

Quiescent Interplay Between Inducible Nitric Oxide Synthase and Tumor Necrosis Factor- α : Influence on Transplant Graft Vasculopathy in Renal Allograft Dysfunction

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A healthy endothelium is essential for vascular homeostasis, and preservation of endothelial cell function is critical for maintaining transplant allograft function. Damage to the microvascular endothelial cells is now regarded as a characteristic feature of acute vascular rejection, an important predictor of graft loss. It is also linked with transplant vasculopathy, often associated with chronic allograft nephropathy. Large bursts of nitric oxide in infiltrating monocytes/macrophages modulated by inducible nitric oxide synthase are considered pivotal in driving this mechanism. Indeed, it has been shown recently that increased circulating levels of tumor necrosis factor- α in the rejecting kidneys are largely responsible for triggering inducible nitric oxide synthase expression. This in turn suggests that several structural and functional features of graft rejection could be mediated by tumor necrosis factor- α . Despite the large body of evidence that supports immunologic involvement, knowledge concerning the cellular and biochemical mechanisms for nephritic cell dysfunction and death is incomplete. The role of tumor necrosis factor- α in mediating pathophysiological activity of inducible nitric oxide synthase during transplant vasculopathy remains contentious. Here, we discuss the effect of inducible nitric oxide synthase and tumor necrosis factor- α interaction on progressive damage to glomerular and vascular structures during renal allograft rejection. Selective inhibition of inducible nitric oxide synthase and tumor

necrosis factor- α as a potential therapy for ameliorating endothelial dysfunction and transplant graft vasculopathy is also discussed.

Key words: Cytokines, Circulating endothelial cells, Transplantation, Macrophages, TNF- α , iNOS, Endothelial dysfunction, Pharmacological inhibition

Damage of microvascular endothelial cells is a characteristic feature of acute vascular rejection and chronic allograft nephropathy, an important predictor of graft loss [1]. One prevalent manifestation of chronic allograft nephropathy is the salient vascular lesion, often referred to as *transplant vasculopathy*, the histopathologic examination of which reveals intimal hyperplasia in the arterial walls, accompanied by infiltration of the vessel wall by macrophages, foam cells, and T cells [2]. Yet the exact mechanisms leading to this neointimal formation and chronic rejection remain unclear. According to the now recognized *response-to-injury hypothesis* [3], it is thought that endothelial damage in the graft vessel wall results in the secretion of various growth factors and cytokines that interact with smooth muscle cells thus inducing phenotypic transformation [4].

Among cytokines, it is believed that tumor necrosis factor-alpha (TNF- α) is implicated in the development and progression of vasculopathy [5]. TNF- α initiates an inflammatory response, leading to endothelial cell activation [6] and free radical formation [7], which consequently induce transient and reversible endothelial dysfunction and vessel occlusion [8]. Circulating increased levels of TNF- α trigger inducible nitric oxide synthase (iNOS) expression, which are responsible for producing large bursts of nitric oxide (NO); this has been postulated as enhancing rejection processes [9]. The mechanism by which TNF- α modulates NO production by iNOS in transplant vasculopathy has not yet been fully elucidated.

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In this review, we examine the basic biology of TNF- α in relation to NO-mediated production and aggravation of endothelial cell dysfunction in renal allograft rejection. Recognizing the pathways that activate this pathological state has led to investigating potential therapies that alleviate, modify, or prevent the inflammatory process.

Transplant Vasculopathy in Renal Allografts

The observation by Seron and associates [9] that up to 7.5% of renal transplants have allograft vasculopathy, which may account for a decrease in long-term graft survival [approximately 41% reduction], supports the hypothesis that chronic allograft nephropathy with or without vasculopathy may have a significant deleterious impact on graft dysfunction. One of the mediators of long-term graft survival is a reduction in oxygen and nutrient delivery to the allograft kidney, which leads to hypoxia-related down-stream ischemia of the glomerular and tubulo-interstitial lesions. This process leads to infiltration of the vessel wall by inflammatory cells [2], impairment of NOS [10], and production of superoxide ($O_2^{\cdot-}$) [11], factors capable of causing thrombotic microangiopathy.

