Kidney International, Vol. 60 (2001), pp. 1173–1181

Prolonged cold preservation augments vascular injury independent of renal transplant immunogenicity and function

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Prolonged cold preservation augments vascular injury independent of renal transplant immunogenicity and function.

Background. While prolonged cold ischemia has detrimental effects on graft survival, the mechanisms remain unclear. We tested whether or not cold preservation enhances intragraft inflammatory responses and vascular injury.

Methods. Rat renal grafts were cold preserved in University of Wisconsin solution for 2, 4, 6, 12, 24, and 48 hours, and then transplanted into syngeneic recipients and harvested after 24 hours. Frozen sections were examined histologically and stained for vascular cellular adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), major histocompatibility complex (MHC) class II, tissue factor, leukocyte function associated molecule-1 (LFA-1), very late antigen-4 (VLA-4), as well as for inflammatory cells.

Results. Function did not differ between isografts preserved for shorter (2 to 6 hours) or longer times (12 to 24 hours). Neutrophil influx and that of LFA-1–positive cells showed similar increases in all groups. Compared with short preservation groups, the long preserved grafts had more VLA-4–positive ED-1+ monocytic infiltrates adjacent to vessels expressing VCAM-1 ($P \leq 0.001$). Increased preservation duration had no effect on infiltration with recipient ED-2+ macrophages, MHC class II-positive cells, or dendritic cells. Decreased color intensity and continuity of PECAM-1 staining indicated loss of endothelial integrity in grafts preserved for longer than six hours. Intensity in VCAM-1 staining increased progressively in grafts preserved for more than six hours and was localized predominantly on the endothelium of elastic vessels. Endothelial cells, vascular smooth muscle cells, and monocytes expressed increasingly more tissue factor in grafts preserved for more than six hours, revealing enhanced intragraft procoagulant capacity. Furthermore, grafts with preservation times of more than six hours developed more severe vascular endothelial injury and worse tubular necrosis scores ($P \leq 0.001$) compared with grafts with shorter preservation times.

Conclusions. Because of the prominent vascular injury, strategies for endothelial protection should be attempted in grafts with long preservation times in clinical renal transplantation.

Key words: endothelial protection, cold ischemia, adhesion molecules, inflammation, monocytes, tissue factor, ischemia-reperfusion injury.

Received for publication December 29, 2000
and in revised form March 21, 2001
Accepted for publication April 3, 2001

Delayed graft function remains the most important complication in the early post-transplant period. Delayed graft function may occur for a variety of reasons, including intraoperative complications, donor age-related decreased renal mass, acute drug toxicity, or cold ischemic injury due to long cold preservation times [1]. Grafts exposed to prolonged cold preservation are at permanent risk for short- or long-term deterioration [2]. We reasoned that such grafts might be at a higher risk for injury during reperfusion. Reperfusion features an acute inflammatory response that involves proadhesive activated endothelium and subsequent tissue infiltration with leukocytes [3]. Furthermore, recent reports by our and other laboratories implicate the importance of major histocompatibility complex (MHC) class II-positive cells and monocytes/macrophages [4, 5]. The relative importance of the cold ischemia time on inflammation in the course of reperfusion has not been studied. We tested the hypothesis of whether prolonged cold preservation times amplify the acute inflammatory response, vascular injury, and functional and morphological features of post-transplant reperfusion injury. The impact of cold ischemia time on graft immunogenicity also was tested. We employed an isogeneic rat renal transplantation model, where renal grafts were exposed either to short (2 to 6 hours) or long (12 to 24 hours) cold preservation periods. To test the hypothesis, first neutrophil-endothelial interactions were studied as the major mechanism responsible for the functional deterioration in the postischemic tissues. We then investigated the endothelial markers and effectors of vascular injury in kidneys with long compared with short preservation times. Finally, the graft infiltration was assessed with recipient cells that are capable of antigen presentation via an indirect recognition pathway.

METHODS

Animals and grafting technique

Inbred male Lewis rats (Lew, RT1) weighing 150 to 200 g were purchased from Harlan-Winkelman (Sulzbach,

Germany). The animals had free access to tap water and a standard rat diet (No. C-1000; Altromin, Lage, Germany). They were kept under regular lighting conditions (lights on at 6 a.m. and off at 6 p.m.) at a constant temperature 24°C. All surgical procedures were approved by local authorities (Permit G 0406/95) according to guidelines corresponding to the American Physiological Society. The rats were fasted overnight before surgery. Lewis (Lew) rat kidneys were transplanted into Lew recipients.

Donor animals were prepared as previously described [6]. Briefly, the left renal artery and vein were separated from each other, and their collateral branches were divided with an electrocautery device. The ureter was freed from the surrounding fibrotic tissue and cut in the proximity to the bladder. After the placement of microaneurysm clips, the renal 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 2, 4, 6, 12, 24, and 48 hours. Recipient Lewis rats were anesthetized with ketamine. Anastomoses of the vessels and the ureter were performed end-to-end using 10-0 Prolene. The anastomosis time averaged 30 minutes. Upon the completion of anastomoses, the right native kidney was removed as well. Both native kidneys were preserved for later use as controls. Four to six animals per preservation time were killed 24 hours after transplantation. Animals did not receive immunosuppression or any other medication during the study. Venous blood was obtained from the retrorobital plexus for serum creatinine and urea concentrations determined with automated methods.

