EXPERIMENTAL STUDIES

Crosstalk of Endothelin-1 and Platelet-Derived Growth Factor in Cardiac Allograft Arteriosclerosis

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OBJECTIVES
In this study, we investigated the crosstalk of endothelin-1 (ET-1) and platelet-derived growth factor (PDGF) in coronary artery smooth muscle cell (SMC) proliferation in the rat cardiac allograft model.

BACKGROUND
Previous studies have suggested an independent role of ET-1 and PDGF in the development of cardiac allograft arteriosclerosis (i.e., chronic rejection).

METHODS
Heterotopic heart transplantations were performed from Dark Agouti to Wistar Furth rats. Grafts were harvested after five days in an acute rejection model and after 60 days in a chronic rejection model. In the in vitro part of the study, SMC proliferation and migration were quantitated, as well as messenger ribonucleic acid (mRNA) levels of ET-1 and PDGF ligands and receptors after growth factor stimulation.

RESULTS
Acute rejection induced both ET-1 receptors in the arterial wall. On linear regression analysis of chronically rejecting cardiac allografts, a strong correlation between intimal thickening and immunoreactivity of ET-1 and ET receptors A and B (ETA and ETB) in the arterial walls was observed. Treatment with Bosentan, a mixed ET-1 receptor antagonist, significantly reduced the incidence and intensity of arteriosclerotic lesions in rat cardiac allografts, as well as total intragraft ETA and ETB mRNA expression and intimal cell ET-1 and receptor immunoreactivity. This was associated with significantly reduced intragraft PDGF beta-receptor (PDGF-Rbeta) mRNA expression. In contrast, CGP 53716, a protein tyrosine kinase inhibitor selective for the PDGF receptor, did not reduce intragraft ET-1, ETA or ETB mRNA expression. In rat coronary artery SMC cultures, ET-1 stimulation significantly upregulated PDGF-Ralpha and -Rbeta mRNA expression and augmented PDGF-BB–mediated SMC proliferation as well as PDGF-AB– and PDGF-BB–mediated SMC migration.

CONCLUSIONS
Our results suggest that the ET-1/PDGF-Rbeta/PDGF-BB axis may operate in SMC migration and proliferation in cardiac allograft arteriosclerosis, thus explaining the marked beneficial effects of blocking the signaling downstream of ET-1 receptors. (J Am Coll Cardiol 2002;39:710–7) © 2002 by the American College of Cardiology

Active endothelin-1 (ET-1) is a 21-amino acid peptide proteolytically generated from its precursor preproET (1). Endothelin-1 is the most potent known vasoconstrictor released from endothelial cells (ECs) to act on the underlying vascular smooth muscle cells (SMCs) (2,3). The effects of ET-1 are mediated through two G-protein–coupled receptors—the ET receptors A and B (ETA and ETB). Ligand binding results in activation of intracellular phospholipase C and release of calcium from sarcoplasmic reticulum, leading to its biologic responses (1). Both ETA and ETB subtypes are expressed by vascular SMCs and mediate vasoconstriction (4). In ECs, ETB is the predominating subtype and mediates vasodilation through release of vasoactive factors such as nitric oxide (5).

In addition to its vasoactive functions, ET-1 acts as a growth factor for SMCs (6). The proinflammatory cytokines interleukin-1-beta (IL-1-beta) and tumor necrosis factor-alpha (TNF-alpha) upregulate ET-1 mRNA levels in ECs (7). In vascular SMCs, platelet-derived growth factor-AA (PDGF-AA) induces production of ET-1 at protein and mRNA levels (8,9). Through these vasoactive and mitogenic effects, activation of ET-1 expression is connected to many vascular disorders (10,11).

PDGF-BB is the most potent mitogen for SMCs in vitro and is expressed in macrophages of intimal lesions of ordinary atherosclerosis (12,13). PDGF-AA is a weak mitogen for SMC proliferation in vitro, but our previous results suggest that it plays a major role in the development of intimal lesions in a macrophage-derived cytokine microenvironment in cardiac allograft arteriosclerosis (14,15). Recent studies indicate that ET-1 may also regulate SMC proliferation in arteriosclerosis, but the role of ET-1 as a direct SMC mitogen remains controversial (6,16). In this study, we investigated the interaction between ET-1 and PDGF in rat coronary artery SMC cultures in vitro and in the cardiac allograft model in vivo.
Abbreviations and Acronyms

