Correlation of Vascular Reaction in Endomyocardial Biopsies with Micro- and Macrovascular Graft Vessel Disease after Heart Transplantation

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Correlation of Vascular Reaction in Endomyocardial Biopsies with Micro- and Macrovascular Graft Vessel Disease after Heart Transplantation

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Graft Vessel Disease (GVD) is the predominant long-term complication after heart transplantation. The study tests the correlation of vascular reaction with micro- and macrovascular evidence of GVD in cardiac transplants. The authors studied 41 heart transplant patients (9 women, 32 men, mean survival 58 months), 15 with and 26 without any angiographic signs of GVD. Paraffin-embedded right ventricular endomyocardial biopsies (n = 272) were graded for vascular reaction (H&E staining), that is, endothelial cell swelling and vascular wall thickening (both grade 0-2). Immunohistochemical investigations for vascular smooth muscle cells (SMCs) (α actin, clone 1A4) were performed and evaluated by light microscopy. Patients with angiographic evidence of GVD had more microvascular endothelial cell swelling than patients without macrovascular GVD ($P < 0.05$). There was a positive correlation of α-actin-positive microvessels with early posttransplant endothelial cell swelling ($P < 0.05$). In contrast, there was a positive correlation of α-actin-positive microvessels early after heart transplantation, with endomyocardial vessel wall thickening in the later postoperative period ($P < 0.05$). Development of GVD in large and small coronary arteries may coincide. Proliferation of medial SMCs plays the dominant role in the pathogenesis of small vessel disease. The serial evaluation of microvascular status provides early evidence of pathologic alterations associated with GVD.

Introduction

Graft vessel disease (GVD) is the predominant, lethal long-term complication after heart transplantation.[1-7] It is characterized by diffuse stenosis of large and small blood vessels, due to an intimal thickening and a proliferation of smooth muscle cells (SMCs).[5,7-20] Many immunological[3,21-23] and nonimmunological pathogenetic factors[19,24-27] may be involved. The roles of acute myocardial rejection episodes and of the severity of vascular reaction are unclear as of yet.[4,28-32]

It is well accepted that endothelial cells (ECs) and vascular smooth muscle cells play a central role in the pathogenesis of this disease.[1,7-16,32-36] The aim of the study was to investigate whether severity of vascular reaction in endomyocardial biopsies is associated with vasculopathy of large coronary arteries and may predict small vessel disease after heart transplantation.

Materials and Methods

We studied 41 heart transplant patients (9 women and 32 men) who underwent transplantation because of dilated cardiomyopathy (n = 26), coronary artery disease (n = 14), and Eisenmenger's syndrome (n = 1). According to their angiographic results, patients were divided into 2 groups: 15 patients with angiographic signs of GVD (4 women,

Nicola E. Hiemann, MD
Deutsches Herzzentrum Berlin
Deptartment of Cardiothoracic and Vascular Surgery
Augustenburger Platz 1
13353 Berlin, Germany
Tel: +49.304.5930
Fax: +49.304.593.2040
email: hiemann@dhzb.de

Table 1 | **Demographical Data of Patients with and without Macrovascular GVD**

Parameter	GVD Patients (Mean ± SD)	Controls (Mean ± SD)	*P* Value
Age at HTx/years	50 ± 4.9	47 ± 7.6	ns
Mean survival time/months	67 ± 22.4	60 ± 27.2	ns
Age at exitus letalis/years	55 ± 5.2	52 ± 7.9	ns
Ischemic time/min	159 ± 23.3	150 ± 66.0	ns
Reperfusion time/min	56 ± 20.6	51 ± 18.9	ns
Donor age/years	36 ± 11.7	37 ± 11.3	ns

SD = standard deviation, ns = not significant.

Table 2 | **Classification of Vascular Reaction, Cardiovascular Pathology Study Group, Deutsches Herzzentrum Berlin**

Grade	Definition
0/0	Endothelial cells inconspicuous, no vessel wall thickening, no proliferation
0/1	Endothelial cells inconspicuous, mild vessel wall thickening by proliferation
0/2	Endothelial cells inconspicuous, intensive vessel wall thickening by proliferation
1/0	Endothelial cells prominent (not swollen), vessel wall inconspicuous
2/0	Intensive endothelial cell swelling, vessel wall inconspicuous
2/1	Intensive endothelial cell swelling, mild vessel wall thickening by proliferation
1/1	Mild endothelial cell swelling, mild vessel wall thickening by proliferation
1/2	Mild endothelial cell swelling, intensive vessel wall thickening by proliferation
2/2	Intensive endothelial cell swelling, intensive vessel wall thickening by proliferation

ETIOPATHOGENESIS:

The doctrine of the causes and development of diseases.