Antibodies

The following antibodies (Abs) were used: monoclonals for PECAM-1 (CD31, clone TLD-4E8), leukocyte function associated molecule-1 (LFA-1; CD11a), α_4 integrin (CD49d, clone TA-2), neutrophils (His 48), monocytes-macrophages (ED-1), macrophages with activated phenotype (ED-2), dendritic cells (OX-62), MHC class II (clone OX-18), all of which were obtained from Serotec (Oxford, UK). Vascular cellular adhesion molecule-1 (VCAM-1; CD106, clone 51-1069) and Ab for tissue factor (TF) were from Becton-Dickinson (San Diego, CA, USA).

Immunohistochemistry

Immunohistochemistry was carried out as previously described [6]. Briefly, for immunohistochemical staining, the sections were incubated with the monoclonal or polyclonal Ab (discussed previously in this article) and diluted in RPMI (Seromed, Heidelberg, Germany) for 60 minutes at room temperature in a humid chamber. After washing with TBS, the sections were incubated with a bridging Ab (Dako, Hamburg, Germany). The immunoreactivity was visualized with an alkaline phosphatase antialkaline phosphatase (APAAP) complex (Dako). For detection and development, the neufuchsin-naphtol-As-Bi-phosphate substrate (Merck, Darmstadt, Germany) was used as described. Negative control stainings were performed by incubation with corresponding isotype controls instead of primary Ab.

Semiquantitative data on the infiltration of neutrophils, ED-1+/ED-2+ monocytes, dendritic cells, MHC class II-positive cells, as well as for LFA-1 and α_4 integrin were determined using the KS 300 3.0 imaging system and Axioplan 2 microscope (Zeiss, Jena, Germany). The sections were digitized via color video camera (Sony 3CCD, Tokyo, Japan) on a screen. Positively labeled cells in the each section were counted in 15 randomly chosen fields corresponding to an area of 75,274 μm^2. The means for four rats at any one time point were then grouped together to obtain a final mean and SEM. Tissue specimens were scored independently by two observers in a blinded manner. Minor differences in the scoring were resolved by joint discussion.

Renal histology

The kidneys preserved in 10% buffered formalin were dehydrated and then embedded in paraffin. Sections (3 μm) were stained with hematoxylin and eosin and periodic acid-Schiff (PAS) using standard procedures. Examination and scoring of the sections of each kidney for hallmarks of acute tubular necrosis (ATN) were conducted on a blinded basis by a renal pathologist (W.S.) as previously described [4]. The degree of renal damage was semiquantitatively evaluated using tubular necrosis score based on a scale in which 0 represented no abnormalities, and 1+, 2+, 3+, and 4+ stood for slight (up to 20%), moderate (20 to 40%), severe (40 to 60%), and total necrosis affecting more than 80% of renal parenchyma, respectively.

Statistical analysis

Statistical analysis was carried out on a G3 Macintosh computer (Apple Inc., Cupertino, CA, USA) with a commercially available program (Statview; Cricket Software Inc., Philadelphia, PA, USA). All values are reported as means ± SEM. The data within groups were compared using nonparametric Kruskal-Wallis and Mann-Whitney-U test. Statistical significance was set at $P \leq 0.001$.

RESULTS

Renal function parameters in rats receiving isografts with different cold preservation times are shown in Figure 1. Creatinine (Fig. 1A) and urea (Fig. 1B) concentrations in serum were increased 24 hours after transplantation in all groups studied, defining the extent of acute renal failure. In contrast to our expectations, there were

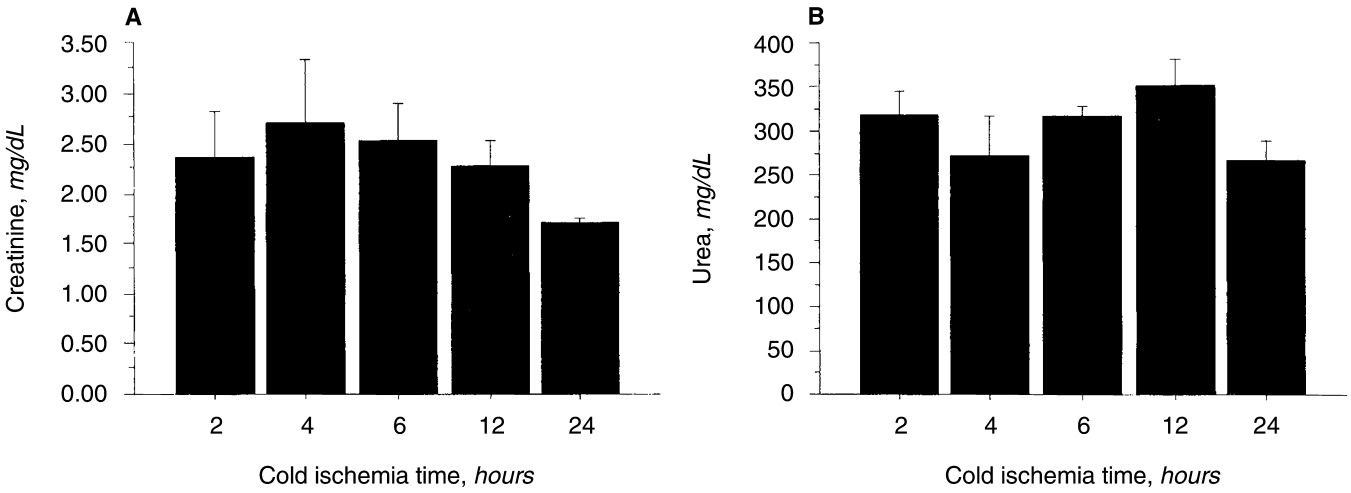


Fig. 1. Renal function parameters of rats with different cold ischemia times 24 hours after transplantation. (*A*) Serum creatinine concentration. (*B*) Serum urea concentration. Both parameters expressed in mg/dL as means ± SEM.

