Animal Model

Increased Venous Proinflammatory Gene Expression and Intimal Hyperplasia in an Aorto-Caval Fistula Model in the Rat

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We hypothesized that the venous limb of an arteriovenous (AV) fistula would evince up-regulation of genes relevant to vascular remodeling along with neointimal hyperplasia and relevant histological changes. Using the aorto-caval model of an AV fistula model in the rat, we demonstrate marked up-regulation in such proinflammatory genes as monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and endothelin-1, 2 weeks after the creation of the fistula. Neointimal hyperplasia occurred in variable degrees by 5 weeks after establishing the fistula, and by 16 weeks, such neointimal hyperplasia was progressive and pronounced; at this time point, abundant extracellular matrix was also observed. Smooth muscle cells were present in the hyperplastic neointima as evidenced by staining for α -smooth muscle actin; ultrastructurally, smooth muscle cells with a synthetic as well as a contractile phenotype were readily observed. Accumulation of extracellular matrix in the model at 16 weeks was accompanied by increased expression of transforming growth factor- β 1 mRNA, the latter finding contrasting with the suppression of transforming growth factor- β 1 mRNA observed in this model at 2 weeks. In summary, we describe marked up-regulation in proinflammatory genes and progressive neointimal formation in the venous vasculature in an AV fistula model in the rat. We suggest that such alteration in gene expression and histological injury, in conjunction with the relative simplicity of this model, offer a new approach in the study of such timely biological and clinically relevant phenomena as differential gene expression in response to hemodynamic forces, processes involved

in vascular remodeling, mechanisms of injury in venous bypass grafts, and mechanisms of dysfunction of AV fistulae used in hemodialysis. (*Am J Pathol* 2003, 162:2079–2090)

An arteriovenous (AV) fistula predisposes to cardiac hypertrophy and cardiac failure,¹ and the fashioning of an AV fistula in rats is widely used to study these conditions.^{2–14} Fistulae in rats may be established by microsurgical techniques that anastomose neighboring arterial and venous segments such as the descending aorta to the inferior vena cava, the latter providing an aorto-caval fistula.^{2–8} Such a fistula may be created more simply by inserting a small-gauge needle directly through the descending aorta and inferior vena cava; this communication between these vessels persists after the needle is removed and hemostasis is secured, thereby providing a chronic AV fistula.^{9–14} This model has provided novel insights regarding the pathogenesis of cardiac hypertrophy and congestive heart failure.^{2–14}

In this model, the inferior vena cava as well as the heart are subjected to enhanced hemodynamic stress. The fistula exposes the inferior vena cava to the higher pressures present in the arterial limb of the circulation, and, by diverting a substantial portion of aortic blood flow into the inferior vena cava, the fistula markedly augments the coursing of blood through the inferior vena cava. Whether the inferior vena cava in this model is altered, be it functionally or structurally, in response to such heightened pressures and flows, to the best of our knowledge, has never been examined. Understanding the nature of alterations in the venous limb of such an AV fistula is relevant, in general, to the overarching issue of vascular adaptation to hemodynamic stress, and, in particular, to the

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pathogenesis of venous injury in arterial bypass grafts^{15–17} and in hemodialysis AV fistulae.^{18–20}

We hypothesized that the inferior vena cava in this model, exposed as it is to heightened pressures and flows, would evince up-regulation in genes incriminated in the pathogenesis of vascular injury, and ultimately, the appearance of venous histological changes. We evaluated this hypothesis by examining expression of monocyte chemoattractant protein (MCP)-1, a gene widely incriminated in the pathogenesis of acute and chronic vasculopathies;^{21–24} plasminogen activator inhibitor-1 (PAI)-1, a procoagulant and proinflammatory gene;^{25,26} endothelin (ET)-1, an inflammatory vasoconstrictive peptide;²⁷ transforming growth factor (TGF)- β 1, a fibrogenic gene responsible for matrix accumulation in vascular and other types of tissue injury;²⁸ additionally, we analyzed the histological changes that evolve after the creation of the AV fistula.

