Chronic Rejection: Prospects for Therapeutic Intervention in Fibroproliferative Vascular Disease

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Vascular disease, manifesting as either transplant arteriopathy or native atherosclerosis, is currently the main obstacle to successful transplant outcome. In addition, vascular restenosis following balloon angioplasty or stenting continues to limit the long-term efficacy of these procedures. Neointimal hyperplasia is refractory to conventional immunosuppression although newer agents, such as rapamycin, have shown considerable promise in controlling it. By allowing large-scale study of gene expression during vascular remodelling, the emerging field of genomics is poised to revolutionise the drug discovery process. Here we summarise our initial experience using genomic methods to identify new targets for therapeutic intervention in vascular disease.

Keywords: *Genomics; graft; atherosclerosis; vascular restenosis; therapy*

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Experimental and Clinical Transplantation (2003) 1: 35-38

Chronic rejection - what is it and why is it a problem?

Advances in graft procurement, preservation and matching, as well as in post-transplant immunosuppression, have reduced the incidence of acute rejection and increased one-year graft survival rates to over 90% for most types of transplanted organs. However, long-term transplant success is still limited by patient mortality and chronic rejection. Death with a functioning graft, primarily due to cardiovascular causes, is currently the leading cause of renal graft loss [1].

The term "chronic rejection" refers to an unfavourable transplant outcome occurring as a result of chronic inflammatory injury to the graft. Such cumulative, insidious injury is refractory to conventional immunosuppression and progressively undermines graft function, culminating in rejection. The causes, incidence and manifestations of chronic rejection vary considerably, depending on the type of transplanted organ [2]. Transplant arteriopathy is the principal manifestation in cardiac grafts, with a reported incidence of up to 60% at one year post-transplant, directly contributing to declining graft function [3]. Conversely, although acute vascular rejection is a leading predictor of chronic allograft nephropathy [4], fibroproliferative vascular changes are less frequent in renal and liver grafts where their relationship to interstitial changes and functional

deterioration remains unclear.

Overall, regardless of the role of transplant arteriopathy in chronic graft nephropathy, vascular complications are the foremost cause of late graft loss. These are thought to arise secondary to endothelial dysfunction, whether related to oxidative stress alone (native atherosclerosis) or to more complex pathology (transplant arteriopathy). Neointimal hyperplasia is initiated by leukocyte infiltration into the vascular wall and requires the presence of functional macrophages [5-7]. In native atherosclerosis, lesions typically evolve as fatty streaks stabilised by fibromuscular caps (atherosclerotic plaques); plaque rupture precipitates infarction. In organ grafts, the vascular response to injury progresses through a proliferative stage, followed by intimal fibrosis and constrictive vascular remodelling, all of which contribute to lumen loss and downstream tissue ischaemia. Recent progress in understanding the molecular basis of neointimal hyperplasia is now raising new prospects for effective management of vascular disease in the clinic.

Genomics as a tool for guiding therapeutic intervention in transplant arteriopathy

The vascular response to injury entails differential regulation of gene expression. Accordingly, our laboratory has taken a genomic approach to studying the development of neointimal hyperplasia, using oligo microarrays alongside conventional histology (Figure 1). In order to dissociate the molecular pathways of vascular injury and remodelling, focusing on the latter, we have used catheter-mediated endothelial denudation injury to model the vascular response to injury. Based on the hypothesis that at least some of the differentially regulated genes would be rate-limiting for the development of neointimal hyperplasia, our overall aim has been to identify these genes and develop novel therapies that intercept the relevant molecular pathways.

We have now mapped gene expression in the course of vascular remodelling after endothelial denudation (Aavik *et al*, manuscript in preparation). Approximately 1,000 out of 48,000 gene transcripts were found to be differentially regulated in at least one time-point following arterial injury. When transcripts were grouped according to putative function, those pertaining to cell locomotion, proliferation and intracellular signalling were upregulated in the initial stages of the vascular response; while transcripts encoding extracel-