11 men, mean survival 57 months) and 26 patients without (5 women, 21 men, mean survival 59 months). All patients were treated with cyclosporine 2–4 mg/kg, prednisolone 0.1–0.5 mg/kg, and azathioprine 1–5 mg/kg. For further demographical data, see Table 1.

Two hundred seventy-two consecutive serial right ventricular endomyocardial biopsies, harvested during the first 14 months after heart transplantation, were subjected to retrospective investigations. All biopsies were classified for acute myocardial rejection according to the Working Formulation of the International Society for Heart and Lung Transplantation.[37] The evaluation of vascular reaction was done by light microscopy at × 200, according to the classification shown in Table 2.

The immunohistochemical preparation of the tissue sections was based on the avidin-streptavidin method.[38] We used as the immunohistochemical marker for SMCs α-actin (clone 1 A4, Dako Industries®).[39] Histomorphometric analysis of immunohistochemical sections was done by light microscopy at × 500, counting antibody-positive blood vessels in a test field of 100 compartments.

All data were analyzed using nonparametric tests; a *P* value of < 0.05 was considered to be significant.

Results

There were no significant differences between the 2 groups concerning their demographical data (Table 1). According to the results of acute myocardial rejection, there was a trend toward a higher number of mild acute rejection episodes in GVD patients.

There was a positive immunohistochemical reaction in all studied endomyocardial biopsies, and all biopsy specimens were subjected to histological, immunohistochemical, and histomorphometric evaluations. In all observed intervals, patients with angiographic evidence of GVD had significantly more α-actin-positive microvessels than patients without macrovascular disease. Microvascular endothelial cell swelling was more pronounced in GVD patients in the 3rd, 5th, and 11th months after heart transplantation than in patients without evidence of macrovascular GVD (Fig 1). Microvascular vessel wall thickening was more pronounced in GVD patients in the 5th month after

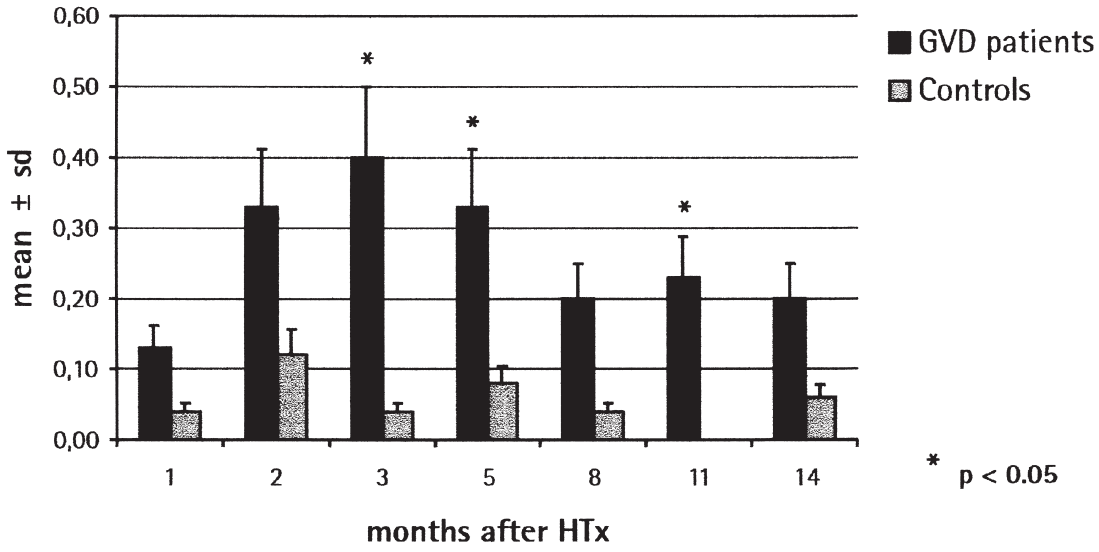

Figure 1. Endothelial cell swelling in patients with and without macrovascular graft vessel disease (GVD).