Materials and Methods

The AV Fistula Model in the Rat

The AV fistula model was fashioned in rats anesthetized with methohexital sodium (50 mg/kg i.p.), as previously described.^{9–14} After a midline abdominal incision, the inferior vena cava and aorta were exposed, and vascular clamps were placed across the aorta and the inferior vena cava, one clamp positioned just above the aortic bifurcation and the other just below the origin of the renal vessels. The lower lumbar aorta was punctured with an 18-gauge disposable needle approximately one-third the length of the lumbar aorta above the aortic bifurcation; the site of puncture was on the side of the aorta not in contact with the inferior vena cava. The needle was then progressively introduced up and across the aorta such that the lateral aortic wall and the neighboring wall of the inferior vena cava were penetrated; the needle was then gently withdrawn.⁹ The point of entry of the needle into the aorta was sealed with a drop of cyanoacrylate glue, and the vascular clamps removed; cyanoacrylate glue was thus applied only to the needle entry site on the side of the aorta not in contact with the inferior vena cava. The abdominal wall was closed in two layers and the rats allowed to recover. Rats in the control group underwent sham operation in which they were subjected to anesthesia with methohexital sodium, laparotomy, cross clamping of the aorta and inferior vena cava for 30 seconds (the time taken for needle insertion and removal, and application of cyanoacrylate glue) without insertion of an 18-gauge needle into the aorta and inferior vena cava, or the application of cyanoacrylate glue.

The inferior vena cava in rats was harvested at 2, 5, and 16 weeks after the creation of the AV fistula or sham operation. In harvesting the inferior vena cava, vascular clamps were placed across the aorta and the inferior vena cava, one clamp positioned just above the aortic bifurcation, and the other above the origin of the renal vessels. The inferior vena cava was dissected free of the aorta, and transected, approximately, through the mid

level of the lumbar portion of the inferior vena cava; both segments of the inferior vena cava were embedded in paraffin such that mid sectional profiles of both segments were cut and prepared for histological analysis.

Histological Studies

Histological analyses were conducted on tissue sections stained with hematoxylin and eosin, and on survey sections (prepared for electron microscopy studies) that were cut at 1 μ m and stained with toluidine blue.^{29,30} The inferior vena cava was prepared for electron microscopy by fixation for 24 hours in Trump's fixative (1% glutaraldehyde and 4% formaldehyde in 0.1 mol/L phosphate buffer, pH 7.2),³⁰ followed by rinsing for 30 minutes in three changes of 0.1 mol/L phosphate buffer, pH 7.2. The tissue was postfixed for 1 hour in 1% OsO₄ and stained with 2% uranyl acetate for 30 minutes at 60°C. The tissue was dehydrated in progressive concentrations of ethanol and propylene oxide and embedded in Spurr's resin. Thin (90 nm) sections were cut on a Reichert Ultracut E microtome, placed on 200-mesh copper grids, and stained with lead citrate. Micrographs were taken on a JEOL 1200 EXII operating at 60 KV.

Northern Analyses

To examine mRNA expression of relevant genes, Northern analyses were conducted on RNA extracted from veins using the Trizol method (Invitrogen, Carlsbad, CA). Ten μ g of total RNA from each sample were separated on an agarose gel and transferred to a nylon membrane. Membranes were hybridized overnight with ³²P-labeled probes for rat cDNAs for MCP-1, PAI-1, ET-1, and TGF- β 1, as described previously.^{29,31} Autoradiograms were evaluated for loading and transfer by assessing the density of the 18S rRNA on an ethidium bromide-stained membrane, as previously described.^{29,31}

Immunoperoxidase Studies

Immunoperoxidase studies for the evaluation of expression of α -smooth muscle actin in the venous limb of the AV fistula were performed as previously described.^{32,33}