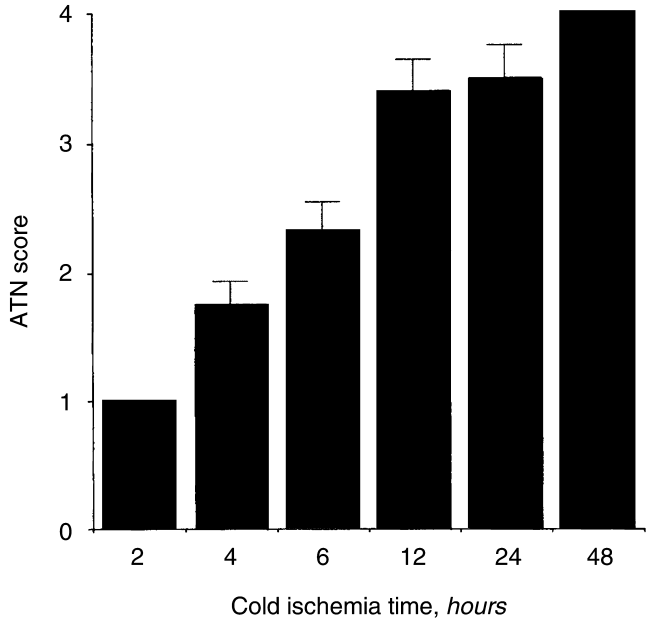


Fig. 2. Acute tubular necrosis (ATN) score in renal grafts exposed to different cold ischemia times 24 hours after transplantation. All results are expressed as means ± SEM. The incremental increases between 2 and 12 hours are significant at every step.

no significant differences in serum creatinine and urea concentrations between the isografts exposed to short or long cold preservation times.

Figure 2 shows a semiquantitative grading scale considering ATN (comparing 4 kidneys from each group) 24 hours after transplantation. We observed typical changes for ATN as described previously [4]. Morphological changes did not correspond to the clinical course in experimental groups. There was a direct strong correlation between cold preservation time and tubular necrosis score, as shown in Figure 2. Grafts with a two-hour preservation time had only mild changes (range 1). Grafts with four- and six-hour preservation times had moderate 1.8 to 2.3 (range 2) changes. Grafts with longer preservation times of 12 and 24 hours developed severe 3.6 (range 3 to 4) changes, while grafts with 48 hours of preservation showed complete necrosis and could not be considered for the immunohistochemical studies.

Enhanced inflammatory cell infiltration during reperfusion injury after transplantation relates to changes in surface adhesive phenotypes on both the endothelium and leukocytes. Neutrophil and monocyte infiltration were analyzed together with LFA-1 and VLA-4 integrins, as depicted in Figure 3. Neutrophils are believed to be the major effector cells responsible for functional deterioration due to the reperfusion injury. To investigate whether prolonged cold preservation times augment inflammatory responses during reperfusion injury, we studied spatial distribution of neutrophil infiltrates and quantified the cells in the experimental groups. In all groups, neutrophils were mainly found within the glomeruli and in perivascular and interstitial spaces (data not shown), as recently reported [4]. Long cold preservation times (12 to 24 hours) did not increase the neutrophils compared with short preservation times (2 to 6 hours), as shown in Figure 3A. These results were consistent with the functional parameters.

Leukocyte function associated molecule-1 is a predominant neutrophil integrin. Figure 3B shows the rate of LFA-1–positive cell influx in isografts exposed to different cold preservation periods. LFA-1–positive cells were found either in interstitial and perivascular areas or within the glomeruli (data not shown). LFA-1+ cells colocalized predominantly with neutrophils in isografts preserved for two hours. In grafts that were preserved for four hours or longer, LFA-1–positive cells colocalized with ED-1 infiltrates. Influx rate of LFA-1+ cells was

