplan-Meier survival analysis in rats receiving kidneys treated with antisense oligonucleotides (ODN; ●), reverse ODN (■), or saline vehicle (▲). By week 6, half the rats in the latter two groups were dead. Rats receiving antisense ODN-treated kidneys had improved surivival. Only two rats in this group died, soon after the operation.

described [13, 14]. For immunohistochemical staining, the sections were incubated with the monoclonal antibody anti rat monocytes, clone ED-1, (Camon, Wiesbaden, FRG), monoclonal antibody anti-rat lymphocyte CD4, clone OX-35, (Dianova, Hamburg, FRG), monoclonal antibody antibody to MHC II, clone OX-18, and diluted in RPMI (Seromed, Heidelberg, FRG) for 30 minutes at room temperature in a humid chamber. After washing with TBS, the sections were incubated with a rabbit anti-mouse bridging antibody (Dako, Hamburg, FRG) followed by an incubation with alkaline phosphatase anti alkaline phosphatase (APAAP) complex (Dako). For detection and development we used the neufuchsin-naphthol-As-Bi-phosphate substrate (Merck, Darmstadt, FRG) per the manufacturer's instructions.

Half kidneys from each group were sectioned, fixed in formalin, and stained with hematoxylin and eosin as described for our laboratory previously [13, 14]. Without knowledge of the regimens, sections from each kidney were evaluated by a pathologist. Statistical analysis was carried out on a Macintosh II computer (Apple Inc. Cupertino, CA, USA) with a commercially available program (Statview; Cricket Software Inc., Philadelphia, PA, USA). We performed a Kaplan-Meier survival analysis on the three treatment groups. Numerical results are presented as mean ± SEM. Nonparametric (Kruskal-Wallis) and parametric (2 way ANOVA) analyses were used as appropriate. $P \leq 0.05$ was considered significant.

RESULTS

Figure 1 shows the Kaplan-Meier survival analysis in the three groups of rats. The group with antisense ODN-treated kidneys lost two rats in the first two weeks after treatment and thereafter had no mortality. In the reverse and saline vehicle kidney treated groups, the mortality was approximately 50% by six weeks and continued so that by week 20, only two rats in the group receiving saline vehicle-treated kidneys and four rats in the group receiving reverse-treated kidneys were still living. Survival was significantly better in the antisense kidney treated group compared to reverse or saline vehicle kidney treatment. These two control groups did not differ from one another in terms of survival. Figure 2 shows the serum creatinine concentrations from the survivors at the measurement time points. Rats that had died of uremia or for other reasons were not included. The serum creatinine values of rats with antisense ODN-treated kidneys were significantly lower than the other two groups at every timepoint. Reverse ODN and saline vehicle kidney treated rats did not differ from one another. Figure 3 shows the 24-hour urine protein excretion of the three groups. At week 2, protein excretion in the survivors of all three groups was low and did not differ. Thereafter, the protein excretion of rats with antisense treated kidneys was consistently lower than that of the other two groups.

Figure 4 shows the morphology of surviving rats at week 20. Hematoxylin and eosin-stained thin sections of fixed, paraffin-embedded, transplanted rat kidneys are shown. Native rat kidneys (A), rats with kidneys receiving saline vehicle treatment (B), rats receiving kidneys treated with antisense ODN (C), and animals with kidneys treated with reverse sense ODN (D) are shown (representative of 30 sections). Inflammatory infiltrate and tubular atrophy manifested by dilated tubules with flattened epithelial cells are apparent in sections (B) and (D), which are indistinguishable from each other. A marked increase in interstitial matrix with round cell infiltrate is evident. Cross sections through blood vessels show increased intimal and medial thickness. In contrast, a section from an animal receiving an antisense treated kidney (C) shows only minimal evidence of increased interstitial infiltrate and the glomeruli are hardly distinguishable from the normal kidney shown in section (A). A quantitative grading scale considering glomerulosclerosis (comparing 4 kidneys from each group) verified these findings statistically; rats with saline-treated kidneys had 3.3 (range 2 to 4), rats with antisense ODN-treated kidneys had 1.2 (range 1 to 2), and rats with reverse sense ODN-treated kidneys had 3.4 (range 3 to 4).

Figure 5 is a periodic acid-Schiff stain of the same kidneys shown in Figure 4. Glomerular damage was evident in the reverse sense and saline vehicle-kidney treated groups (B and D). Focal glomerular sclerosis is evident, with substantial glomerular basement membrane thickening, hyalinization, and glomerular obsolescence. These changes were not present in native kidneys (A) or in antisense treated organs (C). The same interstitial and vascular changes visible with hemotoxylin and eosin were also observed.