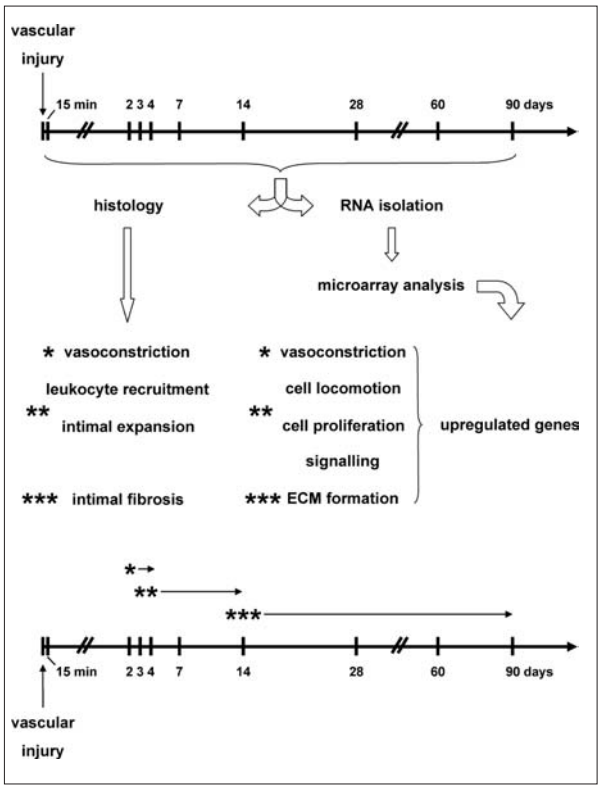

Figure 1. Genomic analysis of catheter-injured arteries reveals strong correlation between patterns of gene expression and histological features of the vascular response to injury.

lular matrix components were abundant at later stages and correlated with intimal fibrosis.

Modulating intimal expansion by growth factor receptor inhibition

Growth factors are thought to play a central role in driving cell proliferation in the expanding neointima. Among other genes known to be rate-limiting for the development of transplant arteriopathy and chronic graft nephropathy, such as endothelin-1 and its receptor [8, 9], our primary genomic screen identified the insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF) agonist-receptor pairs as potential targets for therapeutic intervention (Table 1). Our group has previously shown that inhibition of any of these two growth-signalling pathways inhibited neointimal hyperplasia in rat models of arterial injury [10-12]; these findings were recently corroborated by others [13]. Hence, the functional inhibition of these two growth factor receptors, achieved by inhibiting their expression, kinase activities or through the use of synthetic receptor antagonists, appears to have significant therapeutic potential.

Hormone receptor agonists: the key to refined control of vascular reactivity?

Endocrine regulation of inflammatory responses is a well-documented phenomenon and the vascular wall may be exquisitely sensitive to hormonal stimulation. Previous studies have shown that estrogen suppresses transplant arteriopathy in a rat transplant model [14]. We recently demonstrated that vascular expression of estrogen receptor beta (ERβ), but not ERα, increases acutely following endothelial denudation injury [15] and also in transplant arteriopathy [16] in the rat. ERβ mRNA and protein co-localised with both medial vascular smooth muscle cells and neointimal cells in affected vessels. Similar findings were documented in a baboon model of endothelial denudation injury [17]. Estrogen administration to ovariectomised rats suppressed neointimal hyperplasia but induced uterine hypertrophy. Conversely, administration of genistein, an ERβ-selective phytoestrogen, suppressed neointimal hyperplasia at doses below its receptor tyrosine kinase inhibitory activity, whilst having no effect on uterine tissue [15]. Thus, modulation of neointimal cell proliferation by activating ERβ may represent a highly targeted approach to managing neointimal hyperplasia.

Early results from our laboratory show that BIM23014C, a somatostatin analogue, also suppresses neointimal hyperplasia by specifically

Table 1. Kinetics of baboon gene expression after carotid denudation injury

Functional cluster	Example genes	Fold induction over baseline					
		D2	D3	D4	D14	D30	D90
Vasoconstriction	*ECE1*	***3.1***	1.5	***1.8***	***1.9***	***2.0***	0.4
	EDN1	***1.8***	***1.8***	***1.7***	1.5	1.5	***2.5***
	EDNRB	1.1	***2.3***	1.4	1.0	0.8	1.1
Signalling	*RGS1*	***3.6***	***3.4***	***8.6***	***3.4***	***3.5***	***2.9***
	RGS2	1.4	***4.8***	1.8	***2.2***	1.1	1.2
Cell activation /	*TLR7*	***4.7***	***3.3***	***4.3***	***2.5***	***2.6***	0.6
locomotion /	*ADAM1 2*	***2.8***	***2.0***	1.4	***2.1***	1.6	1.4
proliferation	*TREM1*	***2.5***	***1.8***	***1.9***	0.8	1.1	1.0
	FLT1	***1.6***	***1.6***	***1.7***	1.2	1.3	1.3
	AIF1	0.5	***2.5***	***1.4***	***1.6***	1.2	1.2
	IGF1	1.5	***1.9***	***1.6***	***2.4***	***2.1***	***2.6***
	IGF1R	***1.8***	1.3	1.0	1.2	1.1	1.2
	PDGFB	***1.4***	1.2	***2.0***	***1.4***	1.1	0.9
	PDGFRA	1.4	***2.6***	***1.6***	***1.8***	1.5	1.4
ECM deposition	*FN1*	1.5	***3.2***	***3.0***	***2.4***	***2.3***	***3.2***
	DPT	***3.6***	***3.6***	***2.5***	***5.2***	***3.5***	***2.9***
	DSG3	***4.8***	***2.0***	***4.8***	***3.7***	***3.2***	***3.4***
	COL3A1	0.5	1.4	1.5	1.4	***1.6***	***2.6***

inhibiting neointimal cell proliferation [18]. Somatostatin, a neuroendocrine hormone produced in the brain and pancreas, signals via five different cellular receptors, designated SSTR-1 through 5. We recently showed, using SSTR subtype-selective compounds, that the vasculoprotective effect of somatostatin in the rat is mediated through SSTRs 1 and 4 [19]. Preliminary data suggest that these SSTRs are expressed at low levels in the vascular wall and are upregulated following injury. Ongoing work in our laboratory aims to further characterise SSTR expression following injury to the vascular wall.