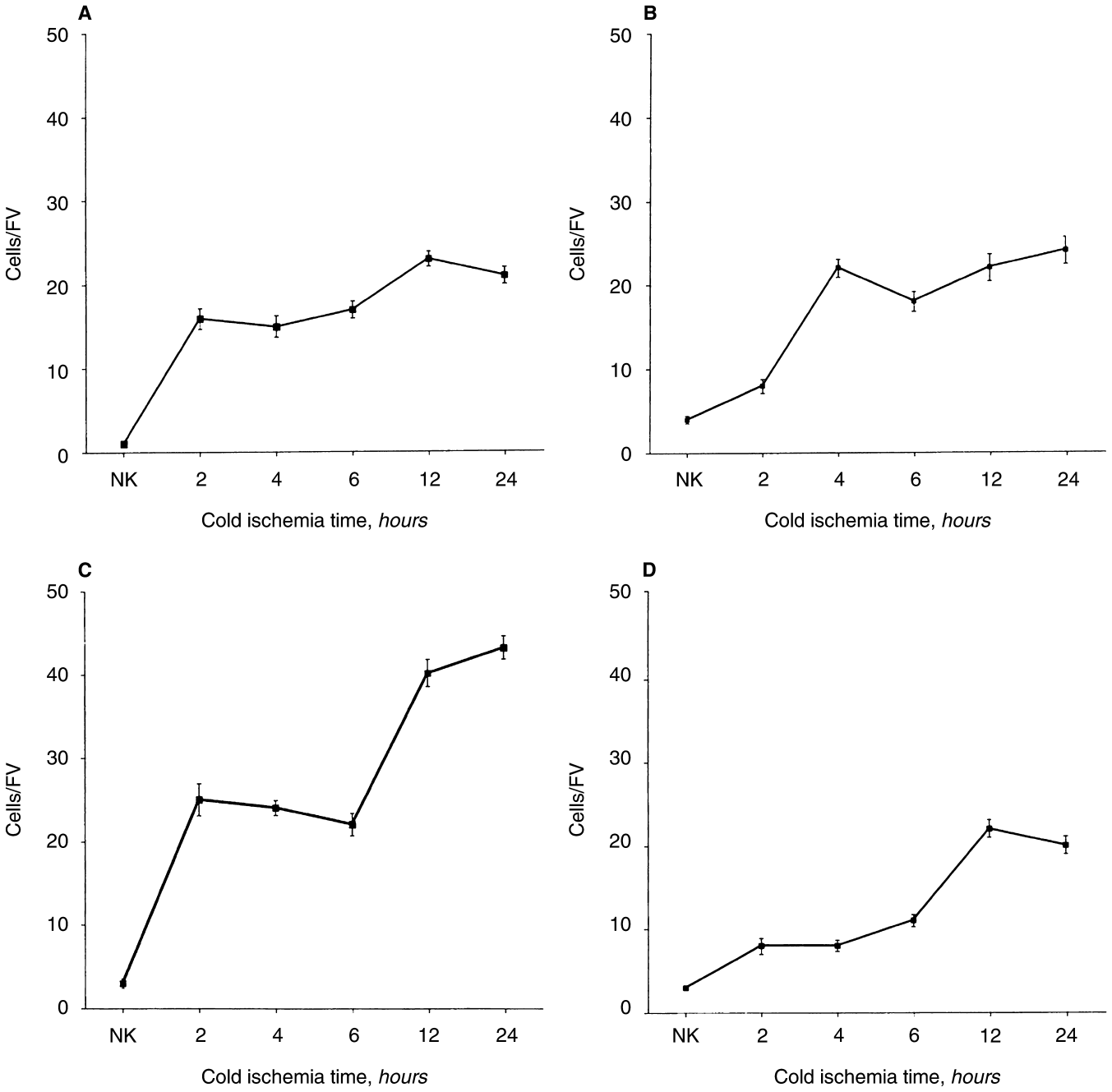


Fig. 3. Effect of cold preservation times and subsequent reperfusion injury on amount of different leukocyte populations and corresponding leukocyte integrins. Data are expressed as a number of positively labeled cells counted in 15 randomly chosen fields of view in sections of grafts exposed to different cold ischemia times. (*A*) Number of infiltrating neutrophils (■; granulocytes). (*B*) Number of LFA-1+ cells (■). (*C*) Number of ED-1+ monocytes/macrophages (■). (*D*) Number of VLA-4+ cells (■). All results are expressed as means ± SEM. *$P \leq 0.001$. NK, native kidney.

significantly higher in grafts with four-hour cold ischemia compared with two hours ($P \leq 0.001$), although grafts with longer ischemia times (6 to 24 h) showed no further increase compared with four hours of cold ischemia. Native kidneys had no neutrophils or LFA-1–positive cells.

Monocytes/macrophages are important effector cells mediating both injury and regeneration [4, 7]. As depicted in Figure 3C, ED-1+ monocytes/macrophages represented the predominant cell population of all infiltrating cells counted in all groups. ED-1–positive cells were found in all groups within glomeruli, in peritubular spaces, and around the elastic vessels. The majority of monocytic perivascular infiltrates in grafts with shorter cold ischemia times (2 to 6 hours) were found adjacent to vessels expressing

VCAM-1. A dramatic increase in ED-1–positive cell influx was found in grafts that were preserved 12 or 24 hours ($P \leq 0.001$) compared with short cold ischemia times (Fig. 3C). The infiltrates increased predominantly in peritubular spaces and around elastic vessels, while the intraglomerular fraction was not affected.

VLA-4 was studied as an integrin associated with monocytes/macrophages. As depicted in Figure 3D, infiltration with VLA-4–positive cells was less than ED-1+ cells seen in Figure 3C. There was only moderate infiltration with VLA-4–positive cells in grafts with shorter cold ischemia times (2 to 6 hours), while grafts with long cold ischemia (12 to 24 hours) exhibited significantly greater peak infiltration ($P \leq 0.001$) at 24 hours after transplantation. VLA-4–positive cells were distributed either in perivascular-peritubular spaces or were a part of the intraglomerular infiltrate. Peritubular fraction of VLA-4–positive cells was expanded in grafts with 12 to 24 hours of cold ischemia.