CsA = cyclosporin A
DMEM = Dulbecco’s Modified Eagle Medium
EC = endothelial cell
ET = endothelin
FBS = fetal bovine serum
IL = interleukin
mRNA = messenger ribonucleic acid
PDGF = platelet-derived growth factor
RT-PCR = reverse transcription polymerase chain reaction
SMC = smooth muscle cell
TNF = tumor necrosis factor

METHODS

Heterotopic heart transplantation. Inbred Dark Agouti (AG-B4, RT1a) and Wistar Furth (AG-B2, RT1u) rat strains (weighing 200 to 300 g) (Laboratory Animal Center, University of Helsinki, Helsinki, Finland) were used for heterotopic heart transplantsations, as described (17). In the acute rejection model, the recipients were not immunosuppressed, and the grafts were removed five days after transplantation. In the chronic rejection model, the allografts were treated with Bosentan, 100 mg/kg body weight per day, or vehicle by orogastric tube and cyclosporin A (CsA), 2 mg/kg per day subcutaneously for the first week and 1 mg/kg per day thereafter, and the grafts were removed 60 days after transplantation. To analyze the correlation between the development of intimal thickening and ET-1 ligand and receptor expression, the recipients were given CsA at a dose of either 1.0 mg/kg per day, 1.5 mg/kg per day or 2 mg/kg per day for two weeks, followed by one week of CsA at 1 mg/kg per day. We have previously demonstrated an inverse correlation between CsA levels and intimal thickening (18).

To investigate the effects of PDGF inhibition on ET-1 ligand and receptors in the chronic rejection model, the rats received CGP 53716, 50 mg/kg per day intraperitoneally by a single injection starting 24 h before transplantation. As CGP 53716 interacts with CsA metabolism, CsA was administrated according to blood trough levels (14). The administration of CsA, 2 mg/kg for the first week, followed by 1.5 mg/kg, yielded blood trough levels in vehicle-treated rats that matched those in rats treated with CsA, 1 mg/kg per day, and CGP 53716 (14).

Drug regimens. Cyclosporin A (Sandimmune; Novartis, Basel, Switzerland) was dissolved in Intralipid, 200 mg/ml (KabiVitrum, Stockholm, Sweden) to a final concentration of 1 mg/ml. Whole blood CsA 24-h trough levels were determined once a week using radioimmunoassay (Sandimmune kit; Novartis). Bosentan (Ro 47-0203/029), an orally active nonpeptide ET antagonist (kindly provided by Dr. M. Clozel, Actelion Ltd., Allschwil, Switzerland) (19), was suspended in 5% gummi arabicum to a concentration of 25 mg/ml. CGP 53716, a protein tyrosine kinase inhibitor selective for the PDGF receptor (Novartis), was dissolved in dimethyl sulfoxide to a concentration of 200 mg/kg, diluted 1:20 with 1% Tween in 0.9% NaCl, and sonicated.

Histologic findings. The grafts embedded in paraffin were examined histologically after sectioning and staining with hematoxylin-eosin and resorcin-fuchsin (internal elastic lamina). The changes in intimal thickness of each vessel were scored from 0 to 4, as previously described (14). As a final score for intimal thickening, the mean score of epicardial arteries and intramyocardial arterioles per cross section was given.

Immunostaining. Immunohistochemical analyses were performed using the ABC method (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, California). The following polyclonal antibodies were applied: rabbit anti–ET-1 (porcine/human) serum (dilution of 1:50; Peninsula Laboratories Inc., San Carlos, California), sheep anti-rat ETA and sheep anti-rat ETB (dilution of 16 µg/ml; U.S. Biological, Swampscott, Massachusetts). Immunoreactivity was scored from 0 to 3, as previously described (14). Specificity control stainings included omission of primary antibody and staining with the same immunoglobulin concentration of species- and isotype-matched antibodies. None of these control stainings showed any immunoreactivity.