References:

1. Ojo AO, Hanson JA, Wolfe RA, Leichtman AB, Ogodoa LY, Port FK. Long-term survival in renal transplant recipients with graft function. Kidney Intl 2000; 57: 307-313
2. Vamvakopoulos J, Aavik E, du Toit D, Häyry P, Sarwal M. Transplant arteriopathy: pathology, pathogenesis and prospects for treatment. In: Runge M, Patterson C, ed. Principles of Molecular Cardiology. New Jersey: Humana Press, 2002 (in press)
3. Julius BK, Attenhofer Jost CH, Sutsch G, Brunner H-P, Kuenzli A, Vogt PR. Incidence, progression and functional significance of cardiac allograft vasculopathy after heart transplantation. Transplantation 2000; 69: 847-853
4. Van Saase JLCM, van der Woude FJ, Thorogood J et al. The relation between acute vascular and interstitial renal allograft rejection and subsequent chronic rejection. Transplantation 1995; 59: 1280-1285
5. Shi C, Lee W-S, He Q et al. Immunologic basis of transplant-associated arteriosclerosis. Proc Natl Acad Sci USA 1996; 93: 4051-4056
6. Qiao JH, Tripathi J, Mishra NK et al. Role of macrophage colony-stimulating factor in atherosclerosis: studies of osteopetrotic mice. Am J Pathol 1997; 150: 1687-1699
7. Rajavashisth T, Qiao JH, Tripathi S et al. Heterozygous osteopetrotic (op) mutation reduces atherosclerosis in LDL receptor- deficient mice. J Clin Invest 1998; 101: 2702-2710
8. Braun C, Conzelmann T, Vetter S et al. Prevention of chronic renal allograft rejection in rats with an oral endothelin A receptor antagonist. Transplantation 1999; 68: 739-746
9. Simonson MS, Herman WH, Robinson A, Schulak J, Hricik DE. Inhibition of endothelin-converting enzyme attenuates transplant vasculopathy and rejection in rat cardiac allografts. Transplantation 1999; 67: 1542-1547
10. Häyry P, Myllärniemi M, Aavik E et al. Stable D-peptide analog of insulin-like growth factor 1 inhibits smooth muscle cell proliferation after carotid balloon injury in the rat. FASEB J 1995; 9: 1336-1344
11. Myllärniemi M, Calderon Ramirez L, Lemström K, Buchdunger E, Häyry P. Inhibition of platelet-derived growth factor receptor tyrosine kinase inhibits vascular smooth muscle cell migration and proliferation. FASEB J 1997; 11: 1119-1126
12. Sihvola R, Koskinen P, Myllärniemi M et al. Prevention of cardiac allograft arteriosclerosis by PDGF-R protein-tyrosine kinase inhibitor. Circulation 1999; 99: 2295-2301
13. Noiseux N, Boucher CH, Cartier R, Sirois MG. Bolus endovascular PDGFR-beta antisense treatment suppressed intimal hyperplasia in a rat carotid injury model. Circulation 2000; 102: 1330-1336
14. Motomura N, Lou H, Hong M, Tsutsumi Y, Mayumi T, Foegh ML. Local administration of estrogen inhibits transplant arteriosclerosis in rat aorta accelerated by topical exposure to IGF-I. Transplant Proc 1997; 29: 1118-1120
15. Makela S, Savolainen H, Aavik E et al. Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to estrogen receptors alpha and beta. Proc Natl Acad Sci USA 1999; 96: 7077-7082
16. Savolainen H, Frosen J, Petrov L, Aavik E, Hayry P. Expression of estrogen receptor sub-types alpha and beta in acute and chronic cardiac allograft vasculopathy. J Heart Lung Transplant 2001; 20: 1252-1264
17. Aavik E, du Toit D, Myburgh E, Frosen J, Hayry P. Estrogen receptor beta dominates in baboon carotid after endothelial denudation injury. Mol Cell Endocrinol 2001; 182: 91-98
18. Mennander A, Raisanen A, Paavonen T, Hayry P. Chronic rejection in the rat aortic allograft: mechanism of the angiopeptin (BIM23014C) effect on the generation of allograft arteriosclerosis. Transplantation 1993; 55: 124-128
19. Aavik E, Luoto NM, Petrov L, Aavik S, Patel YC, Hayry P. Elimination of vascular fibrointimal hyperplasia by somatostatin receptor 1,4-selective agonist FASEB J 2002; 16: 724-726