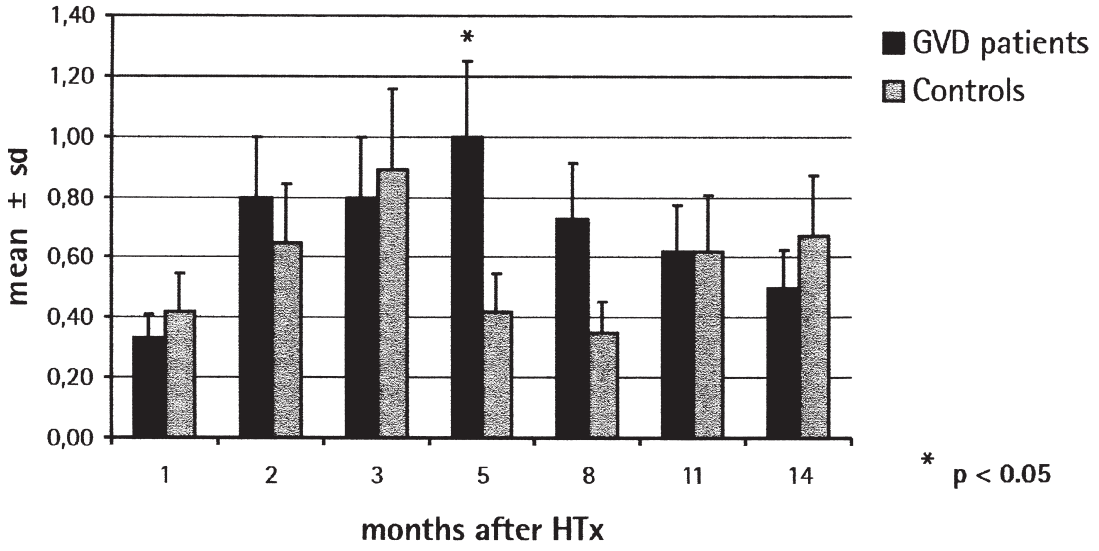

Figure 2. Vessel wall thickening in patients with and without macrovascular graft vessel disease (GVD).

heart transplantation than in patients without GVD of the large coronary arteries (Fig 2). There was a positive correlation of α-actin-positive microvessels in the later period after heart transplantation, with early posttransplant endothelial cell swelling. In contrast, there was a positive correlation of α-actin-positive microvessels early after heart transplantation, with endomyocardial vessel wall thickening in the late postoperative period (see Table 3).

Table 3 | **SPEARMAN'S CORRELATION—VASCULAR REACTION AND α-ACTIN-POSITIVE MICROVESSELS**

Endothelial Cell Swelling				
	Months after HTx	**1**	**2**	**11**
α-actin-positive microvessels	8	ns	ns	0.333*
	14	0.378*	0.356*	ns
Vessel Wall Thickening				
	Months after HTx	**5**		
α-actin-positive microvessels	1	0.317*		
	5	0.329*		
	8	0.368*		
Endothelial Cell Swelling				
	Months after HTx	**1**	**2**	**8**
Vessel wall thickening	1	0.363*	ns	ns
	2	0.395*	0.533**	ns
	8	ns	ns	0.402**

ns = not significant, $*P < 0.05$, $**P < 0.01$.

VASCULAR REACTION:

The morphological appearance of the small intramyocardial blood vessels, that is, the morphology of the endomyocardial microvascular endothelial cells (i.e. not swollen, prominent or swollen), and the morphology of the medial vessel wall (i.e. absence of thickening, mild thickening by proliferation or strong thickening by proliferation).