According to the "injury hypothesis," the immune response correlates with the severity of the injury [8]; however, the effect of cold ischemia on recipient antigen-presenting cells has not yet been investigated. We studied the effect of different cold preservation periods on infiltration with MHC class II-positive cells, dendritic cells, and ED-2–positive macrophages with an activated dendritic phenotype. As depicted in Figure 4A, in grafts exposed to prolonged cold ischemia (12 to 24 hours) compared with those with shorter cold (2 to 6 hours) preservation, the amount of MHC class II-positive cells increased numerically ($P < 0.1 > 0.05$). MHC class II+ cells were distributed predominantly in peritubular areas and sparsely around larger vessels. Figure 4B shows that cold preservation and subsequent reperfusion injury did not induce infiltration with dendritic cells 24 hours after transplantation compared with native kidneys. To characterize the phenotype of macrophage infiltrate better, we stained for the ED-2+ dendritic macrophage subpopulation, which was believed to play an important role in antigen presentation and processing [9]. The proportion of ED-2+ cells comprised only one third of the total monocyte/macrophage population, as seen in Figures 3C and 4C. There was no difference in the amount of ED-2+ infiltrates between grafts with short or long cold ischemia times.

Platelet-endothelial cell adhesion molecule-1 is localized to junctions in quiescent endothelium and plays an important role in physiological transmigration of the leukocytes by the process of homophilic adhesion [10]. Inflamed endothelium changes the distribution of molecules comprising cell-cell junctions, which results in permeability changes and local leakage into tissue [11]. The native kidneys had a strong basal level of uniformly distributed PECAM-1 on endothelium of intraglomerular and peritubular capillaries, as well as larger vessels seen in Figure 5. There was no difference in PECAM-1 staining between native kidneys and isografts with shorter cold preservation times for instance at two hours, as shown in Figure 5A. In contrast, isografts with longer cold preservation times displayed gradual losses in staining continuity and color intensity for PECAM-1. Figure 5B shows PECAM-1 expression and distribution in grafts exposed to six hours, Figure 5C for 12 hours, and Figure 5D in grafts with 24 hours of cold ischemia. The patchy distribution of pale PECAM-1 staining was not limited to the endothelium and affected neighboring interstitial cells, which may be a feature of increased permeability and loss of endothelial barrier integrity in groups with long cold ischemia times. The reduction of staining intensity affected arterial vessels and peritubular capillaries to the same extent.

Staining for VCAM-1 exclusively exhibited an endothelial pattern. A weakly positive staining reaction appeared after 6 hours and progressively increased to reach the maximal intensity in both allografts and isografts at 24 hours. VCAM-1 mAb predominantly stained the intima of small arteries and arterioles of all groups. No VCAM-1 was detected on the capillary endothelium in grafts with cold ischemia times of two to four hours. In Figure 6, representative photomicrographs show strong VCAM-1 expression at 24 hours post-transplant on the endothelium of arterioles in isografts with short 2-hour ischemia and longer 6-hour cold ischemia times. The degree of VCAM-1 staining increased with the length of cold preservation. VCAM-1 positivity was also observed in almost all glomerular vascular poles of isografts preserved for six hours or longer (data not shown). Although this finding was consistent with findings in allografted kidneys from our previous study [4], as yet we can offer no functional explanation. Native control kidneys were entirely negative for VCAM-1, as recently described [4]. TF is an important regulatory molecule bridging coagulation cascade with the inflammatory response. Figure 7 shows vascular, intraglomerular, and peritubular immunostaining for TF. Endothelial TF expression followed the pattern of VCAM-1 expression primarily on elastic vessels, affecting peritubular capillaries as well. Vascular smooth muscle cells stained for TF, as well. Monocytic infiltrates adjacent to vessels expressing TF and VCAM-1 and within the glomeruli also expressed TF in significant amounts. The degree of TF staining correlated with the length of cold ischemia time.

DISCUSSION

Experimental and clinical studies indicate an increasing importance of alloantigen-independent mechanisms in the pathogenesis of delayed graft function and chronic graft failure [12, 13]. Among the alloantigen-independent factors, cold ischemic time has a significant negative ef-