Cell cultures. A rat coronary artery SMC line (kindly provided by Dr. D. Leszczynski, Finnish Center for Radiation and Nuclear Safety, Helsinki, Finland), was cultured in 10% fetal bovine serum (FBS) Dulbecco’s Modified Eagle Medium (DMEM), supplemented with 10 mmol/l HEPES, 100 IU/ml penicillin, 100 µg/ml streptomycin and 1% glutamine (GIBCO BRL, Rockville, Maryland). For proliferation analysis, the cells were trypsinized and seeded on microwells in culture medium containing 10% FBS at a concentration of 25,000 cells/ml and allowed to adhere for 24 h. After starvation in serum-free medium for 72 h, quiescent cells were either incubated with 20 ng/ml of PDGF-AA, -AB or -BB (Upstate Biotechnology Inc., Waltham, Massachusetts) for 24 h or pre-incubated with 10 ng/ml of ET-1 (Peninsula Laboratories Inc.) in 0.5% FBS DMEM, supplemented with 0.1% bovine serum albumin for 24 h and then with PDGF ligands for another 24 h. 3H-TdR incorporation (1 µCi/ml) was measured during the last 24 h of incubation. For migration analysis, Transwell culture chambers (Costar, Acton, Massachusetts) were coated with collagen (Upstate Biotechnology Inc.), and 50,000 cells were seeded in the upper chamber. After 2 h, ET-1, PDGF-AA, PDGF-AB and PDGF-BB were added to the lower chamber and incubated for 24 h. Filters were fixed in methanol, stained with Mayer’s hemalum and removed. Migrated cells on the lower side of the filter were quantitated by counting cross-sectional fields with a light microscope using ×400 magnification. For mRNA analysis, the cells were starved for 72 h in 0.5% FBS DMEM, supplemented with 0.1% BSA. Quiescent cells were challenged with 20 ng/ml of ET-1 in a serum-free medium,
washed with phosphate-buffered saline (PBS) and harvested in buffer at 4 h.

Reverse transcription polymerase chain reaction (RT-PCR). Relative preproET-1, ET_A, ET_B and PDGF-A, PDGF-B, PDGF-alpha and PDGF-beta mRNA transcript levels were measured with RT-PCR after normalization against levels of the reference gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Total RNA was extracted from fresh heart tissue samples and cell culture samples, as described (20,21). After deoxyribonucleic acid treatment, the total RNA yields were estimated by spectrophotometry and confirmed by 1% agarose/Tris-borate-EDTA gel electrophoresis. Reverse transcription PCR was performed as described (22). The sense and antisense primers for GAPDH (accession M17701) were: 5'-GTCCTCACCAACCATGAGAAAGCCT-3' and 5'-TGTTAGCCCCAGATGCCCTTTAGTG-3' for preproET-1 (M64711); 5'-TGCTCTCGTCTCCTCTCTTGGATGAT-3' and 5'-CACCACGGGCGCTCTGTAGTC-3' for ET-RA (M60786): 5'-GAGTCTGTCCTCTGTGGCATCA-3' and 5'-CTGTGCTCTCGCCCTTGT-3'; for ET-B (Z14117): 5'-CTGAGCTGACTTTGAAGATGA-3' and 5'-GAGAAGATTGTGCCGCTGAGT-3'; for PDGF-alpha (Z14118): 5'-GAGAAGATTGTGCCGCTGAGT-3' and 5'-CACACTGAGAAGGACATGAGAT-3'; for PDGF-B (Z14119): 5'-CTGAGCTGACTTTGAGATGA-3' and 5'-CACCACGGCGCTCTGTAGTC-3'; and for PDGF-beta (Z14121): 5'-TCACCCTCACCCTCCTCGAGG-3'. Gene transcript levels for a single gene were amplified simultaneously, and each PCR analysis was completed in triplicate. For each experiment, negative control studies were performed using water instead of complementary deoxyribonucleic acid (cDNA) or omitting reverse transcription during cDNA synthesis. None of these control studies showed positive signals. The mean values of three determinations were used for the final analysis, and the normalized gene mRNA transcript levels were derived by dividing the mean value of the gene mRNA by the mean value of GAPDH for each sample. The results are given as optical density values, with the control group given the numeric value of 1.

Statistical analyses. All data are given as the mean value ± SEM. The Mann-Whitney U test, z-corrected for ties (Statview program, version 4.1; Abacus Concepts, Berkeley, California), was used to evaluate the differences between two groups. For multiple comparisons, the Kruskal-Wallis and Dunn tests were applied (Medstat, Astra Group A/S). In addition, linear regression analysis was applied to evaluate the possible relationship of growth factor ligand and receptor expression to intimal thickening. A p value <0.05 was regarded as statistically significant.

RESULTS

Bosentan reduces arteriosclerotic lesion formation in cardiac allografts. The mean numbers of arteries and arterioles in the cardiac cross sections analyzed were 45 ± 5 in allografts of vehicle-treated rats and 53 ± 7 in allografts of bosentan-treated rats. In cardiac allografts of vehicle-treated rats, 90 ± 4% of vessels were affected by intimal thickening, and the mean grade of intimal thickening was 1.8 ± 0.3. Treatment with bosentan significantly reduced the amount of affected vessels to 53 ± 7% (p < 0.01) and the mean grade of intimal thickening to 0.8 ± 0.2 (p < 0.01) (Fig. 1).

