Blockade of Growth Factor Synthesis and Growth Factor Action: Two Possible Sites of Interference in Allograft Vessel Disease and Coronary Bypass or Balloon Injury

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When injured, vascular endothelial cells produce growth factors that cause smooth muscle cells (SMC) to migrate from the media to the intima of the vessel wall, replicate in the intima, and stimulate arteriosclerotic changes. Interference with the actions of growth factors in allograft arteriosclerosis was explored. The somatostatin analog angiopeptin was administered to allograft-recipient rats after transplantation of aortic allografts between major and minor histoincompatible rat strains. Levels of epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and platelet-derived growth factor (PDGF) in grafts from angiopeptin-treated recipients were 35% to 75% of levels in grafts from nontreated recipients. Replication of SMC in the media and intima was reduced by 30% to 90% and intimal thickening by approximately 50%. The effect of blockade of IGF-1 receptors (IGF-1R) on the intimal response was also investigated. SMC cultures were serum-deprived of growth factors, then stimulated to replicate by addition of PDGF-B and EGF. Anti–IGF-1 and anti–IGF-1R antibodies reduced SMC replication by 50% and 90%, respectively. A D-amino acid analog of IGF-1, JB3, inhibited SMC replication and dose-dependently inhibited insulin receptor substrate 1 (IRS-1) and IGF-1R phosphorylation in vitro. Infusion of JB3 into rats undergoing balloon dilatation injury inhibited SMC replication in the injured vascular area by nearly 70%, but inhibited intimal thickening by only 30%. In conclusion, interference in the growth factor response may be one way of reducing/preventing vascular injury. However, blockade of more than one growth factor may be needed to achieve an optimal effect.

ALLOGRAFT ARTERIOSCLEROSIS, a concentric, generalized, fibroproliferative disorder affecting all intragraft arteries, is the common factor in chronic rejection of all organs. It is the end result of a complex biological process beginning with the activation of graft endothelium by a variety of nonimmunological factors, such as ischemia or reperfusion injury, as well as of immunological factors, such as acute rejection episodes that damage a transplant. Histologically, the disorder manifests as low-grade inflammation of the graft vascular wall, focal media necrosis, focal breaks in the internal elastic lamina, and concentric intimal thickening.

Allograft arteriosclerosis is also related to patterns of vascular injury following, for example, coronary ballooning and venous bypass surgery. In these situations, the initiating factor is mechanical, rather than immunological in nature. Consequently, balloon restenosis is focal, whereas, in venous grafts, the whole length of the transplanted vessel is affected.

Under both of these circumstances, endothelial cell activation obviously occurs, in addition to morphological changes, and there is also upregulation of class II major histocompatibility complex (MHC) antigens in endothelial cells and expression of a variety of binding molecules for recirculating leukocytes. According to the "response-to-injury" hypothesis of Ross,[1] endothelial cells respond to injury by producing a variety of growth factors, which, in turn, induce smooth muscle cells (SMC) to migrate from the media to the intima and continue their replication in the intima. These events result in intimal thickening and remodeling of the vascular wall. An outline of the molecular events is illustrated in Fig 1.

When considering therapeutic interference with the intimal response, prophylactic measures appropriate to the allograft type are of paramount importance. The preservation time of an allograft should be kept to a minimum, the recipients should be properly immunosuppressed to avoid acute episodes of rejection, and prophylactic measures should be applied to avoid cytomegalovirus (CMV) infection, which is the single most important infection contributing to allograft arteriosclerosis. With regard to ballooning reinjury and complications of venous grafts, no therapeutic measures have been available until recently.

In this communication, we will not deal with immunosuppression, inhibition of alloimmune response, or CMV prophylaxis; instead, we will deal with the possibility of interfering with the growth factor response and/or the effect of growth factors on the SMC, which are common to all these forms of vascular complications.