Figure 6 shows immunohistochemical staining with the

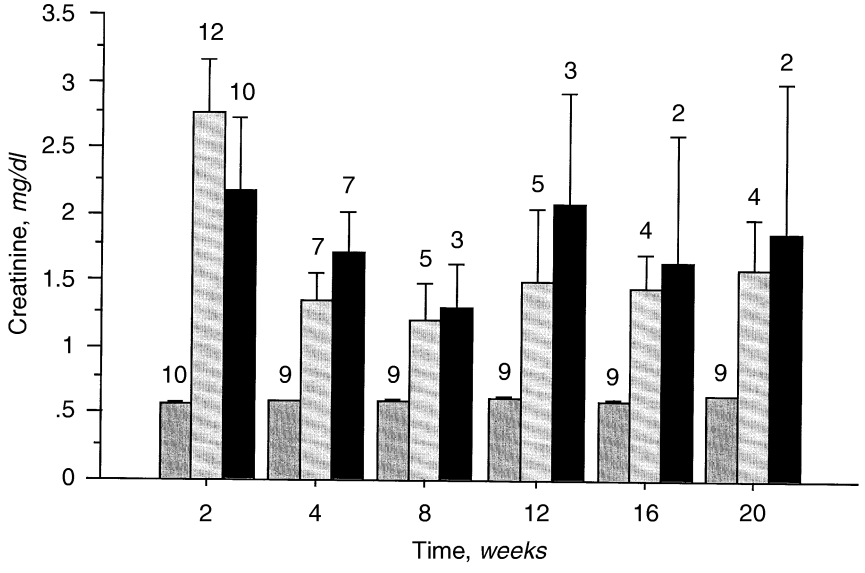

Fig. 2. Serum creatinine concentrations of surviving rats at the given time points. The numbers of rats at these time points were as follows: Rats receiving reverse treated kidneys or saline vehicle-treated kidneys had significantly higher creatinine concentrations than rats receiving antisense ODN-treated kidneys at every time point. Symbols are: (▨) antisense ODN-treated, $N = 10$; (□) reverse ODN-treated, $N = 12$; (■) vehical treated, $N = 10$.

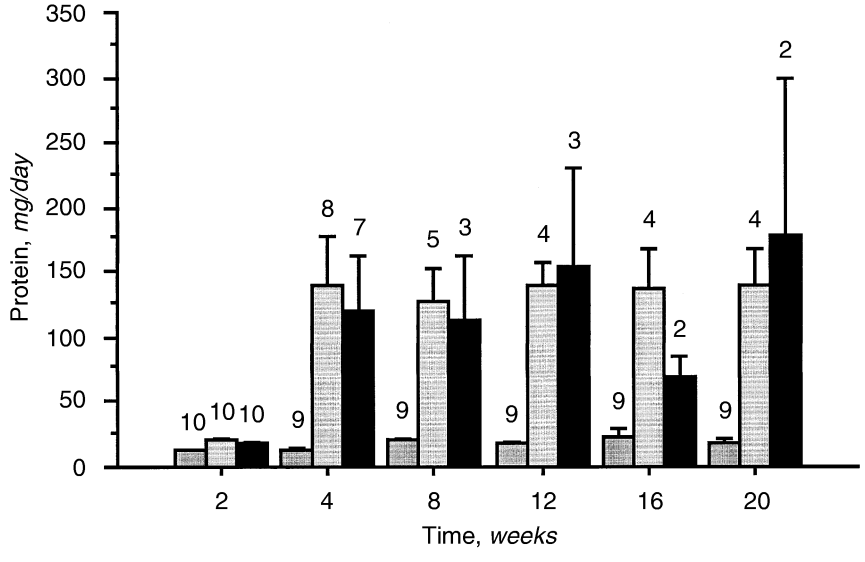

Fig. 3. Twenty-four-hour urine protein excretion of surviving rats at the given time points. Rats receiving reverse treated kidneys or saline vehicle-treated kidneys had significantly higher urinary protein excretion than rats receiving antisense ODN-treated kidneys at every time point. Symbols are: (▨) antisense ODN-treated, $N = 10$; (□) reverse ODN-treated, $N = 10$; (■) vehical treated, $N = 10$.