Results

Two weeks after establishing the AV fistula, marked changes in gene expression were observed in the venous limb. As shown in Figure 1, mRNA expression of MCP-1 and PAI-1 were markedly increased, 15-fold and 10-fold, respectively, in the venous segment of the AV fistula as compared to expression of these genes in the sham-operated rats; thus expression of a prototypic proinflammatory chemokine (MCP-1) and a procoagulant, proinflammatory gene (PAI-1) was vigorously induced. Expression of ET-1 mRNA was also increased threefold in the AV fistula whereas TGF- β 1 mRNA was

Figure 1. Northern analysis for expression of PAI-1 (top) and MCP-1 (bottom) in the inferior vena cava in rats subjected to an AV fistula (AVF) or sham operation (Sham); each lane represents RNA extracted from the inferior vena cava of an individual rat. In this and subsequent Northern analyses, equivalency of loading and transfer of RNA during the Northern analysis was assessed by expression of 18S rRNA; the numbers below each Northern analysis represent the individual standardized densitometric reading and the mean values for each group.

markedly suppressed 2 weeks after establishing the fistula (Figure 2).

At 2 weeks, there was little evidence by light microscopy of histological alterations in the AV fistula; however, throughout subsequent weeks, venous histological changes, characterized by neointimal hyperplasia and thickening of the venous wall in varying severity, appeared in rats with an AV fistula but not in sham-operated rats. Figure 3 shows sections from the venous limb of the fistula in a cohort of rats studied 5 weeks after fashioning of the AV fistula (Figure 3; D to F), and sections of the

Figure 2. Northern analysis for expression of ET-1 (top) and TGF- β 1 (bottom) in the inferior vena cava in rats subjected to an AV fistula (AVF) or sham operation (Sham); each lane represents RNA extracted from the inferior vena cava of an individual rat.

inferior vena cava after sham operation (Figure 3; A to C); these representative sections demonstrate the normal appearance of the inferior vena cava in sham-operated rats (Figure 3; A to C), and varying amounts of venous histological injury in the AV fistula studied at this time point (Figure 3; D to F). Intimal hyperplasia in the AV fistula ranged from modest thickening of the venous wall (Figure 3D), to venous hyperplasia extending in a papilliform manner into the lumen (Figure 3E), and finally to exuberant hyperplastic responses with papillomatous protrusions into the lumen and in contiguity with the opposite venous wall (Figure 3F). In addition to heterogeneity in neointimal hyperplasia at this time point in the model, venous histological injury was also characterized by focality. For example, in another rat subjected to the placement of the AV fistula and studied 5 weeks thereafter, standard sectioning of the inferior vena cava (as described in Materials and Methods) failed to reveal evidence of intimal hyperplasia (Figure 4A); however, further sectioning along the length of the inferior vena cava in this harvested specimen revealed focal proliferation and intimal hyperplasia, and within the hyperplastic focus, there were areas of necrosis, dystrophic calcification, and deposition of hemosiderin (Figure 4B). Serial sectioning of the inferior vena cava in a sham-operated rat showed normal histological appearance of the venous wall in all serial sections (data not shown).

Venous histological injury tended to progress with time, and by 16 weeks, all veins examined showed marked venous thickening, neointimal hyperplasia, and elaboration of extracellular matrix (Figure 5; E to H), whereas the histological appearance of the inferior vena cava in sham-operated rats remained normal (Figure 5; A to D). At this time point other histological features, as observed at earlier time points in the AV fistula model, were again noted, including central necrosis of the hyperplastic polypoidal protrusions of the neointima, and dystrophic calcification in these areas of necrosis (Figure 6). Insights regarding the pathogenesis of these lesions may be derived from the survey sections undertaken for EM studies and stained by toluidine blue (Figure 7). Larger polyps resulting from neointimal hyperplasia in the AV fistula, as shown by the central lesion in Figure 7B, exhibited two distinct regions: an inner, lighter staining region in which cells were relatively few and separated by abundant extracellular matrix, the latter heaped up into nodules in the more acellular regions; an outer darker staining rind in which cells were more compacted and the extracellular matrix was quite sparse (Figure 7B). It is possible that these inner regions, as shown in Figure 7B, experienced progressive ischemia, and ultimately, necrosis and sloughing, thereby giving rise to the appearance of these polyps shown in Figure 6. Survey sections undertaken for electron microscopy studies of the venous limb of the AV fistula also revealed hemorrhage and thrombus formation in the venous wall surrounded by organization and repair (Figure 8); that thrombogenesis occurred in the venous segment of the AV fistula was indicated by ultrastructural studies demonstrating the presence of platelet-fibrin thrombi (Figure 9). Thus, hemosiderin, as observed in the thickened, organized ve-