Discussion

GVD after heart transplantation affects large and small coronary arteries,[1,8-16] based on morphological intimal and medial alterations.[6-20] The dominant feature of these changes is a proliferation of SMCs and a transformation of capillaries to blood vessels that display morphological characteristics of arterioles.[8,9,11,15]

According to our results, there were no significant differences between the 2 groups with regard to their demographic data. This situation has also been described by others,[1,13,40-42] whereas some investigations revealed an increased risk for the development of GVD in association with older donor age,[43,44] increased ischemic time, and increased reperfusion time.[26,27] In view of the association between the development of GVD with the number, duration, and grade of acute rejection episodes, the literature presents conflicting results,[4,28-30] which may result from different immunosuppressive regimens.[45]

The differentiation between cell-mediated responses to allograft parenchyma and those to allograft vasculature has been suggested by Hosenpud and others[31] and may explain progressive vascular disease, despite the absence of acute cellular rejection. The importance of diagnosis and grading of vascular rejection has been published by Hammond and others.[32] In this article, endothelial cell swelling and/or vasculitis on light microscopy provided clinical impact and prognostic value on outcome of cardiac recipients. We complemented the classification of morphological features of the terminal vascular system with evaluation of the vessel wall, preferring the term vascular reaction rather than rejection, because what kind of etiology these morphological changes have, and at what time they should be considered disease, is still unknown.

In our own, as in other studies,[1,8-16,36] GVD occurred in large extramural and small intramural blood vessels, whereas some published data failed to find development of GVD within the terminal vascular system.[14,46]

It is well accepted that ECs play a central role in the pathogenesis of GVD after heart transplantation,[7,32-35] by secretion of several mediators that regulate vascular tone[30] and influence proliferation[23,33,47] or phenotypic appearance[48] of SMCs. They also act as antigen-presenting cells[32] and may sustain ongoing immune stimulation by the graft.[47] According to our results, endothelial cell swelling preceded evidence of SMC proliferation as indicated by immunohistochemical investigations and may reflect endothelial activation and/or endothelial damage.

In summary, these data underline the important role of EC-SMC interaction in the development of GVD after heart transplantation, and we suggest that serial investigations of endomyocardial biopsies of cardiac transplant recipients provide impor-

tant information with impact for the diagnosis of GVD in small coronary arteries.