Alloimmune response induces ET-1 receptor activation in the arterial wall, which may be inhibited by bosentan.

PROTEIN EXPRESSION. In nontransplanted DA rat hearts, mild to moderate ET-1 expression was localized to media cells of arteries and cardiomyocytes. In syngeneic grafts, ET-1 expression did not differ from that of nontransplanted hearts. Acute rejection induced mild ET-1 immunoreactivity in the interstitial mononuclear inflammatory cells (Table 1). In syngeneic grafts 60 days after transplantation, ET-1 expression was nonexistent in the media cells of arteries and moderate in cardiomyocytes. Expression of ET-1 was increased in the intima and media cells of arteries in chronically rejecting allografts, compared with syngeneic grafts. Treatment with bosentan significantly reduced ET-1 expression in the intima and media cells, compared with vehicle treatment (Table 2).

Mild to moderate ET_A immunoreactivity was recorded in the media cells and cardiomyocytes of normal hearts. In syngeneic grafts, moderate ET_A expression was detectable in cardiomyocytes. Expression of ET_A was significantly upregulated in the media cells of arteries and in the interstitial mononuclear inflammatory cells in the acute rejection model, compared with syngeneic grafts (Table 1). In syngeneic grafts 60 days after transplantation, ET_A expression was detectable in the media cells and cardiomyocytes. In chronically rejecting allografts, ET_A immunoreactivity was significantly increased in the intima and media cells of the arterial wall, compared with syngeneic grafts. In bosentan-treated allografts, there was a reduction in ET_A immunoreactivity in intimal cells, compared with vehicle-treated allografts (Table 2).

In nontransplanted DA rat hearts, mild to moderate ET_B expression was localized to endothelial and media cells and cardiomyocytes. In syngeneic grafts, ET_B expression was undetectable in ECs but was otherwise similar to that seen in nontransplanted DA rat hearts. During acute rejection, ET_B immunoreactivity was upregulated in the media cells of allograft arteries, compared with syngeneic graft arteries (Table 1). In syngeneic grafts 60 days, ET_B immunoreactivity was not detectable in ECs and was mild in the media cells of arteries. In chronically rejecting allografts, the expression of ET_B was increased in the intima and media cells of arteries. In bosentan-treated allografts, a clear
reduction in ET_B expression was recorded, compared with vehicle-treated allografts (Table 2).

EXPRESSION OF mRNA IN THE ACUTE REJECTION MODEL. In syngeneic and allogeneic grafts, there was a 2.9 ± 0.4- and 3.6 ± 0.1-fold increase, respectively, in optical density in total intragraft preproET-1 mRNA expression, compared with normal hearts (p < 0.01). At the receptor level, no difference in total intragraft mRNA expression was observed between the groups.

EXPRESSION OF mRNA IN THE CHRONIC REJECTION MODEL. Sixty days after transplantation, no significant difference in preproET-1 mRNA expression was observed between syngeneic and allogeneic hearts. However, expression of both ET_A and ET_B mRNA was significantly increased (1.8 ± 0.1- and 1.9 ± 0.2-fold increase in optical density, respectively) in chronically rejecting allografts as compared with syngeneic grafts (p < 0.05). Bosentan treatment reduced total intragraft ET_A mRNA expression by 39 ± 5% (p < 0.01), compared with vehicle treatment.

Intimal cell ET-1 ligand and receptor immunoreactivity correlates with arteriosclerotic lesion formation. As shown in Figure 2, linear regression analysis revealed that ET-1 immunoreactivity in the intima cells correlated with the severity of chronic rejection. At the receptor level, the expression of ET_A in the intima cells and that of ET_B in the intima and media cells of arteries correlated with enhanced intimal thickening.

Endothelin-1 regulates SMC proliferation and migration downstream of PDGF-beta. There were 2.2 ± 0.2- and 1.6 ± 0.1-fold increases in optical density of PDGF-alpha and -beta mRNA expression, respectively, in cultured SMCs stimulated with ET-1, but the mRNA expression of PDGF-A and -B ligands remained unchanged (p < 0.05). On stimulation with ET-1, PDGF-AA, PDGF-AB and PDGF-BB alone, there were 1.5-, 2-, 3- and 4-fold increases, respectively, in SMC 3H-TdR incorporation (Fig. 3). When SMCs were prestimulated with ET-1 for 24 h, only PDGF-BB, but not PDGF-AA nor -AB, significantly (p < 0.001) upregulated 3H-TdR incorporation in SMCs (Fig. 3).