Figure 3. Histological changes in the inferior vena cava in sham-operated rats (A–C) and rats subjected to an AV fistula (D–F), 5 weeks after placement of the AV fistula. Sections are stained with H&E. Original magnifications, $\times 40$.

nous wall of the AV fistula, shown in Figure 10, likely reflects previous hemorrhage, the entrapment of red blood cells with thrombi, and the subsequent repair of these lesions.

We confirmed that smooth muscle cells were present in the hyperplastic neointima in the AV fistula as evidenced by staining for α -smooth muscle actin (Figure 11). Such positivity for α -smooth muscle actin tended to

Figure 4. Histological changes in the inferior vena cava in a rat subjected to an AV fistula, 5 weeks after placement of the AV fistula. **A:** The appearance of the inferior vena cava with standard sectioning of the paraffin block is demonstrated. **B:** A deeper section in the block. Sections are stained with H&E. Original magnifications, $\times 100$.

occur away from the region where extracellular matrix accumulated. On ultrastructural analyses, proliferating smooth muscle cells surrounded by abundant basal lamina were commonly observed in the AV fistula (Figure 12). At least two smooth muscle phenotypes were observed in the AV fistula: smooth muscle cells with a contractile phenotype, as evidenced by abundant myofibers (Figure 13), a phenotype also observed in the sham-operated rat (data not shown); smooth muscle cells with a synthetic phenotype, the latter characterized by abundant endoplasmic reticulum (Figure 14), and representing a phenotype not observed in the sham-operated rat.

Given the prominent accumulation of matrix observed at 16 weeks in this model, and the importance of TGF- β 1 as a fibrogenic cytokine, we assessed the expression of TGF- β 1 at this time point. Expression of TGF- β 1 mRNA was increased almost twofold at this stage in the model when significant accumulation of matrix occurred in this model (Figure 15A), and contrasts with the suppression of TGF- β 1 mRNA observed at 2 weeks (Figure 15B).

Discussion

This study demonstrates that the venous limb of an AV fistula in the rat exhibits marked up-regulation of proinflammatory genes (MCP-1, PAI-1, and ET-1), all broadly relevant to mechanisms underlying vascular injury. These changes in gene expression were accompanied by the evolution of progressive histological venous injury. Such injury was characterized by neointimal hyperplasia that markedly thickened the venous wall and protruded in a papillomatous manner into the venous lumen. In certain instances, the venous wall appeared laminated by cycles of thrombosis and organization of thrombus, whereas in other instances, dystrophic calcification and necrosis of the hyperplastic intima were observed. Smooth muscle cells in the AV fistula exhibited not only the contractile phenotype (the phenotype expressed by smooth muscle

cells in healthy veins) but also a synthetic phenotype, which may contribute to the elaboration of large amounts of extracellular matrix observed in advanced lesions.

