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Direct or indirect endothelial cell transforming growth factor-β receptor activation initiates arteriolar hyalinosis

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Calcineurin inhibitor nephrotoxicity is one of the leading causes of chronic allograft dysfunction.[1–3] Unfortunately; increased serum creatinine lags well behind the appearance of significant histologic damage to the kidney seen, in part, as hyalinosis. This hyalinosis is focal and beaded in appearance as opposed to the circumferential hyalinosis produced by diabetes or hypertension. Due to the focal nature, histologic examination of serial sections from two different biopsy cores is recommended for accurate identification and diagnosis. The molecular and cellular mechanisms contributing to the overall pathologic process of hyalinosis is the subject of the present study.[4]

The macrolid lactone tacrolimus (also known as FK-506) is an immunosuppressant that decreases the risk of organ rejection by binding to the immunophilin FK-506 binding protein (FKBP12). In this study, Chiasson and coworkers hypothesized that in addition to its known effect of increasing TGF-β levels, tacrolimus relives a tonic inhibition of TGF-β signaling as it interacts with FKBP12 ultimately boosting activation of the transcription cofactor Smad2/3 and extracellular matrix protein production thus causing smooth muscle injury and eventual hyalinosis.[4] In order to mechanistically dissect the process and determine the contribution of endothelial cells, the authors developed endothelial-specific FKBP12 knockout mice by crossing animals containing lox P sites flanking either side of the FKBP12 gene with mice expressing Cre driven by the endothelial-specific Tie2 promoter. These mice were found to be deficient in FKBP12 in isolated endothelial cells, did not have elevated levels of angiotensin II (a driver of TGF-β production in renal diseases) or TGF-β in their serum, or increased TGF-β mRNA expression in isolated aortas. In this study it was assumed that the aortas reflect the biology of the renal arterioles. Wild type mice, treated with 10 mg/kg/day tacrolimus for 1 week, exhibited histologic evidence of arteriolar hyalinosis in kidney sections. Similar hyalinosis was seen in the kidneys of untreated 12 week old endothelial-specific FKBP12 knockout mice. In the aortas of the wild type mice there was activation (phosphorylation) of the TGF-β-linked transcription factors Smad2/3, and increased production of the extracellular matrix proteins collagen I and fibronectin. All these effects were also seen in the aortas of the untreated endothelial-specific FKBP12

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knockout mice. In aortas isolated from wild type mice, treatment with tacrolimus caused Smad2/3 activation and increased expression of collagen I and fibronectin; effects inhibited *in vitro* by prior endothelial cell denudation of the aortas or inhibition of the TGF-β receptor by the small molecule SB-505124, thus confirming the involvement of endothelial cells and TGF-β receptor signaling in events leading up to hyalinosis. The effect of tacrolimus in vitro was not prevented by incubation with calcineurin autoinhibitory peptide, further indicating the TGF-β pathway and not calcineurin inhibition as the mechanistic conduit leading to hyaline deposition. The authors do not know whether this same pathway is responsible for ciclosporin-induced arteriolar hyalinosis.

Although the study strongly implicates indirect TGF-β type I receptor activation as an initiating event in the overall biologic process of tacrolimus-induced arteriolar hyalinosis, the authors rightly caution about the concentration of tacrolimus used in their studies both *in vivo* and *in vitro*. To achieve effects of tacrolimus in short order, doses many times higher than those seen clinically were used. Although there was successful knockout of FKBP12, the authors found expression of FKBP12.6. This is not unanticipated since they are separate gene products with the FKBP12 located on mouse chromosome 2 (human chromosome 20) while FKBP12.6 is located on mouse chromosome 12 (human chromosome 2). While both FKBP12 and FKBP12.6 regulate intracellular calcium levels through ryanodine and inositol 1, 4, 5-triphosphate receptors, FKBP12.6 does not seem interact with type 1 receptors of the TGF-β family.[5–7] As mentioned before, a major consideration is the assumption that isolated aortas reflect the biology of renal arterioles, and this needs more rigorous investigation.