Involvement of TNF- α in Rejection

Today, TNF- α is recognized as a pleiotropic cytokine functioning within a complex and tightly regulated cytokine network. It activates multiple transduction pathways, inducing or suppressing a wide variety of genes, including those encoding the production of cytokines, adhesion molecules, and iNOS [12], and it helps orchestrate the rejection response [13]. The rejection response is initiated by activating CD4+ T-helper cells via alloantigen, either through direct stimulation by donor antigen presenting cells, or indirectly via recipient antigen presenting cells. Activated T-helper cells release initiator cytokines such as IL-1 β , IL-2, and interferon γ , which in turn activate macrophages to release TNF- α [14]. TNF- α then participates in the initiation response through the up-regulation of major histocompatibility complex expression and activation of inflammatory cells [15].

Early reports showing that TNF- α may play a role in the rejection response came from 2 sources: First, observations were made that renal [16] and liver [17] transplant recipients had increased serum levels of TNF- α following rejection episodes, although no causal relationship had been established. Second, evidence came from animal models of acute allograft rejection [18]. In addition, localization of the protein

and mRNA transcripts within the rejection infiltrate of the renal allograft [19] provided supporting evidence for the role of TNF- α in rejection.

Inducible Nitric Oxide Synthase (iNOS) Activity in Transplant Vasculopathy

Allograft-associated vasculopathy is considered a form of chronic rejection, and transplant-associated arterial disease tends to be diffuse [20]. There is now increasing evidence that NO biosynthesis is involved in the initiation and progression of transplant arteriosclerosis via upregulation of iNOS expression [21]. However, it is unclear whether iNOS expression plays a role in the pathogenesis of vascular disease (eg, by increasing oxidant stress and the expression of oxidant sensitive genes) or whether it protects against vascular disease. Data from a study by Aji and colleagues have demonstrated that administering L-arginine supplements to low-density-lipoprotein receptor knockout mice fed on a high-cholesterol diet reduces xanthoma formation and the extent of atherosclerotic aortic lesions by 40% [22]. This beneficial effect does not occur when an NOS inhibitor is coadministered with the L-arginine supplement, suggesting that NO produced by iNOS is responsible for lesion reduction. Therefore, inhibiting NO production by iNOS results in inhibition of platelet and white cell adhesion and transmigration of white blood cells across the endothelium, thus blocking smooth-muscle-cell proliferation.

Systemic Activation of TNF- α and iNOS in Allograft Rejection

Synthesis of iNOS is responsible for producing large bursts of both NO and superoxide ($O_2^{\cdot-}$) that lead to peroxynitrite-mediated nitrotyrosine formation [23-24] and is expressed in several cells of the kidney, particularly during acute rejection of a renal allograft. Moreover, infiltrating TNF- α and iNOS expression reportedly have been co-localized in a kidney transplant model [25]. Similarly, TNF- α released by macrophages is reported as being present in vessels undergoing allograft rejection [26]. This therefore suggests that several structural and functional features of graft rejection could be mediated by TNF- α . Despite the large body of evidence that supports immunologic interactions, knowledge concerning the cellular and biochemical mechanisms for nephrotic cell dysfunction and death remains incomplete. Hence, the role of NO production by iNOS and TNF- α as a pathophysiological mediator

of infiltrating mononuclear cells during acute graft rejection has not yet been specifically examined. Nevertheless, there is evidence to suggest that TNF- α produced by activated macrophages plays an important role in inducing iNOS mRNA, protein, and enzyme activity in macrophages and vascular smooth muscle cells [26]. It is also plausible that the interaction between the TNF- α ligand expressed on CD4+ T lymphocytes actually plays a part by interacting between the CD40 ligand on T cells and CD40 on macrophages, thus inducing iNOS [27].