We suggest that this model provides an *in vivo* approach by which to examine gene expression in the venous vasculature in states of increased venous blood flow rates and increased hydrostatic pressure. Much of the current understanding of differential gene expression in the vasculature in response to hemodynamic alterations is derived from *in vitro* approaches in which cellular components of the vasculature are subjected to biomechanical forces.^{34–36} However, the nature of expression of specific genes in the *in vivo* circumstance may differ quite appreciably from perspectives derived from *in vitro* studies. In this regard, our findings demonstrating down-regulation of TGF- β 1, when examined at 2 weeks in this model, merit comment. Derived primarily from findings *in vitro*, TGF- β 1 is regarded as a shear stress-inducible gene, and shear stress response elements are recognized in the TGF- β 1 gene,^{37–39} yet, surprisingly, in our model, TGF- β 1 expression was suppressed quite significantly at 2 weeks, the time point at which proinflammatory genes were induced. Thus the behavior of genes in *in vivo* settings of hemodynamic stress may not be accurately predicted from the responses of such genes observed *in vitro*. Besides providing a novel *in vivo* model for the examination of gene expression in response to hemodynamic stress, an added attribute of this model, in contrast to other *in vivo* models used to explore this question, is that it avoids the confounding and artifactual effects of foreign body-instigated, inflammation-mediated gene expression arising from sutures used to anastomose venous grafts to arterial segments.

A particularly marked expression of MCP-1 was observed in the venous segment of the AV fistula at an early time point (2 weeks). MCP-1 is expressed in diverse vasculopathies including atherosclerosis, restenosis injury, assorted vasculitides, hypertensive arteriosclerosis,

Figure 5. Histological changes in the inferior vena cava from sham-operated rats (**A–D**) and rats subjected to an AV fistula (**E–H**), 16 weeks after the placement of the AV fistula. Sections are stained with H&E. Original magnifications, $\times 40$.

Figure 6. Histological changes in the inferior vena cava from a sham-operated rat (**A**) and a rat subjected to an AV fistula (**B**), 16 weeks after the placement of the AV fistula. Sections are stained with H&E. Original magnifications, $\times 100$.

and venous intimal hyperplasia.^{21–24} Regulation of the expression of MCP-1 in health and disease is complex, influenced as it is by hemodynamic stress, nitric oxide, angiotensin II, reactive oxygen species, thrombin, and a large number of cytokines including interleukin (IL)-1 α , tumor necrosis factor- α , platelet-derived growth factor, M-CSF, IL-6, interferon- γ .^{21–24} Given the importance assigned to this chemokine in the initiation and progression of diverse vasculopathies, we suggest that this model provides a hitherto unrecognized approach in examining mechanisms underlying up-regulation and pathogenetic significance of MCP-1 in vascular injury, especially when the latter is attended by hemodynamic stress. Interest-

ingly, as shown in previous studies in this AV fistula model, marked up-regulation of MCP-1 also occurs in the heart,¹⁴ and this latter organ, like the inferior vena cava, is subjected to marked hemodynamic stress as imposed by the AV fistula.

It is notable that at 2 weeks after the formation of the fistula, MCP-1 and other proinflammatory cytokines such as PAI-1 and ET-1 were induced whereas TGF- β 1 was suppressed. TGF- β 1 is a potent anti-inflammatory cytokine that is considered part of the anti-inflammatory responses in the vasculature,⁴⁰ by virtue of such actions as the deactivation of macrophages and the suppression of cytokine-driven up-regulation of selectins, adhesion mol-

Figure 7. Histological changes in the inferior vena cava from a sham-operated rat (**A**) and a rat subjected to an AV fistula (**B**), 16 weeks after the placement of the AV fistula. This EM survey section is stained with toluidine blue. Original magnifications, $\times 100$.

Figure 8. Histological changes in the inferior vena cava from a rat subjected to an AV fistula, 16 weeks after the placement of the AV fistula. This EM survey section, stained with toluidine blue, demonstrates hemorrhage and thrombus formation in the venous walls surrounded by organization and repair. Original magnification, $\times 40$.

ecules, IL-8, and MCP-1.⁴⁰ We speculate that whatever the mechanism accounting for the suppression of TGF- β 1 at 2 weeks, this reduction in expression of TGF- β 1 in the venous wall may facilitate the expression of such proinflammatory cytokines as MCP-1; at later stages in the model increased TGF- β 1 expression promotes extracellular matrix expansion, the latter observed at 16 weeks in this model. It should be pointed out that cytokine expression was evaluated in our study by mRNA expression on Northern analysis; it would also be of interest to determine whether these changes in gene expression are accompanied by analogous alterations in protein expression for these specific cytokines.