There are other considerations to be gleaned from this study. Of importance to this study is that in the absence of ligands such as tacrolimus, FKBP12 also interacts with the intracellular glycine-serine rich domain (GS-motif) of TGF-β receptor I (TGF-βRI) stabilizing it in an inactive conformation[6,7] (Figure 1). This stabilization of TGF-βRI prevents spontaneous interaction with the type II receptor (TGF-βRII), even in the absence of TGF-β, and prevents low level activation of the signaling system by phosphorylation of transcription factors Smad2/3. In essence, FKBP12 prevents leakiness of the TGF-β signaling system.[6,7] Both activin-like kinase-I (another type I receptor that interacts with the TGF-βRII receptor in endothelial cells), TGF-βRI and presumably all type I receptors with a GS-motif interact with FKBP12 thereby preventing a leakiness in receptor activation and subsequent biologic responses.[6,7] The authors are correct that “future immunosuppressive drugs that do not increase TGF-β levels or lead to TGF-β receptor activation” need to be developed to eliminate or minimize arteriolar hyalinosis. These future drugs need to be TGF-β type I-specific to eliminate unintended side effects involving other type I receptors of the extended TGF-β superfamily that may be coupled to beneficial homeostatic pathways.

Traditionally, hyalinosis is thought to result from excessive extracellular matrix production by smooth muscle cells and as now, indicated by Chiasson and coworkers, endothelial cells[4] (Figure 1). Additionally, leakage of plasma proteins across the damaged endothelia is thought to contribute to the protein build up characterized as homogeneous pink-staining material (Figure 1). This reduces the flexibility of the renal arterioles and decreases renal function. Thus, direct (through ligand) or indirect (through macrolid) endothelial cell

transforming growth factor-β type I receptor activation is sufficient to initiate arteriolar hyalinosis.

Acknowledgments

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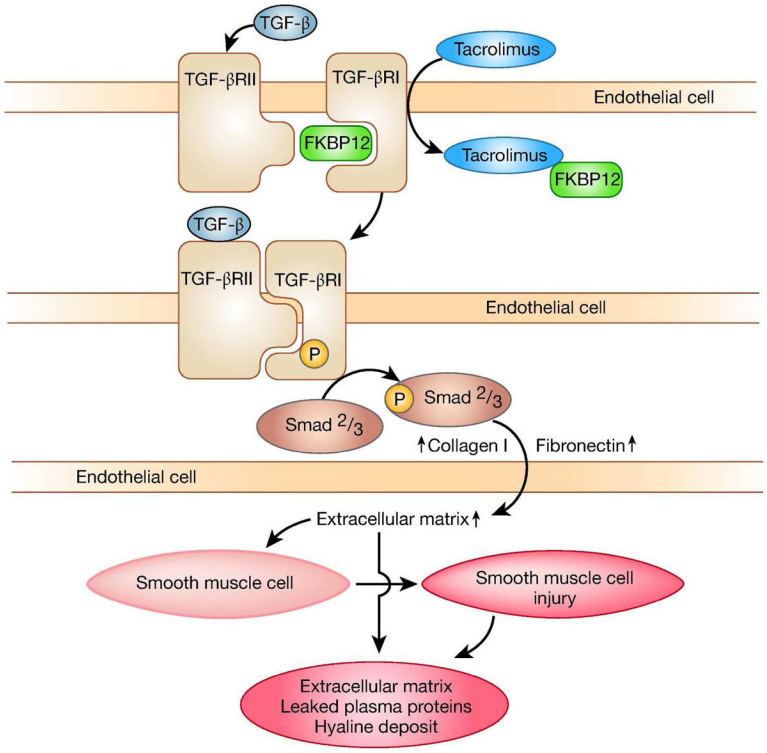

Figure 1.

Direct and indirect activation of the transforming growth factor-β (TGF-β) type I receptor by TGF-β or tacrolimus, respectively leads to arteriolar hyalinosis. Receptor activation leads to activation of the Smad2/3 transcription factor and increased synthesis of the extracellular matrix proteins collagen I and fibronectin by endothelial cells. This, in turn, induces smooth muscle cell injury and eventual hyaline deposition. Another contributor to the hyaline deposits are plasma proteins leaked across damaged endothelium.