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References

1. Billingham ME. Graft coronary disease: the lesions and the patients. **Transplant Proc 1989;21:3665-6**.
2. Gallo P, Baroldi G, Thiene L, Agozzino L, Arbustini E, Bartoloni G, et al. When and why do heart transplant recipients die? A 7 year experience of 1068 cardiac transplants. **Virchows Archiv A Pathol Anat 1993;422:453-8**.
3. Weis M, von Scheidt W. Cardiac allograft vasculopathy—a review. **Circulation 1997;96:2069-77**.
4. Winters GL, Kendall TJ, Radio SJ, Wilson JE, Costanzo-Nordin MR, Switzer BL, et al. Posttransplant obesity and hyperlipidemia: major predictors of severity of coronary arteriopathy in failed human heart allografts. **J Heart Lung Transplant 1990;9:364-71**.
5. Pethig K, Dengler TJ, Mohacsi P, Deng MC, Wahlers T. Langzeitbetreuung nach Herztransplantation—Eine Bestandsaufnahme. **Z Kardiol 2000;89:93-9**.
6. Pethig K, Besser K, Heublein B, Wahlers T, Harringer W, Haverich A. Koronare Vaskulopathie nach Herztransplantation—Einfluss von zeitlichem Auftreten, Schweregrad und Progredienz im Langzeitverlauf. **Z Kardiol 1999;88:498-506**.
7. Mancini MC, Evans JT. Role of plateled-derived growth factor in allograft vasculopathy. **Ann Surg 2000;231:682-8**.
8. Hiemann NE, Musci M, Wellnhofer E, Meyer R, Hetzer R. Light microscopic biopsy findings after heart transplantation and possible links to the development of graft vessel disease. **Transplant Proc 1999;31:149-51**.
9. Hiemann N, Musci M, Wellnhofer E, Meyer R, Hetzer R. Histomorphometrische Untersuchungen von intramyokardialen Blutgefäßen in rechtsventrikulären Biopsien nach Herztransplantation. **Z Herz Thorax Gefäßchirurgie 1998;12:111-16**.
10. Arbustini E, Roberts WC. Morphologic observations in the epicardial coronary arteries and their surroundings late after cardiac transplantation (allograft vascular disease). **Am J Cardiol 1996;78:814-20**.
11. Aziz S, McDonald TO, Gohra H. Transplant arterial vasculopathy: evidence for a dual pattern of endothelial injury and the source of smooth muscle cells in lesions of intimal hyperplasia. **J Heart Lung Transplant 1995;14:S123-36**.
12. Billingham ME. Does intracoronary ultrasound conflict with pathology? Comments from a panel discussion. **J Heart Lung Transplant 1995;14:S211-4.**
13. Clausell N, Butany J, Molossi S, Lonn E, Gladstone P, Rabinovitch M, et al. Abnormalities in intramyocardial arteries detected in cardiac transplant biopsy specimens and lack of correlation with abnormal intracoronary ultrasound or endothelial dysfunction in large epicardial coronary arteries. **J Am Coll Cardiol 1995;26:100-9**.
14. Gao SZ, Alderman EL, Schroeder JS, Silverman JF, Hunt SA. Accelerated coronary vascular disease in the heart transplant patient coronary angiographic findings. **J Am Coll Cardiol 1988;12:334-40**.
15. Tanaka H, Swanson SJ, Sukhova G, Schoen FJ, Libby P. Early proliferation of medial smooth muscle cells in coronary arteries of rabbit cardiac allografts during immunosuppression with cyclosporine A. **Transplant Proc 1995;27:2062-5**.
16. Valantine H, Pinto FJ, St Goar FG, Alderman EL, Popp RL. Intracoronary ultrasound imaging in heart transplant recipients: the Stanford experience. **J Heart Lung Transplant 1992;11:S60-4**.
17. Hosenpud JD, Shipley GD, Wagner CR. Cardiac allograft vasculopathy: current concepts, recent developments, and future directions. **J Heart Lung Transplant 1992;11:9-23**.
18. Liu G, Butany J. Morphology of graft arteriosclerosis in cardiac transplant recipients. **Hum Pathol 1992;23:768-73**.
19. Faulk WP, Labarrere CA, Nelson DR, Pitts D. Hemostasis, fibrinolysis, and natural anticoagulation in transplant vascular sclerosis. **J Heart Lung Transplant 1995;14:S158-64**.
20. Petig K, Klauss V, Heublein B, Mudra H, Westphal A, Weber C. Progression of cardiac allograft vascular disease as assessed by serial intravascular ultrasound: correlation to immunological and non-immunological risk factors. **Heart 2000;84:494-8**.
21. Hruban RH, Beschorner WE, Baumgartner WA, Augustine SM, Ren H, Reitz BA, et al. Accelerated arteriosclerosis in heart transplant recipients is associated with a T-lymphocyte-mediated endothelialitis. **Am J Pathol 1990;137:871-82**.
22. Vassalli G, Gallino A. Endothelial dysfunction and accelerated coronary artery disease in cardiac transplant recipients. **Eur Heart J 1997;18:1712-7**.
23. Libby P, Tanaka H. The pathogenesis of coronary arteriosclerosis ("chronic rejection") in transplanted hearts. **Clin Transplantation 1994;8:313-8**.
24. Gibbons GH. The pathogenesis of graft vascular disease: implications of vascular remodeling. **J Heart Lung Transplant 1995;14:S149-58**.
25. Dong C, Wilson JE, Winters GL, McManus BM. Human transplant coronary artery disease: pathological evidence for fas-mediated apoptotic cytotoxicity in allograft arteriopathy. **Lab Invest 1996;74:921-31**.
26. Ardehali A, Laks H, Drinkwater DC, Kato NS, Permut LC, Grant PW, et al. Expression of major histocompatibility antigens and vascular adhesion molecules on human cardiac allografts preserved in University of Wisconsin solution. **J Heart Lung Transplant 1993;12:1044-52**.
27. Day JD, Rayburn BK, Gaudin PB, Baldwin WM, Lowenstein CJ, Kasper EK, et al. Cardiac allograft vasculopathy: the central pathogenetic role of ischemia-induced endothelial cell injury. **J Heart Lung Transplant 1995;14:S142-9**.
28. Radovancevic B, Poindexter S, Birovljev S, Velebit V, McAllister HA, Duncan JM, et al. Risk factors for development of accelerated coronary artery disease in cardiac transplant recipients. **Eur J Cardiothorac Surg 1990;4:309-12**.
29. Uretsky BF, Murali S, Reddy PS, Rabin B, Lee A, Griffith BP, et al. Development of coronary artery disease in cardiac transplant patients receiving immunosuppressive therapy with cyclosporine and prednisone. **Circulation 1987;76:827-34**.
30. Kobashiwaga JA, Miller L, Yeung A, Hauptman P, Ventura H, Wilensky R, et al. Does acute rejection correlate with the development of transplant coronary artery disease? A multicenter study using intravascular ultrasound. **J Heart Lung Transplant 1995;14:S221-6**.
31. Hosenpud JD, Shipley GD, Mauck KA, Morris TE, Wagner CR. Temporal reduction in acute rejection after heart transplantation is not associated with a reduction in cell-mediated responses to donor-specific vascular endothelium. **J Heart Lung Transplant 1995;14:926-37**.
32. Hammond EH, Yowell RL, Nunoda S, Menlove RL, Renlund DG, Bristow MR, et al. Vascular (humoral) rejection in heart transplantation: pathologic observations and clinical implications. **J Heart Lung Transplant 1989;8:430-43**.
33. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. **Nature 1993;362:801-9**.
34. Deng MC, Plenz G, Erren M, Wilhelm MJ, Moennig G, Rothenburger M, et al. Transplantatvaskulopathie: Ein Modell der koronaren Herzerkrankung? **Herz 2000;25:95-9**.
35. Tayara L, Giaid A. Endothelin and cardiac transplantation. **Z Kardiol 2000;89(Suppl):28-31**.
36. Shirasawa B, Hamano K, Ueda M, Ito H, Kobayashi T, Fujimura Y, et al. Contribution of proliferating leukocytes to phenotypic change in smooth muscle cells during the development of coronary arteriosclerosis in transplanted hearts. **Eur Surg Res 2000;32:30-8**.
37. Billingham ME, Cary NRB, Hammond ME, Kemnitz J, Marboe C, McCallister HA, et al. A working formulation for the standardization of nomenclature