If the quiescent interplay between iNOS and TNF- α is a mediator of the induction and progression of acute rejection, then selective iNOS inhibition may ameliorate histologic lesions and reduce transplant dysfunction, ultimately enhancing long-term graft survival. Theoretically, sustained iNOS inhibition may offer protection against reperfusion-induced tubulointerstitial injury, possibly by curbing excessive NO production and oxidant-induced renal tissue scarring. On the other hand, early iNOS induction may mainly

mediate a cytotoxic inflammatory response to the graft [28]. Independent of the therapeutic aspect, one should consider that these studies strengthen the concept that NO generated by iNOS and TNF- α is significantly involved in acute rejection (Figure 1).

Transplant Graft Vasculopathy: The Need for a Predictor

Transplant graft vasculopathy is an immune-mediated response, hallmarked by cellular infiltration (T lymphocytes and macrophages) and myocyte damage in the transplanted kidney [29]. Both the CD4+ T lymphocytes and macrophages play a major role in directing the rejection response, through elaboration of initiator cytokines, such as TNF- α and the induction of effector molecules such as NO [29].

Recent evidence suggests that TNF- α expression is much higher shortly after transplantation, and that this may not be specific to rejection [30]. Consequently, detecting the TNF- α protein in renal allografts in the absence of histologic or clinical