Systemic arterial pressure is reported as reduced in the AV fistula model fashioned by surgical anastomosis,² whereas in the AV fistula model created by needle insertion (as used in the present study), systemic arterial pressure is reported as unchanged from controls;¹² by either method, cardiac output is significantly increased. Systemic hemodynamic alterations occurring in the AV fistula model may lead to increased circulating levels of cytokines which may contribute to the changes observed

Figure 9. EM studies demonstrating platelet-fibrin thrombi in the inferior vena cava of a rat subjected to an AV fistula, 16 weeks after the placement of the AV fistula. Original magnification, $\times 7500$.

Figure 10. Histological changes in the inferior vena cava from a sham-operated rat (A) and a rat subjected to an AV fistula (B), 16 weeks after the placement of the AV fistula. Sections are stained with H&E. Original magnifications, $\times 100$.

in the venous limb of the AV fistula. Indeed, elevated systemic concentrations of MCP-1 are increasingly recognized in the acute coronary syndrome, restenosis after coronary angioplasty, and congestive heart failure;^{22,24} it is possible that alterations in MCP-1 and other cytokines in the systemic milieu, besides those occurring in the venous wall, in this AV fistula model may be relevant to venous histological alterations described in this model.

The present model complements other *in vivo* models in the study of venous intimal hyperplasia. Intimal hyperplasia is one of the dominant mechanisms underlying the occlusion of saphenous venous segments used in coronary artery bypass surgery. Intimal hyperplasia, driven by cell proliferation and migration, and elaboration of extracellular matrix,^{15–17} predisposes to atheromatous thickening of the venous graft, ulceration of the endothelium, and thrombosis; such alterations, in aggregate, contribute to the relatively high incidence of occlusion of saphenous vein grafts used in coronary artery bypass surgery,

occurring at a rate of $\sim 15\%$ at 1 year and 40% at the first decade after coronary bypass surgery.¹⁵ A variety of experimental models are used in examining the pathogenesis of venous intimal hyperplasia and the efficacy of modalities that may combat such intimal hyperplasia. Each model has its own virtues and limitations. For example, although the study of venous segments *in vitro* readily facilitates the examination of a wide range of mechanisms that may underlie intimal hyperplasia,^{41,42} this approach suffers, obviously and notably, from the absence of the hemodynamic and humoral influences as found in the *in vivo* setting. The anastomosis of venous segments directly into the arterial circulation in rats and other rodents engenders predictable and pronounced venous intimal hyperplasia, but represents a technically demanding model, requiring as it does, microsurgical expertise.^{43–46} Venous intimal hyperplasia is also effectively modeled and studied using similar experimental approaches in larger animals, but these preparations not

Figure 11. Histological appearance of the inferior vena cava in a sham-operated rat (A) and a rat subjected to an AV fistula (B), 16 weeks after the placement of the AV fistula, and stained for α -smooth muscle actin. Original magnifications, $\times 100$.

Figure 12. EM studies demonstrating proliferating smooth muscle cells enmeshed in abundant basal lamina in the inferior vena cava in a rat subjected to an AV fistula, 16 weeks after the placement of the AV fistula. Original magnification, $\times 3000$.

only entail surgical expertise but exact a substantial financial cost and other requirements involved in large animal research.^{47–49} We suggest that the demonstration of marked alterations in gene expression accompanied by intimal hyperplasia in this quite simple model introduces another experimental approach in the study of this disease process.