APAAP method using an anti-ED-1 antibody to identify monocytes. Section (A) from native kidney and section (C) from an animal with an antisense ODN-treated kidney show only occasional monocytes in the glomeruli and interstitium. In contrast, section (B) saline vehicle-treated kidney and (D) reverse ODN-treated kidney show marked monocytic infiltration in the interstitium and around blood vessels. Figure 7 shows immunohistochemical staining with the APAAP method using an antibody directed at CD4 to identify lymphocytes. A prominent lymphocytic infiltration was observed in sections from kidneys receiving saline vehicle treatment (B) and in kidneys pretreated with reverse ODN (D), while native kidneys (A) and antisense-treated kidneys (C) showed very few lymphocytes. Figure 8 shows immunohistochemical staining with the APAAP method to detect cells expressing the MHC Class II (OX-18) antigen. Native kidney showed very little staining (A). Marked staining was observed in rats receiving saline vehicle-treated kidneys (B), especially around glomeruli and within the interstitium. The same was the case for rats receiving kidneys pretreated with reverse sense ODN (D). Rats receiving kidneys with antisense ODN pretreatment revealed a modest degree of staining by basolateral surface of tubular cells (C).

Finally, we considered the possibility that graft necrosis might have been irreversible in reverse sense ODN and vehicle treated rats at 10 days. If this were true, then the present data would confirm that the treatment prevents acute irreversible ischemic injury, but not necessarily show that the treatment prevent chronic graft loss by other

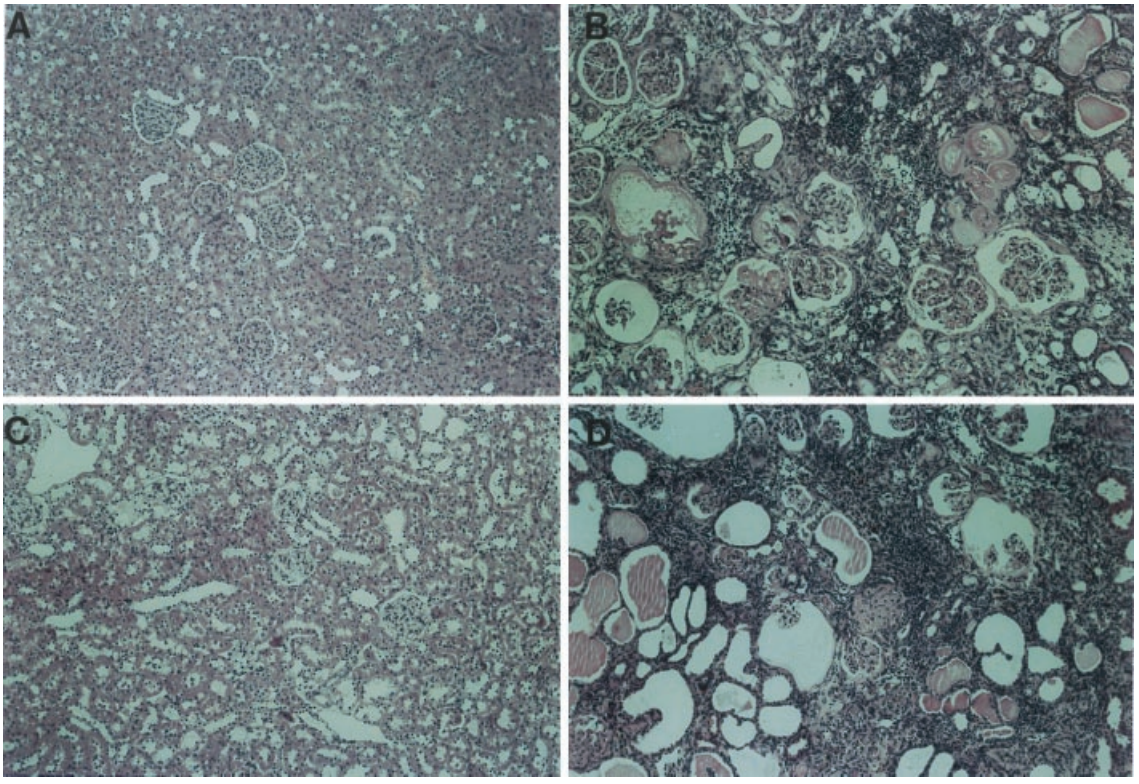

Fig. 4. Renal morphology (hemotoxylin and eosin) at week 20. (*A*) Native kidney. (*B*) Saline vehicle-treated kidney. (*C*) Antisense ODN-treated kidney. (*D*) Reverse ODN-treated kidney. Panels B and D show a marked interstitial fibrosis with the infiltrate. The tubules are generally atrophic. Blood vessels show intimal and medial thickening.