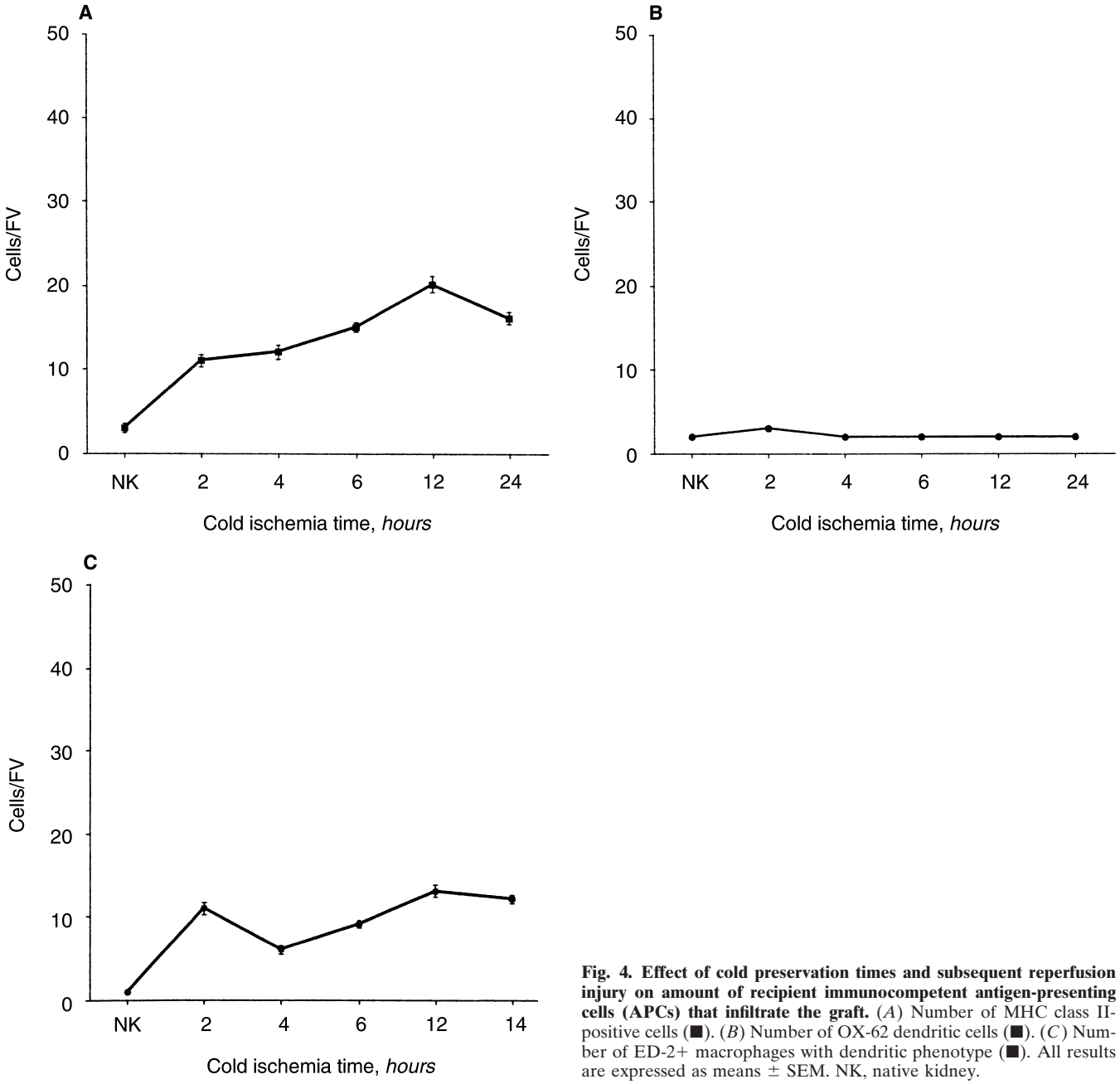


Fig. 4. Effect of cold preservation times and subsequent reperfusion injury on amount of recipient immunocompetent antigen-presenting cells (APCs) that infiltrate the graft. (*A*) Number of MHC class II-positive cells (■). (*B*) Number of OX-62 dendritic cells (■). (*C*) Number of ED-2+ macrophages with dendritic phenotype (■). All results are expressed as means ± SEM. NK, native kidney.

fect on transplant outcome for reasons that are unclear [14, 15]. The "injury response" hypothesis suggests that the immune response is amplified in stressed or injured tissue that secretes immunogenic substances and displays increased antigenicity [8, 16]. We compared the response to injury in syngeneic Lew-to-Lew transplants that were exposed to variable cold preservation times in the UW-solution. This solution was selected because a controlled clinical trial documented its superior efficacy in humans [17]. Comparative studies in rats have not been done to our knowledge. We considered our model well suited because there is no difference in the early inflammatory mechanisms between the syngeneic and low responder allogeneic strain combinations, as documented in our previous study [4]. In high-responder strain combinations, animals develop fulminant alloantibody-mediated vascular acute rejection with fibrinoid necrosis [18]. These changes develop as early as 24 hours post-transplant and would mask changes induced by cold preservation [18]. In nonimmunosuppressed low-responder strain combinations, we found earlier that features of acute rejection occur between day 7 and day 10 post-transplant [4].