We also suggest that this model may be used to explore mechanisms that contribute to the dysfunction and failure of AV fistulae used for vascular access in patients with end-stage renal disease. The AV fistula is the vascular access of choice in the provision of hemodialysis,

Figure 13. EM studies of the inferior vena cava in a rat subjected to an AV fistula, 16 weeks after the placement of the AV fistula, and which demonstrate smooth muscle cells with a contractile phenotype. Original magnification, $\times 10,000$.

Figure 14. EM studies of the inferior vena cava in a rat subjected to an AV fistula, 16 weeks after the placement of the AV fistula, and which demonstrate smooth muscle cells with a synthetic phenotype. Original magnification, $\times 10,000$.

and dysfunction and failure of AV fistulae necessitate less desirable vascular accesses such as polytetrafluoroethylene grafts and central venous catheters. Vascular access-related morbidity severely impairs the welfare of patients with end-stage renal disease and contributes colossally to the cost of their medical care; for example, such morbidity accounts for 20% of all hospitalization of these patients, and in using 15% of the cost of caring for hemodialysis patients, exceeds well more than 1 billion dollars in absolute costs.^{50–52} A major cause for vascular dysfunction is impaired venous blood flow because of intimal hyperplasia and attendant thrombosis occurring in either native AV fistulae or in and around synthetic grafts such as those using polytetrafluoroethylene.^{53–56} Among the cytokines incriminated in the initiation and progression of such injury are ET-1, MCP-1, and TGF- $\beta 1$, all of which are also up-regulated in the model we describe.^{57–61} Current models for the study of mechanisms involved in the dysfunction of hemodialysis vascular access use large animals such as sheep and pigs, thereby entailing extraordinary costs and the need for surgical expertise.^{62–64} We suggest that this relatively simple and inexpensive rodent model, recapitulating as it does, the

Figure 15. **A:** TGF- $\beta 1$ expression by Northern analysis in sham-operated rats (Sham) and rats subjected to an AV fistula (AVF), 16 weeks after the placement of the AV fistula. **B:** Standardized densitometric values of TGF- $\beta 1$ mRNA in rats subjected to an AV fistula, expressed as a percent of values in sham-operated rats, 2 and 16 weeks after the placement of the AV fistula.

basic pathological features of dysfunctional hemodialysis AV fistulae, thus affords a hitherto unexplored approach in the study of mechanisms underlying the dysfunction of hemodialysis AV access.

The presence of cardiac hypertrophy and congestive heart failure, and attendant cumulative mortality of some 40 to 50% throughout 16 weeks,⁶⁵ in this rodent model of an AV fistula also reproduce disease processes and mortality so commonly observed in patients with AV fistulae maintained on hemodialysis. Cardiac hypertrophy and clinical congestive heart failure occur in 75% and 40%, respectively, of hemodialysis patients, whereas mortality from cardiovascular diseases in dialysis patients, ~10% per year, may be increased 30-fold greater than mortality rates observed in the general population.^{66,67} Thus, the pathogenesis of venous injury in the AV fistula in this rodent model, akin to the development of disease in AV fistulae in patients on dialysis, evolves in the presence of cardiac hypertrophy and congestive heart failure. A similar consideration applies to the use of this model in evaluating mechanisms underlying venous injury in arterial bypass grafts used in patients with cardiovascular diseases: the prevalence of cardiac hypertrophy, remodeling, and failure, not unexpectedly, is also significantly increased in this patient population.

In summary, we describe marked up-regulation in proinflammatory genes in the venous vasculature in an AV fistula model in the rat; these changes are accompanied by progressive neointimal proliferation. We suggest that the defined and pronounced alteration in gene expression and histological injury, in conjunction with the relative simplicity of this model, in aggregate, provide a new and appealing approach in the study of such timely biological and clinically relevant phenomena as the differential expression of genes in response to hemodynamic forces, biological processes at work in vascular remodeling, mechanisms of injury to venous grafts used in bypassing occluded arteries, and mechanisms of dysfunction of AV fistulae used in hemodialysis.

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