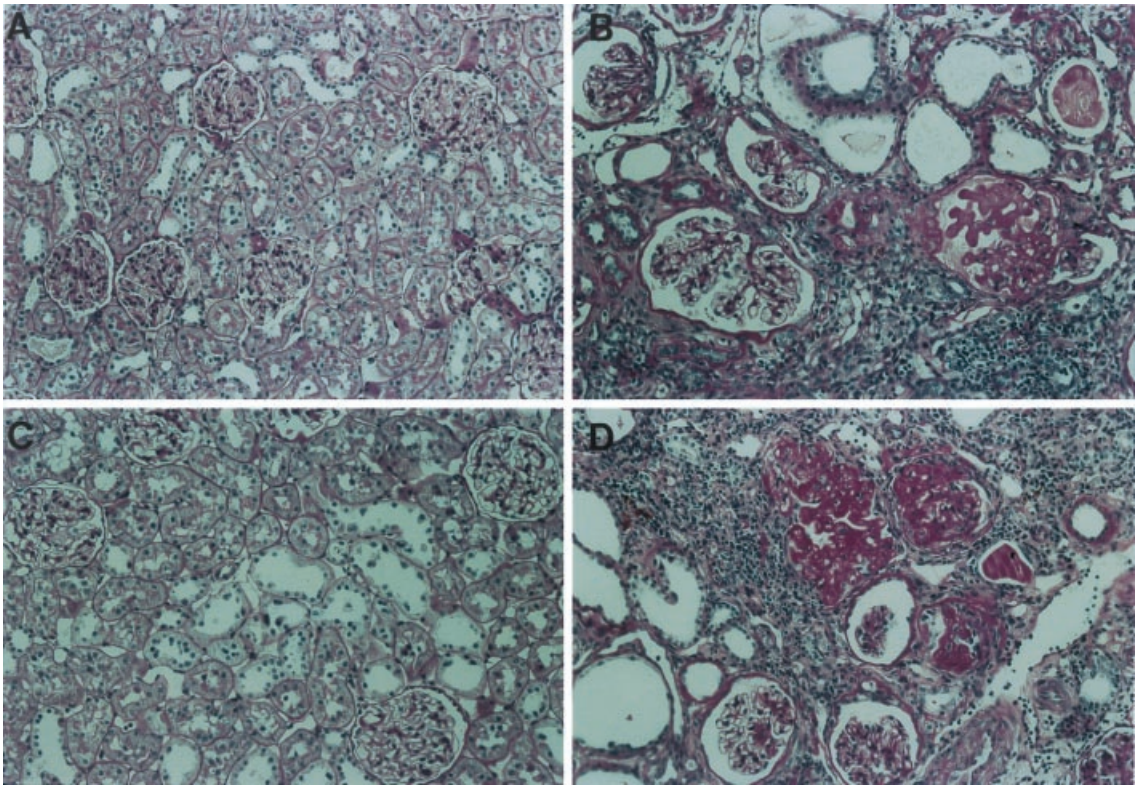

Fig. 5. Renal morphology (periodic acid Schiff) at week 20. (*A*) Native kidney. (*B*) Saline-treated kidney. (*C*) Antisense treated kidney. (*D*) Reverse ODN-treated kidney. Panels B and C show glomeruli with focal sclerosis, basement membrane thickening, and glomerular obsolescence. The same interstitial, tubular, and vascular changes are evident.