Endothelin-1 and PDGF-AA stimulation had no effect on SMC migration, whereas PDGF-AB and PDGF-BB stimulation induced a threefold increase in SMC migration. When SMCs were stimulated with ET-1, PDGF-AB and PDGF-BB significantly upregulated SMC migration (Fig. 3).

Figure 1. Effect of Bosentan (Ro 47-0203), an ET-1 receptor antagonist, on (A) incidence and (B) mean score of intimal thickness in epicardial arteries and intramyocardial arterioles of rat cardiac allografts. Allograft recipients were given Bosentan, 100 mg/kg per day (n = 9), or vehicle (n = 7), and, as background immunosuppression, CsA, 2 mg/kg per day for the first week and 1 mg/kg per day thereafter. The grafts were removed 60 days after transplantation. Data are given as the mean value ± SEM; data were analyzed by using the Mann-Whitney U test. Grade 0 = normal artery with intact internal elastic lamina; grade 1 = <10% occlusion of the lumen by arterial intimal thickening and proliferation, disruption of the internal elastic lamina and the presence of some foam or vacuolated ECs; grade 2 = >10% but <50% occlusion of the lumen; grade 3 = >50% but <100% occlusion of the lumen; and grade 4 = 100% vessel occlusion of the lumen. Photomicrographs of the coronary arteries of vehicle-treated (C) and Ro 47-0203–treated (D) rat cardiac allografts. Original magnification ×200. Hematoxylin-eosin and resorcin-fuchsin staining for internal elastic lamina.
Bosentan reduces intragraft PDGF-beta mRNA expression during chronic rejection. In chronically rejecting cardiac allografts, bosentan treatment decreased the mRNA expression of PDGF-beta by 31 ± 4% (p < 0.05), whereas the mRNA expression of PDGF-A, -B and -alpha remained unchanged 60 days after transplantation.

**Table 1.** Endothelin-1 Ligand and Receptor Protein Expression in Normal Hearts and Syngeneic and Allogeneic Grafts 5 Days After Transplantation

<table>
<thead>
<tr>
<th>Arterial endothelial cells</th>
<th>ET-1</th>
<th>ET_A</th>
<th>ET_B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal DA rat hearts</td>
<td>0.5 ± 0.3</td>
<td>0.5 ± 0.0</td>
<td>1.0 ± 0.0</td>
</tr>
<tr>
<td>DA → DA grafts</td>
<td>0.8 ± 0.5</td>
<td>0.9 ± 0.4</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>DA → WF grafts</td>
<td>0.8 ± 0.3</td>
<td>1.6 ± 0.3*</td>
<td>1.8 ± 0.3*</td>
</tr>
</tbody>
</table>

**Table 2.** Effect of Bosentan (Ro 47-0203) on Endothelin-2 Ligand and Receptor Protein Expression in Cardiac Allografts 60 Days After Transplantation

<table>
<thead>
<tr>
<th>Arterial endothelial cells</th>
<th>ET-1</th>
<th>ET_A</th>
<th>ET_B</th>
</tr>
</thead>
<tbody>
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<td>DA → DA grafts</td>
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<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>DA → WF grafts</td>
<td>0.8 ± 0.3</td>
<td>1.6 ± 0.3*</td>
<td>1.8 ± 0.3*</td>
</tr>
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**DISCUSSION**

The results of the present study demonstrate that during the alloimmune response, ET-1 receptor activation occurs in arterial SMCs. In acutely rejecting cardiac allografts, ET-1 has a significant induction of ET-1 in interstitial mononuclear inflammatory cells and ET_A and ET_B in the media cells of coronary arteries was observed, indicating possible activation and functional change of SMCs early after transplantation. In chronic rejection, a strong correlation between intimal thickening and immunoreactivity of ET-1, ET_A and ET_B in intimal cells was observed. Treatment with bosentan, a mixed ET-1 receptor antagonist, significantly reduced the incidence and intensity of arteriosclerotic lesions in rat cardiac allografts, decreased total intragraft ET_A and ET_B mRNA expression and intimal cell ET-1, ET_A and ET_B immunoreactivity and also reduced PDGF-beta mRNA expression. These and previous results demonstrate a possible functional role for ET-1 in the regulation of intimal thickening in cardiac allograft arteriosclerosis.
ET-1. When evaluating the results of the SMC proliferation and migration studies, the concept of the isoform-specific association of both PDGF receptor subunits is key: binding of PDGF to its receptor requires dimerization and is restricted to a ligand-specific association of the ligand subunits (27). For example, PDGF-AA binds only to the alpha-alpha receptor homodimer, AB to alpha-alpha and alpha-beta receptors and BB to all possible receptor dimers (alpha-alpha, alpha-beta and beta-beta). However, PDGF-AB is able to induce beta-beta receptor homodimers (28) and does not require alpha receptors for functional binding (29).