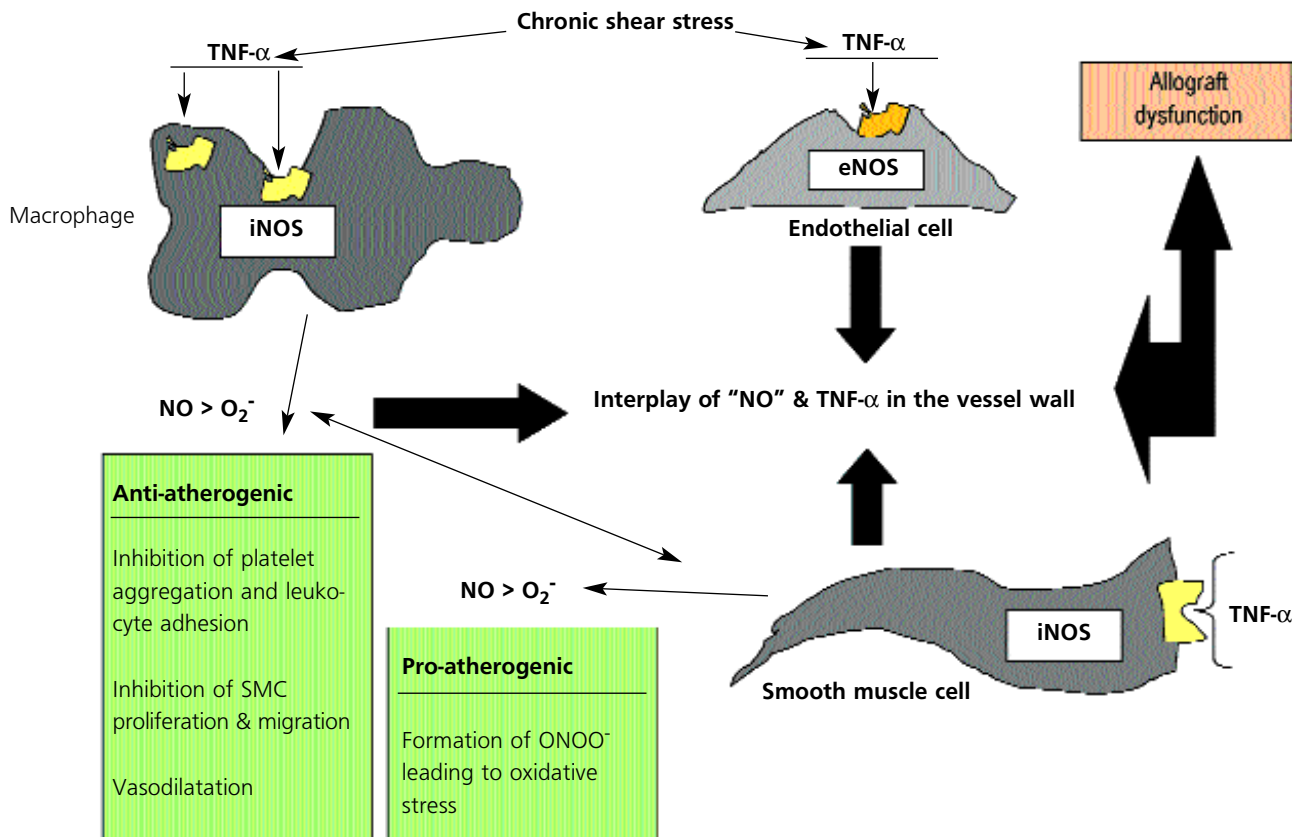


Figure 1. The interaction between nitric oxide (NO) and tumor necrosis factor-alpha (TNF- α) in regulating the initiation of proliferative vessel changes in allograft vasculopathy. This control is modulated in macrophages, endothelial cells, and smooth muscle cells under the influence of the chronic shear stress that leads to renal graft dysfunction. eNOS = endothelial nitric oxide synthase; iNOS = inducible nitric oxide synthase; NO = nitric oxide; O₂⁻ = superoxide; SMC = smooth muscle cell; TNF- α = tumor necrosis factor alpha; ONOO⁻ = peroxynitrite

evidence of rejection cannot safely be considered a contributing factor. Larger numbers of samples must be analyzed to substantiate such findings. Although the studies described in this review do not establish whether increased TNF- α expression is the cause or the effect of rejection, the genetic studies indicate that stimulation of the inflammatory response (by alloantigen or ischemia) results in large amounts of TNF- α being secreted by individuals with a genetic predisposition to producing higher levels of TNF- α [31]. Similarly, apoptosis of nephritic cells in association with iNOS expression in allograft rejection may have several diagnostic implications. In view of this, there is a pressing need to identify a marker that could predict rejection. A noninvasive diagnostic method, such as serum analysis, would be of great benefit.

Possible Therapeutic Potentials Targeting Transplant Graft Vasculopathy

In the past 40 years, our understanding of the mechanisms of chronic rejection in kidney transplantation gradually has grown. Various nonimmunologic factors have been found to be important contributors as well (Figure 2) and since multiple factors play a part in chronic rejection, the more inclusive term *chronic allograft nephropathy* has been introduced. Chronic allograft nephropathy manifests itself clinically

by a gradual decrease in renal function accompanied by hypertension and low-grade proteinuria, usually occurring months or years after transplantation [32]. Therefore, determining the optimal treatment for established chronic allograft nephropathy remains a challenge.

To date, no immunosuppressive regimen has been effective for treating or preventing this condition in humans. Preliminary data suggest that the addition of mycophenolate mofetil, with or without a reduction in the dosage of cyclosporine, might be an effective strategy for stabilizing or improving allograft function in patients with established chronic allograft nephropathy [33]. These observations then led to other avenues that enhance the natural anti-inflammatory response of the patient. Three forms of broad-spectrum immunomodulatory strategies are currently under investigation: (1) intravenous immunoglobulin, (2) immunoabsorption, and (3) immune-modulation therapy [34]. Whether these are useful in chronic rejection is not known, but they are a focus of further investigations in patients with transplant vasculopathy that perhaps will result in better or more intelligent immune modulation in the future. Moreover, new preservation techniques and pharmacologic strategies have the potential to reduce the initial ischemic injury to the allograft [35]. These include optimal selection and treatment of donors before recovery of the organ,