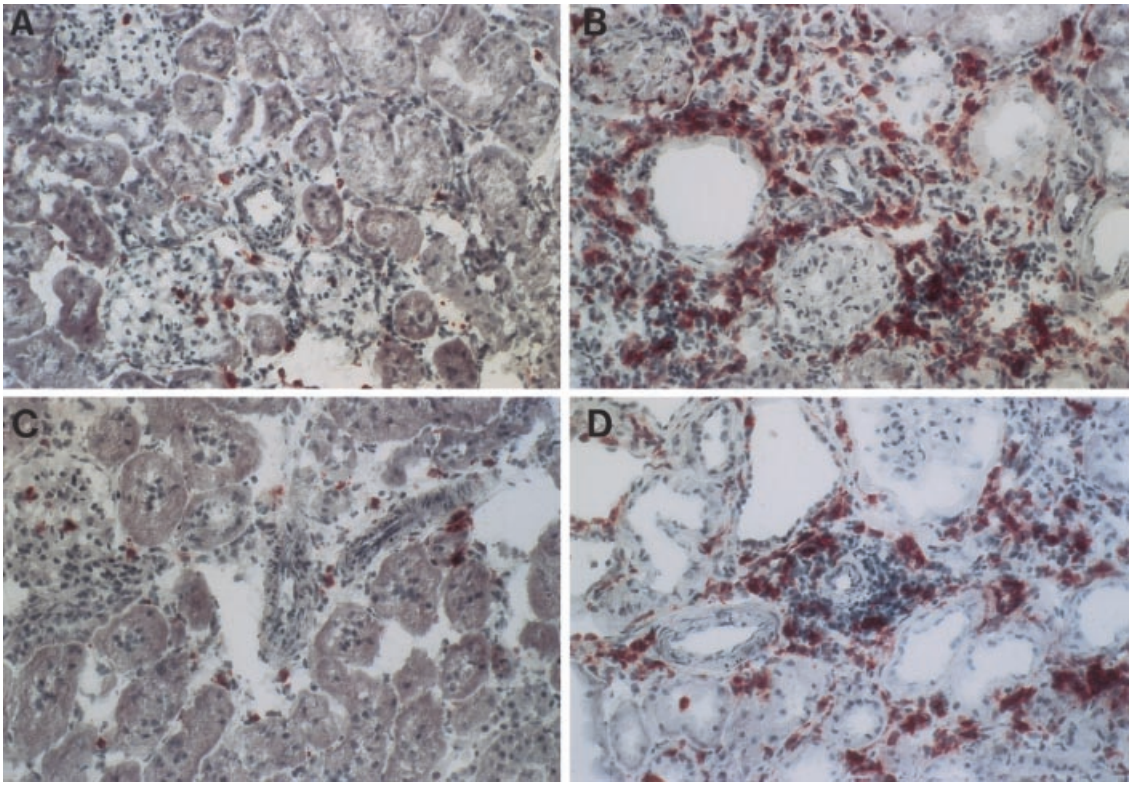

Fig. 6. Immunohistochemistry (anti-ED-1 and anti-alkaline phosphatase) at week 20. (*A*) Native kidney. (*B*) Saline vehicle-treated kidney. (*C*) Antisense ODN-treated kidney. (*D*) Reverse ODN-treated kidney. Panels B and C show ED-1 positive macrophages in copious numbers, particularly within the interstitium.

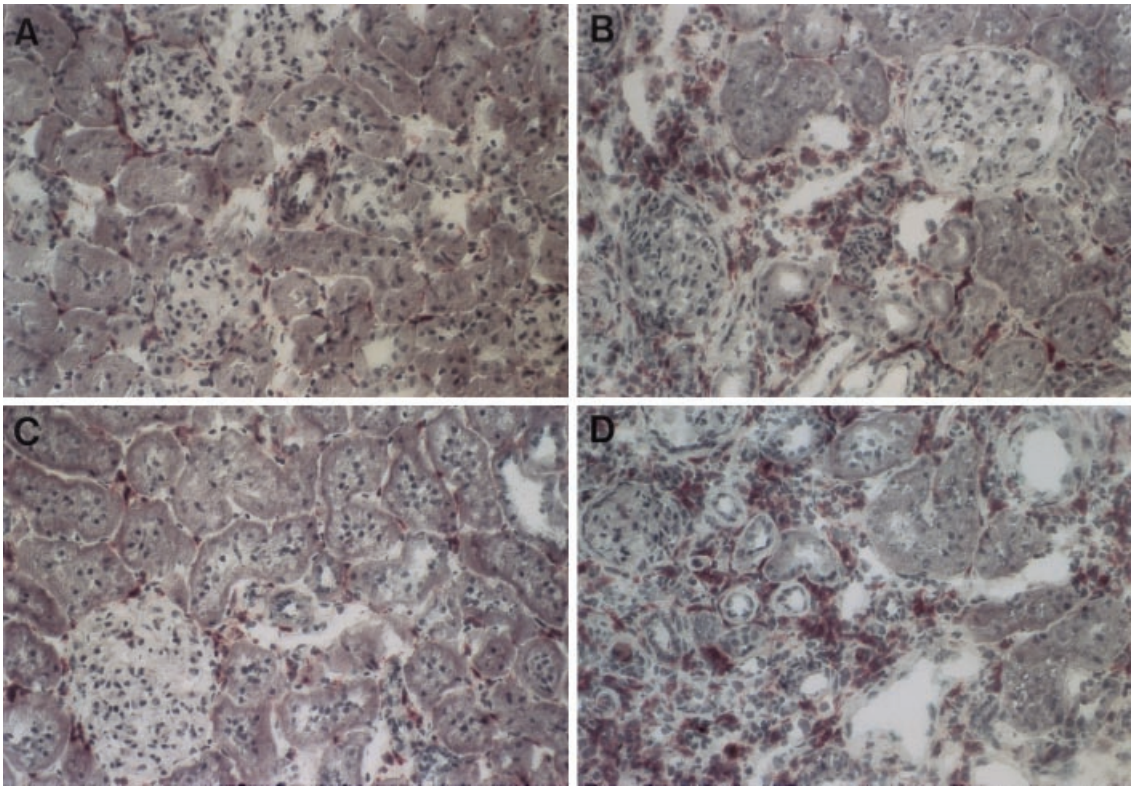

Fig. 7. Immunohistochemistry [anti-CD4 (OX-35) antigen and anti-alkaline phosphatase] at week 20. (*A*) Native kidney. (*B*) Saline vehicle-treated kidney. (*C*) Antisense ODN-treated kidney. (*D*) Reverse ODN-treated kidney. Panels B and C show CD4 positive lymphocytes, particularly within the interstitium.