Blocking of the ET-1 pathway with Bosentan decreases PDGF-beta mRNA expression in cardiac allografts. To test the hypothesis that ET-1 regulates SMC proliferation by induced PDGF receptor activation in vivo, the effects of Bosentan treatment on PDGF ligand and receptor mRNA expression were examined. The results showed that treatment with Bosentan significantly decreased the intragraft preproET-1 mRNA expression. Rather, the mRNA levels of ET_A and ET_B were increased during CGP...
As demonstrated in the in vitro part of the study, ET-1 itself is not a strong mitogen and does not stimulate SMC migration, but it augments these functions through PDGF. Thus, even though ET-1 ligand and receptor expression remains high during CGP 53716 treatment, the net effect is downregulation of arteriosclerosis, as the key mediator, and the PDGF activation pathway is blocked. These in vitro and in vivo results speak for the priming function of ET-1 in PDGF-mediated signaling, suggesting intracellular crosstalk between G-protein–coupled ET receptors and protein tyrosine kinase PDGF receptors in the development of allograft arteriosclerosis. The results also explain the clear beneficial effect of ET-1 receptor antagonism, leading to reduced PDGF-Rbeta expression in cardiac allograft arteriosclerosis, although ET-1 itself, is a weak mitogen for SMCs.

**The ET-1/PDGF-Rbeta/PDGF-BB axis may operate in heart allograft arteriosclerosis.** In vitro studies have shown that activation of PDGF-Ralpha leads to SMC proliferation but inhibits migration, whereas activation of PDGF-Rbeta stimulates both the proliferative and migratory responses of SMCs (30). Although PDGF-BB is a potent mitogen for SMCs in vitro, in vivo studies using rat cardiac allografts have shown that PDGF-Ralpha and -Rbeta expression in intimal cells, as well as PDGF-BB expression only in interstitial mononuclear inflammatory cells, correlates with intimal thickening (15). Blocking of signaling downstream of PDGF receptors by CGP 53716 significantly reduced arteriosclerotic lesion formation (14). When rat coronary SMCs were stimulated with PDGF-AA or -BB in the presence of IL-1-beta or TNF-alpha, the drug significantly inhibited only AA-ligand–induced proliferation. This was explained by the observation that in coronary artery SMC culture, IL-1-beta and TNF-alpha specifically upregulated PDGF-Ralpha mRNA expression, whereas the expression of other PDGF ligands and receptors remained unchanged (14). Endothelin-1, IL-1-beta and TNF-alpha can induce the production of ET-1 from ECs (7), although in our cardiac allograft model, ET-1 immunoreactivity in arterial ECs was nonexistent, and mononuclear inflammatory cells, most likely activated macrophages, demonstrated mild ET-1 immunoreactivity. In addition, preproET-1 mRNA expression in SMCs may be induced by PDGF-AA (8,9). Thus, synthesis of these results implies that ET-1 and PDGF produced by activated macrophages may regulate migratory and proliferative SMC responses by PDGF-Rbeta in intimal lesion development in rat cardiac allograft arteriosclerosis.

**Conclusions.** We show here that ET-1 alone is a weak mitogen for SMCs, but it regulates PDGF receptor expression in SMCs and augments PDGF-BB–mediated SMC proliferation as well as PDGF-AB– and PDGF-BB–mediated SMC migration. The ET-1 receptor antagonist significantly reduced the incidence and intensity of arterio-
sclerotic lesions in rat cardiac allografts, as well as intragraft PDGF-beta mRNA expression. Our results suggest that the ET-1/PDGF-B/ETA/PDGF-BB axis may operate in SMC migration and proliferation in cardiac allograft arteriosclerosis and may explain the marked beneficial effects of blocking the signaling downstream of ET-1 receptors.

Acknowledgments
We thank Ms. M. Anttila, RN, for her excellent technical assistance in the laboratory, and Dr. M. Clozel for providing us with the ET-1 receptor antagonist, bosentan (Ro 47-0203/029).

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