in the diagnosis of heart and lung rejection: Heart Rejection Study Group. **J Heart Lung Transplant 1990;9:587-93**.

38. Naish SJ. Handbuch immunhistochemischer Färbemethoden. **DAKO Corporation, Via Real, CA; 1989**.
39. Shalli O, Ropraz P, Trzeciale A, Benzonana G, Gillessen D, Gabbiani G. A monoclonal antibody against a-smooth muscle actin: a new probe for smooth muscle differentiation. **J Cell Biol 1986;103:2787-96**.
40. Mangiavacchi M, Frigerio M, Gronda E, Danzi GB, Bonacina E, Masciocco G, et al. Acute rejection and cytomegalovirus infection: correlation with cardiac allograft vasculopathy. **Transplant Proc 1995;27:1960-2**.
41. Russell ME, Fujita M, Masek MA, Rowan RA, Billingham ME. Cardiac graft vascular disease—nonselective involvement of large and small vessels. **Transplantation 1993;56:1599-601**.
42. Billingham ME. Cardiac transplant atherosclerosis**. Transplant Proc 1987;19:S19-25**.
43. Gao SZ, Hunt SA, Alderman EL, Liang D, Yeung AC, Schroeder JS. Relation of donor age and preexisting coronary artery disease on angiography and intracoronary ultrasound to later development of accelerated allograft coronary artery disease. **J Am Coll Cardiol 1997;29:623-9**.
44. Costanzo MR, Naftel DC, Pritzker MR, Heilman JK, Boehmer JP, Brozena SC, et al. Heart transplant coronary artery disease detected by angiography: a multi-institutional study. **J Heart Lung Transplant 1996;15:S39**.
45. Hauptmann PJ, Nakagawa T, Tanaka H, Libby P. Acute rejection: culprit or coincidence in the pathogenesis of cardiac graft vascular disease? **J Heart Lung Transplant 1995;14:S173-80**.
46. Demetris AJ, Zerbe T, Banner B. Morphology of solid organ allograft arteriopathy: identification of proliferating intimal cell populations. **Transplant Proc 1989;21:3667-9**.
47. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. **Blood 1998;91:3527-61**.
48. Molossi S, Clausell N, Rabinovitch M. Coronary artery endothelial interleukin-1b mediates enhanced fibronectin production related to post-cardiac transplant arteriopathy in piglets. **Circulation 1993;88:II248-56**.