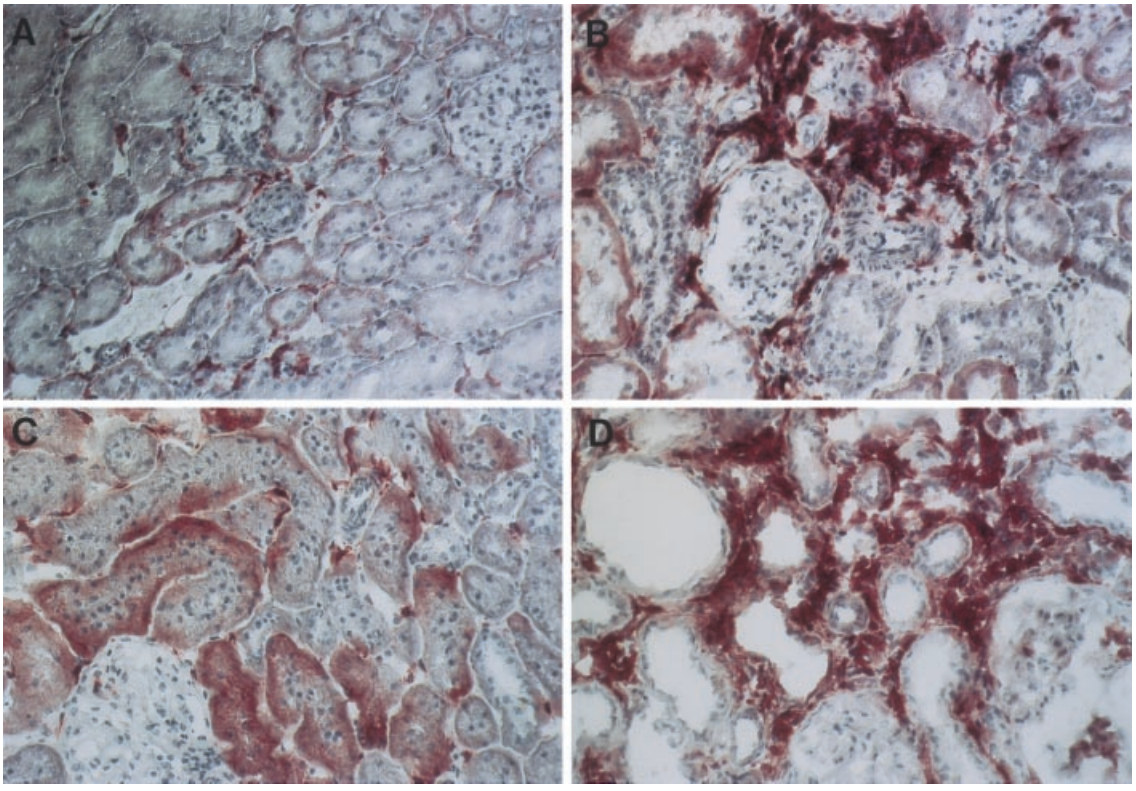

Fig. 8. Immunohistochemistry [MHC Class II (OX-18) antigen and anti-alkaline phosphatase) at week 20. (*A*) Native kidney. (*B*) Saline vehicle-treated kidney. (*C*) Antisense ODN-treated kidney. (*D*) Reverse ODN-treated kidney. Panels B and C show marked staining around glomeruli and within the interstitium. Panel D shows modest staining of the tubular cells.