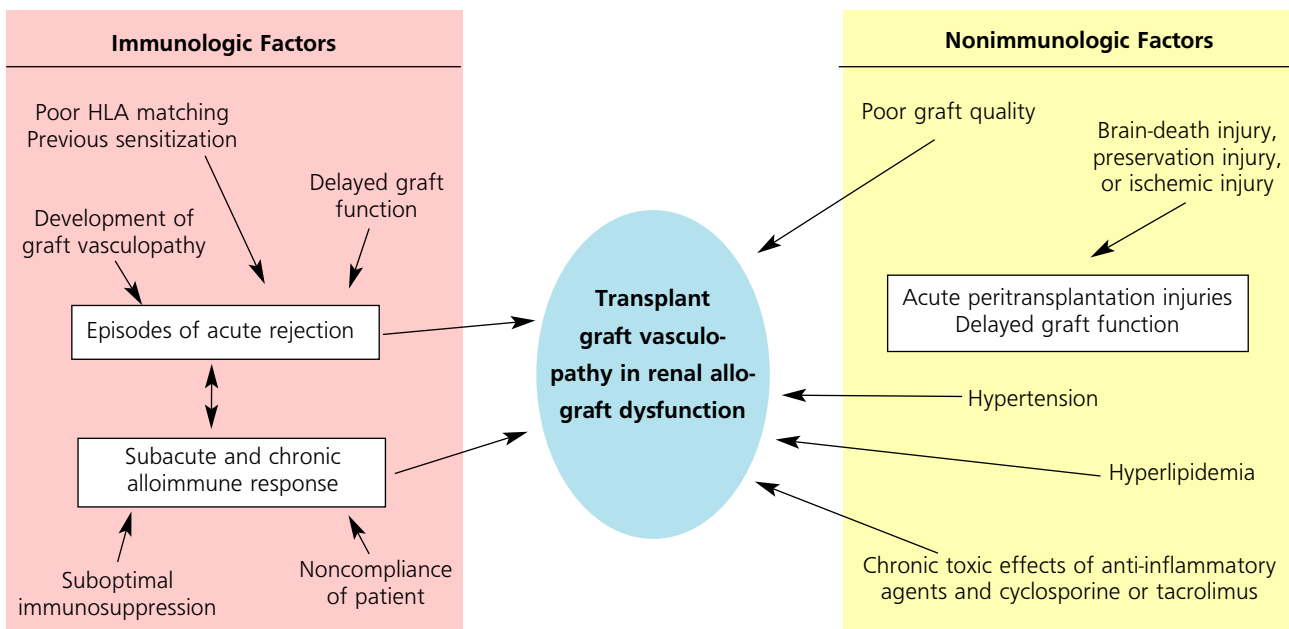


Figure 2. Early allograft damage caused by the acute posttransplantation injuries. The outline also shows that episodes of acute rejection may result in a loss of functional nephrons. Thereafter, both immunologic and nonimmunologic factors contribute to the development of chronic allograft nephropathy.

HLA = Human leukocyte antigen

shortening cold ischemia duration and the preferential use of living donors (related or unrelated).

Conclusions

Renal transplantation generally is the preferred therapy for end-stage renal disease. The outcome of patients is still limited by early graft loss related to chronic allograft nephropathy. An emerging notion in the renal transplantation field is that chronic allograft nephropathy and cardiovascular disease are manifestations of a single disease, and that they share the same pathophysiology such as chronic inflammation and atherosclerosis. It has been established that NO plays an important role in endothelial function, where its production is catalyzed by iNOS isoforms. NO inhibits the expression of many genes thought to be involved in inflammation. Although it is evident that NO production frequently accompanies inflammatory states, it is unclear whether it promotes, inhibits, or has no effect on the inflammatory process itself. This may be explained by varying levels of NO in inflammation that have been reported in the discussed studies. When interpreting the results of such studies, it is important to keep in perspective the disease process being studied. We potentially have presented the very first step toward understanding vasculopathy development and how this correlates with molecular expression of TNF- α and iNOS activity. By doing this, we have taken a very large jump, bypassing other factors that modify cytokine production, modification, release, antagonism, receptor function, and ultimately effect. Further investigation in this field should, therefore, involve immunohistochemical biopsy examination of cytokine expression, allowing more accurate dissection of pathogenic mechanisms. It is likely that randomized clinical trials will be conducted to compare modern maintenance regimens of immunosuppressive agents with built-in protocols that induce tolerance, to define the optimal anti-rejection strategies of the 21st century.

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