INHIBITION OF GROWTH FACTORS AND ALLOGRAFT ARTERIOSCLEROSIS WITH ANGIOPEPTIN, A LANREOTIDE ANALOG OF SOMATOSTATIN

Following the pioneering work of Marie Foegh and Peter Ramwell in a variety of rabbit and rodent models,[2-5] we decided to interfere experimentally in allograft arteriosclerosis using the somatostatin lanreotide analog BIM 23014 (angiopeptin). Aortic allografts, simulating the intragraft arteries of an organ transplant, were transplanted between major and minor histoincompatible rat strains. As shown elsewhere,[6] this kind of transplant undergoes first an acute, mainly adventitial, episode of rejection that is spontaneously reversible, followed by chronic inflammation of the transplant, induction of SMC replication in the media, and migration of SMC into the intima. Angiopeptin, at a dose of 80 μg/kg/d was infused via subcutaneous minipumps into the allograft recipients, and the grafts were harvested at various times thereafter. The levels of three growth factors,

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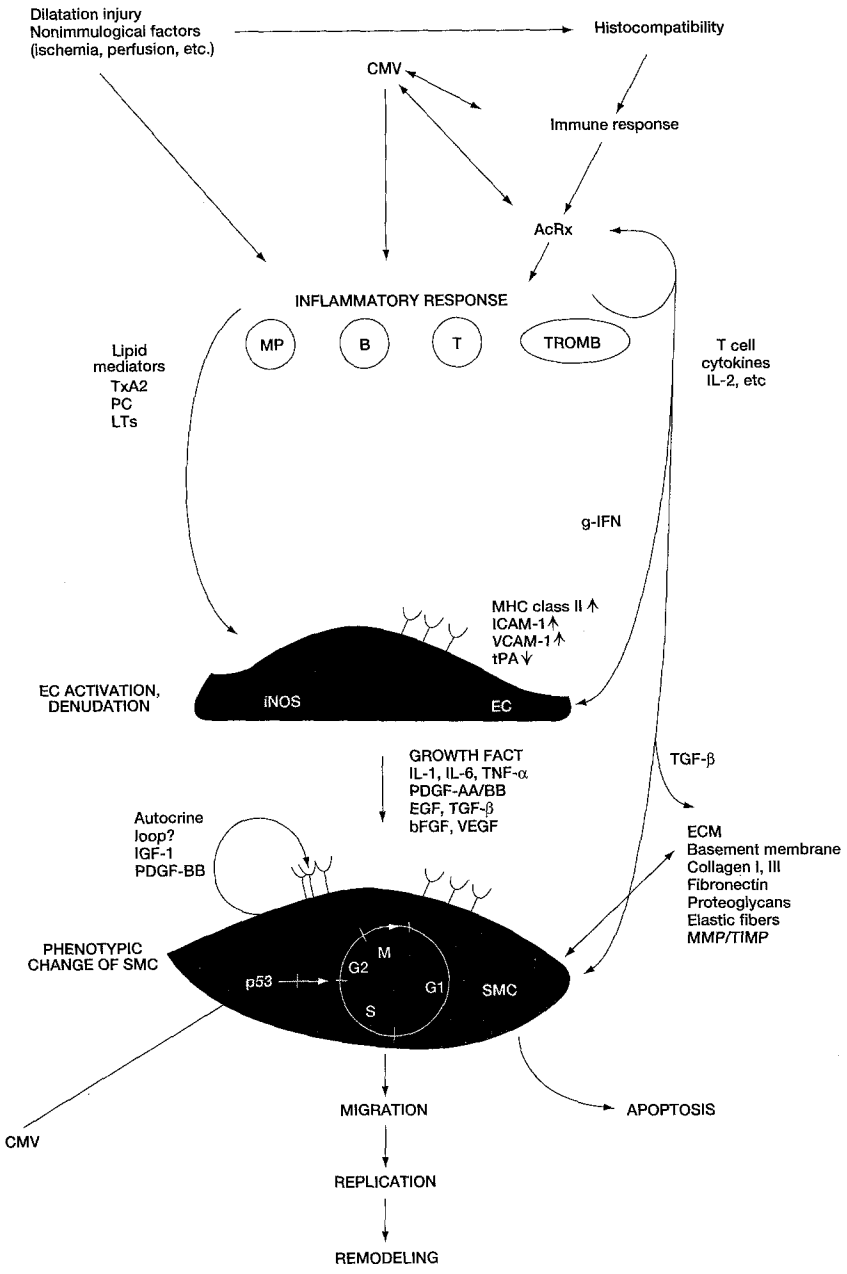