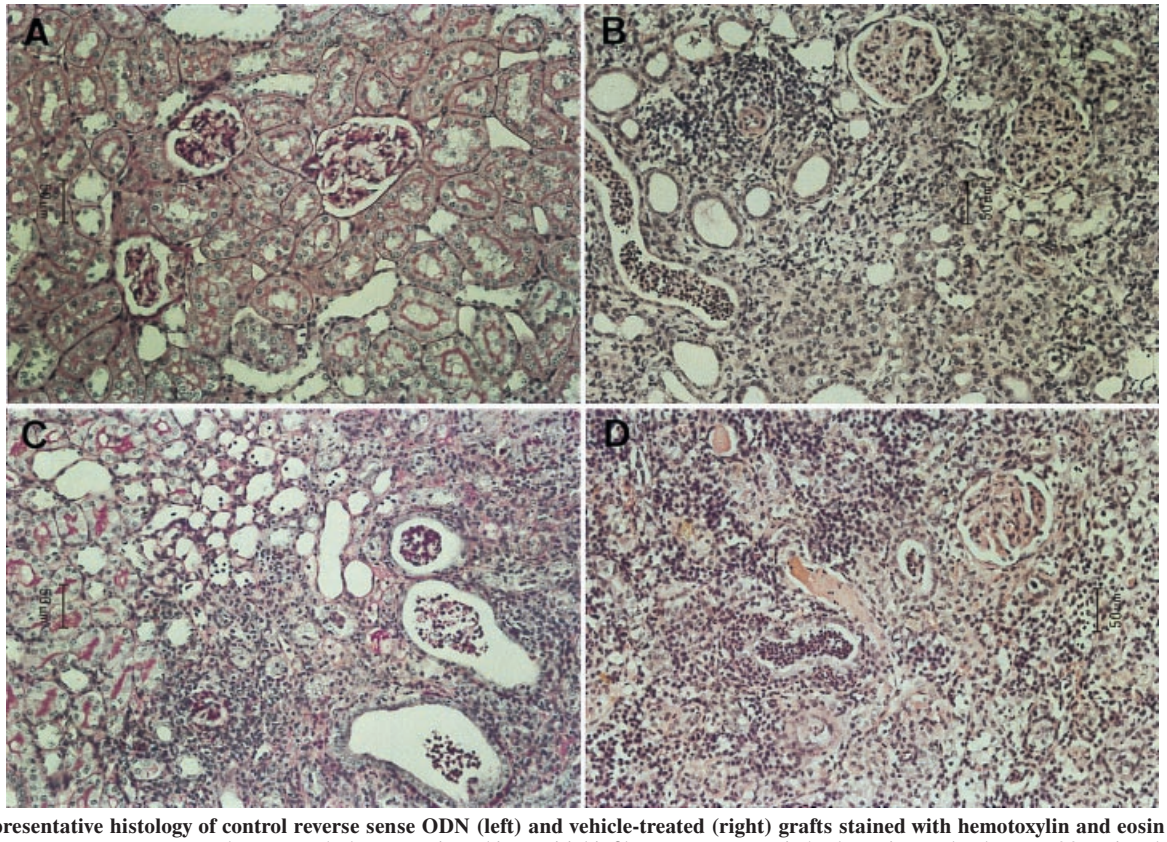

Fig. 9. Representative histology of control reverse sense ODN (left) and vehicle-treated (right) grafts stained with hemotoxylin and eosin at the time of native nephrectomy (day 10). Patchy acute tubular necrosis and interstitial infiltrate are present in both sections. The degree of functional reversibility at this time point is difficult to estimate.

mechanisms. We studied grafts from three reverse sense ODN-treated rats and from three vehicle-treated rats at 10 days post-transplant. The kidneys from these two treatments looked the same and revealed considerable, but patchy evidence of acute tubular necrosis. Figure 9 is a representative section from a reverse sense ODN-treated animal and a vehicle treated animal. The reversibility of the lesions is difficult to discern.

DISCUSSION

In these studies we were able to verify the acute experiments using antisense directed against ICAM-1, which we described earlier [13, 14]. The acute study demonstrated that antisense application markedly attenuated acute reperfusion injury in an autotransplanted model. In the chronic experiment, we used an isotransplanted model and observed the rats for 20 weeks. We employed genetically similar rats from the same Lewis strain to avoid the confounding effects of rejection. Our focal point was reperfusion injury, so that we could examine nonimmunological features of graft injury. We found that antisense treatment had a profound effect on outcome in this model. A significant influence on survival was evident early, already by three weeks. This effect was sustained, so that by 20 weeks the antisense rats that had survived the second week were all doing well and the various control rats instead exhibited chronic renal failure with elevated creatinine values, proteinuria, and corresponding histology.

Our results confirm and extend the work of Tullius et al [7], who examined long-term renal isografts from the same model. Renal isografts or allograft control kidneys were transplanted orthotopically into bilaterally nephrectomized rat recipients and studied functionally, morphologically, and immunohistologically. Rats with allograft control kidneys developed proteinuria with gradual renal failure leading to death by week 16. Rats with isografts developed the same lesions after 24 weeks, which progressed thereafter. In our study, we purposely employed a warm ischemia time of 60 minutes to assure reperfusion injury. Thus, our unprotected isografted rats exhibited changes that occurred significantly earlier than in the study by Tullius et al [7]. However, a similar morphological and functional picture was observed, with infiltrating lymphocytes and macrophages, tubular atrophy, interstitial fibrosis, arteriosclerosis, and glomerular atrophy, sclerosis, and obsolescence. The same antigen-independent functional and morphological changes occurred in both experiments. We showed that antisense treatment directed at ICAM-1 can markedly improve the long-term results in isograft transplanted rats. The cascade of chronic events described by Tullius et al [7] and reviewed by other authors in detail [18] was reiterated in our control groups. Moreover, our data show that these pathologic changes may be ameliorated to a considerable degree. The course of our model differs from that of Tullius et al [7] in that the vehicle and reverse ODN group exhibited an accelerated course. One possible explanation for this result is the lengthy warm ischemia time we employed in order to produce delayed graft function. This procedure likely reduced the nephron mass in our rats not only through adhesion molecule-related events, but also by ischemia-induced tubular necrosis. The effect of reduced nephron mass has been emphasized in both animal experiments [19] and observations in transplanted patients [20].