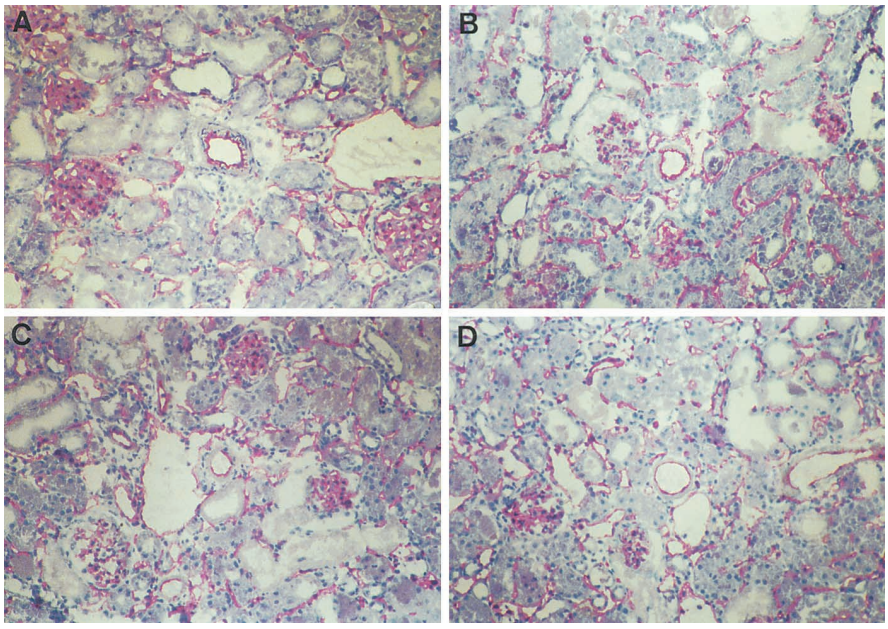


Fig. 5. Expression and distribution of PECAM-1 24 hours after transplantation in: isografts with (*A*) 2 hours, (*B*) 6 hours, (*C*) 12 hours, and (*D*) 24 hours of cold ischemia. Original magnification ×200.

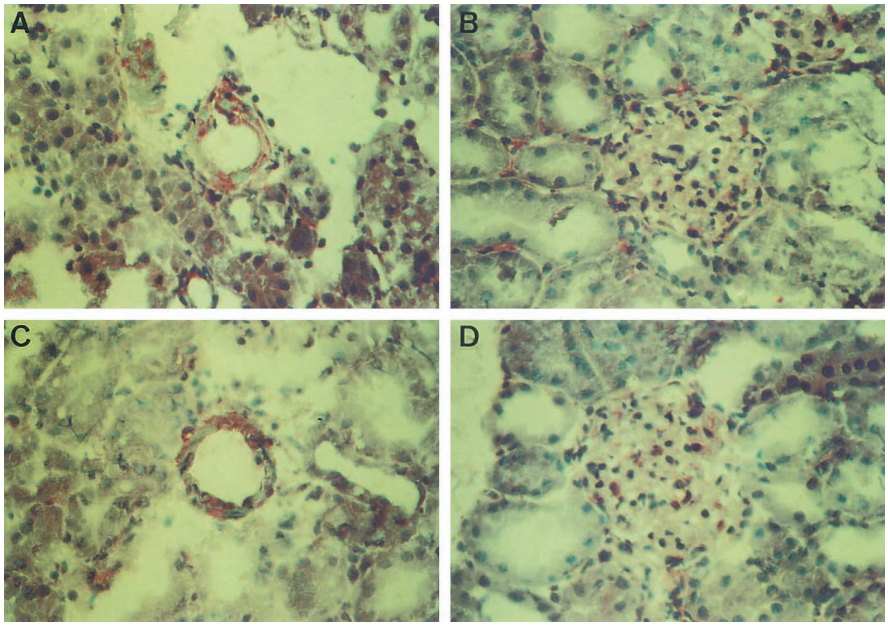
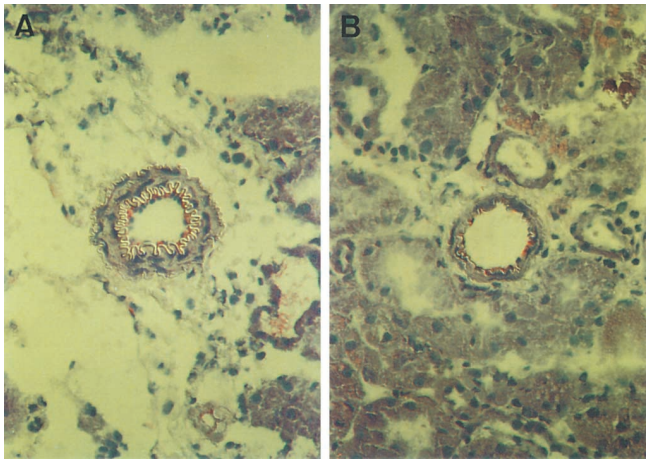


Fig. 7. Immunostaining for tissue factor 24 hours after transplantation in renal isografts. Vascular expression in grafts exposed to (*A*) two hours and (*C*) six hours cold preservation. Intraglomerular and peritubular expression in grafts exposed to (*B*) two hours and (*D*) six hours cold preservation. Original magnification ×400.



We found that long cold ischemia (12 to 24 hours) did not induce further functional deterioration compared with short cold ischemia times (2 to 6 hours), although structural damage worsened in a linear fashion. We were not able to document any significant correlation between cold ischemia time and neutrophil infiltration. Neutrophils are considered to be important effector cells in the reperfusion injury of native and transplant kidneys [19–21]. Furthermore, infiltration with recipient ED-2–positive activated macrophages of the dendritic phenotype, OX-62

Fig. 6. Immunostaining for VCAM-1 24 hours after transplantation in renal isografts exposed to (*A*) 2 hours and (*B*) 6 hours cold preservation. Original magnification ×400.

dendritic, and MHC class II-positive cells did not differ between isografts that experienced short or long cold preservation periods. Thus, cold ischemic trauma apparently did not increase graft immunogenicity 24 hours after transplantation. Our major finding was that grafts exposed to cold ischemia times exceeding six hours developed progressive vascular injury, accompanied with a twofold increase in ED-1–positive monocyte infiltration. This infiltration resulted in progressively severe structural damage. The vascular lesions were characterized by irregularity of endothelial PECAM-1 staining, increasing endothelial expression of VCAM-1, accompanied with densed perivascular monocytic infiltrates. Injured endothelium and infiltrating monocytes also became more thrombogenic and expressed TF.