Fig 1. Summary of the major pathways of SMC in allograft arteriosclerosis. The aetiology of chronic rejection is most likely multifactorial. Nonimmune and immune inflammation induce persistent, low-grade damage to the vascular endothelium, which in turn begins to secrete growth factors and cytokines to repair the damage. This results in neighboring SMC proliferation in the allograft vascular wall, the influx of SMC from the media into the intima, and the generation of an arteriosclerotic lesion. Within the arterial wall, SMC are embedded in and associated with a complex network of noncellular structural elements, called the extracellular matrix (ECM). The ECM is in a steady state of production and degradation. The degradation is mediated by a "gene family" of proteolytic enzymes called matrix metalloproteinases (MMP), which are secreted as inactive zymogens. These are activated by proteolytic cleavage reactions, and their activity is also regulated by a family of specific protein inhibitors, ie, tissue inhibitors of metalloproteinases (TIMP). AcRX, acute rejection; EC, endothelial cells; HB-EGF, heparin-binding–epidermal growth factors; iNOS, inducible nitric oxide synthase; LT, leukotrienes; MMP, matrix metalloproteinases; MP, macrophages; thromb, thrombocytes; PC, prostacyclins and TxA2 thromboxane A_2.

epidermal growth factor (EGF), insulin-like growth factor-1 (IGF-1), and platelet-derived growth factor B (PDGF-B), were determined from lysates of the vascular wall using commercially available enzyme-linked immunoadsorbent assays. The expression levels of these growth factors in angiopeptin-treated recipients were 75% to 35% of the nontreated grafts. Concomitantly, the replication rate of SMC in the media and intima was reduced by 30% to 90%, and the intimal thickening, on average, by 50%.[7]

INTERFERENCE WITH THE IGF-1 RECEPTOR USING PEPTIDE ANALOGS OF IGF-1

In the second part of the study, we focused on the inhibition of the IGF-1 receptor (IGF-1R). Previous studies by Sell et al[8] demonstrated that cell lines deriving from IGF-1R knock-out (life-incompatible) mice did not respond to PDGF-B or EGF by more than 30% of the expected rate, whereas, after transfection of the wild-type IGF-1R gene, the response to these growth factors normalised. As we have found (Aavik, unpublished data) that there is a steep peak of IGF-1R expression coinciding with the induction of SMC replication in the vascular wall, we decided to test the effect of IGF-1R block on the intimal response in the rat denudation model.

First, we attempted to reproduce the observations of Sell et al[8] during in vitro SMC cultures, either in primary cultures or using a rat coronary SMC line, and we obtained compatible results. After the SMC were stimulated, follow-

ing serum deprivation, PDGF-B and EGF were found to be potent stimulators of SMC replication. Application of antibody to IGF-1 reduced the replication rate by 50%, and application of antibody to IGF-1R reduced the replication rate by 90%.

Second, in collaboration with Baserga, Cozzone, and Jameson of Thomas Jefferson University, Philadelphia, PA, we designed analogs of IGF-1 consisting of D-amino acids rather than of L-amino acids. A whole variety of cyclic D-peptides, stable in biological milieu, were designed using technology described in detail by Jameson et al.[9] One of these, JB3 (C-S-K-A-P-K-L-P-A-A-Y-C), was found to be particularly effective in inhibiting SMC replication in vitro, whereas a scrambled control (SC) peptide (C-Y-A-A-P-S-A-U-L-K-P-C) was without effect. In addition to inhibiting the replication of SMC in vitro, administration of peptide JB3 dose-dependently inhibited the phosphorylation of the IGF-1R and the IRS-1 in vitro.[10]

Finally, JB3 was infused into rats undergoing balloon dilatation injury. Administration of 10 to 30 μg/kg/d of JB3 inhibited the replication of SMC in the injured vascular area by nearly 70%, but inhibited the intimal thickening maximally by only 30%.[10]

These findings suggest that blocking the IGF-1R to inhibit SMC replication is not sufficient on its own, but additional factors, such as PDGF-B–dependent regulation of the migration of SMC, possibly plus other unidentified factors, should be considered.

CONCLUSIONS

Since the studies described here were conducted, the clinical efficacy of angiopeptin has been demonstrated in two studies targeted at restenosis after coronary angioplasty, and angiopeptin has been shown to inhibit angiographically demonstrable reocclusion of the lumen and/or the clinical events.[11] Moreover, preliminary results from a third placebo-controlled study, although using a relatively small number of patients, indicate thtat angiopeptin also inhibits the graft vessel disease (chronic rejection) that can occur after heart transplantation in man (Meiser, unpublished data). Taken together, these results, though not impressive at the moment, suggest that interference in the growth factor response, possibly at the transcriptional level of growth factors, is one possible method that may be used to interfere with the progression of the vascular lesion.

To date, there have been no clinical studies investigating inhibition of growth factor receptors. However, from the experimental evidence derived from this and other laboratories, it appears that more than one growth factor receptor must be blocked to achieve an optimal effect.

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