We suggest that antisense ODN treatment promotes chronic graft survival by decreasing not only acute tubular necrosis, but also chronic graft loss. This interpretation would require that the acute renal failure of the reverse sense and vehicle groups was reversible rather than irreversible. For that reason we included a group of control animals with reverse sense and vehicle treatment, in which the transplanted kidneys were examined 10 days later, at the time of native nephrectomy. These kidneys showed patchy evidence of ischemia-induced acute renal failure, and we can only speculate to what degree the damage in these kidneys was reversible. An additional confounding variable is the attenuation of injury due to presence of a functioning kidney. Finn et al showed that postischemic unilateral renal damage is substantially influenced by the presence or absence of the contralateral kidney [21]. When the contralateral kidney is removed prior to unilateral renal ischemia, reflow of blood and recovery are more complete. As a consequence, histological damage with unilateral renal ischemia was less in rats without a contralateral kidney in place. In our model, we deviated from the actual transplant situation in that endogenous renal function was present for 10 days, when the endogenous kidney was removed.

The antisense molecules we employed consist of phosphorothioate ODN, which have the specific advantage of being more resistant to degradation by endogenous nucleases than native phosphodiester oligonucleotides. Thus, they exhibit improved *in vivo* stability [22]. Recently, the organ and cellular distribution of phosphorothioate ODN after intravenous administration was defined in a rat model by Butler, Stecker and Bennett [23]. The renal proximal tubular cells, the hepatic Kupfer cells, and the hepatic endothelial cells were most heavily labeled. In the kidney, the phosphorothioate ODN were also found in glomerular epithelial cells particularly in the renal cortex. The loop of Henle, distal tubules, and the medullary portion of the kidney were less heavily labeled. Interestingly, the endothelial cells within the kidney were hardly labeled at all. The mechanism of cellular phosphorothioate ODN uptake is not clear. Studies indicate that transport into cells occurs via receptor-mediated or absorptive endocytosis [24]. In the endocytosis model, the ODN presumably bind to one or more surface proteins, become internalized into endosomes with the protein, and traffic to intracellular vesicles. Cationic lipids such as lipofectin facilitate cellular uptake and improve subcellular distribution through destabilizing

the endosomal membrane, thereby opening the entry of the ODN to the cytoplasm and the nucleus.

In their pharmacokinetic studies, Butler et al administered the naked phosphorothioate ODN without a facilitatory vector [23]. We employed lipofectin in the present studies. From preliminary *in vitro* experiments, a lipofectin concentration of 0.8 mg/mg DNA and a ODN concentration of 10 mg/kg body wt was chosen for the *in vivo* studies. In our acute *in vivo* study, we found that antisense ODN inhibited the expression of ICAM-1 in renal cortical vessels at 24 hours [14]. We interpret these findings that antisense ODN were effective in the endothelium of the kidney, despite the lack of renal endothelial labeling in the pharmacokinetic studies. Possibly the lipofectin facilitated this uptake, although we have not experimented with phosphorothioate ODN alone. Butler et al found that ODN were detectable at 24 hours in similar concentrations as at two hours, while in the liver the concentrations declined substantially during this time period.

In summary, we succeeded in showing that ICAM-1 suppression with antisense ODN not only obviates delayed graft function, but also improves outcome in a chronic rat model of isograft transplantation. We performed these chronic isograft antisense experiments to underscore the possibility that they will be of clinical utility, and envision the antisense ODN treatment of brain-dead organ donors, possibly those greater than 50 years of age whose organs are more likely to exhibit delayed graft function, prior to the harvesting of the organs. Transplanted hearts and livers also are subject to reperfusion injury, and ICAM-1 seems to play a role in acute and chronic rejection [25–27]. The antisense ODN treatment is not subject to the same immunological problems that accompany the use of antibodies directed against adhesion molecules. We envision a multiple antisense ODN treatment of transplant grafts directed against a variety of inducible adhesion molecules associated with reperfusion injury. We suggest that the time is at hand to consider a controlled, randomized, double-blind trial of antisense ODN therapy to obviate delayed graft function in humans.

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