We believe these observations may have clinical relevance. Graft survival in the recipients of living unrelated kidneys with minimal cold ischemia time is superior to that observed in recipients of cadaveric transplants despite a higher degree of HLA mismatch [13]. There are presently no consistently effective modes to prevent the changes of reperfusion injury or to influence the later detrimental consequences. The underlying cause for the long-term poor prognosis may be related to cellular events occurring in the course of reperfusion injury 12- to 48-hours post-transplant. The pathogenesis of post-transplant reperfusion injury involves a complex interplay of vascular and tubular factors with the interstitium and infiltrating cells [19]. While postischemic tubular morphology had been in the focus of many investigations [20], changes in the vasculature were studied only in immediate postreperfusion biopsies [20, 23]. Our previous results on the early kinetics of endothelial adhesion molecule expression and cellular infiltration demonstrated that major differences in rolling, firm adhesion, and infiltration mechanisms may exist during acute, subacute, and chronic leukocyte recruitment within the same organ and that cellular infiltration is dependent on the nature of the injury [4]. Since core needle biopsies are not routinely performed as early as 24 hours post-transplant, mechanisms of the cold injury in humans remained only speculative. Furthermore, in earlier experimental studies on the synergy of cold ischemia and other alloantigen-independent factors, cold ischemia times of two hours were considered as prolonged periods [24].

Vascular lesions were detected in grafts exposed to the cold preservation for longer than six hours. Cold ischemia disturbed endothelial integrity and increased vascular leakage, as documented by the irregular PECAM-1 staining in affected neighboring interstitial structures. In human renal biopsies of native renal diseases or rat renal allografts, PECAM-1 expression was not affected by normothermic inflammatory processes [4, 25]. The injured arterial endothelium, vascular smooth muscle cells, and neighboring monocytic infiltrates exhibited increased procoagulatory properties and expressed larger amounts of TF. Similar TF expression patterns were described as a hallmark of acute vascular rejection in cardiac xenografts [26]. Whether or not these findings reflect a response to tissue injury rather than a cause of prothrombotic changes due to the cold ischemic trauma requires further study. In human renal allografts, these vessels are targets of cyclosporine toxicity and alloantibodies [27]. These findings may explain why cold ischemic injury is an important predictor for both early and late graft loss [28]. The affected vessels expressed more VCAM-1 and dense monocytic infiltrates. The interaction between endothelial VCAM-1 and monocytes has been recognized as a central adhesion mechanism in atherosclerotic lesions [29]. Monocytes are also capable of reverse transmigration in atherosclerosis. Stimulated monocytes use TF to exit the site of inflammation and migrate to draining lymph nodes [30]. In another setting, the expression of TF leads to thrombosis. In our model, we saw no fibrin thrombi, which increases the likelihood that monocytes used TF for reverse transmigration. We and others have previously discussed the potential roles of monocytes as a possible specific link between alloantigen-independent and alloantigen-dependent process [4, 31]. Monocytes exert tissue damage by releasing death signals or alternatively by disrupting cell-matrix interactions through the secretion of metalloproteinases [32]. Monocytes/macrophages appeared to be major effectors of the vascular injury due to the extended cold preservation in our study. Unlike neutrophils, which predominantly exploit ICAM-1–related adhesion mechanisms, adhesion of monocytes primarily involve VCAM-1/VLA-4 interactions or yet unidentified novel adhesion molecules. Furthermore, chronic rejection in experimental models is considered a local macrophage-dependent event [33].

Our results demonstrate that the neutrophil-mediated acute inflammatory response is not enhanced in tissue exposed to prolonged cold preservation This observation is supported by functional and immunostaining data, showing a similar rate of neutrophil infiltration and functional deterioration in all groups studied. Although grafts that were exposed to prolonged preservation showed the stereotyped "injury response," the infiltration of recipient classic antigen presenting cells was not greater in the grafts that experienced more severe cold trauma. Thus, in this model using long preservation times, "recognition of the injury" is not enhanced compared with other models using shorter preservation times [4, 5]. The degree of monocyte infiltration and the extent of vascular lesions were the major determinants for the tubular necrosis and the nephron loss. We suggest that vascular endothelial injury may be playing a more important role in the pathogenesis of early graft failure in transplants exposed to long cold preservation times than was previously appreciated. Furthermore, our findings may explain the apparent synergy between cold ischemic trauma and re-

jection in predisposing to earlier chronic graft failure. Our results have important clinical implications for the design of future therapies directed at the prevention of delayed graft function.

ACKNOWLEDGMENTS

This study was supported by a grant-in-aid from the Klinisch-Pharmakologischer-Verbund, Berlin-Brandenburg to Duska Dragun, and Hermann Haller. These data were presented in part at the 18th Annual Meeting of American Society of Transplantation in Chicago, IL, USA, 15–19 May, 1999, and they were published in abstract form.

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APPENDIX

Abbreviations used in this article are: Ab, antibody; APAAP, alkaline phosphatase anti-alkaline phosphatase; ATN, acute tubular necrosis; LFA-1, leukocyte function-associated molecule-1; MHC-I, major histocompatibility complex-I; NK, native kidney; PAS, periodic acid-Schiff; PECAM-1, platelet-endothelial cell adhesion molecule-1; UW, University of Wisconsin; VCAM, vascular cell adhesion molecule; VLA-4, very late antigen-4.

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