Cytomegalovirus Infection Enhances mRNA Expression of Platelet-Derived Growth Factor-BB and Transforming Growth Factor-β1 in Rat Aortic Allografts

Possible Mechanism for Cytomegalovirus-Enhanced Graft Arteriosclerosis

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Abstract  We have recently demonstrated that rat cytomegalovirus (RCMV) infection induces an early inflammatory response in the adventitia (perivasculitis) and in the subendothelial space (endothelialitis) as well as doubles smooth muscle cell (SMC) proliferation and intimal thickening in rat aortic allografts performed from the DA (AG-B4, RT1b) to the WF (AG-B2, RT1b) strain. In this study, the impact of RCMV infection on the structure of inflammation in the allograft adventitia and on the expression of SMC growth factors in the allograft vascular wall was investigated. The recipient rats were inoculated with 10⁶ plaque-forming U of RCMV Maastricht strain or left noninfected and used as controls. The allografts were removed at 7 days and 1 and 3 months after transplantation and processed for morphometry and immunohistochemistry. RNA was isolated for reverse transcriptase polymerase chain reaction (RT-PCR). RCMV infection was associated with significantly upregulated presence (P<.05) of T helper (W3/25), T cytotoxic (OX8), and natural killer (3.2.3) cells in the allograft adventitia 7 days after transplantation but not thereafter. More monocyte/macrophages (OX42) were also present in RCMV-infected allografts, but the difference was not significant. Concomitantly, RCMV infection significantly enhanced (P<.05) the expression of major histocompatibility complex class II (OX6) and almost doubled (P=NS) the expression of interleukin-2R (CD25), intercellular adhesion molecule-1 (CD54;1A29), and lymphocyte function-associate 3. CD54 antigen-1 α-chain (CD11a; WT.1) in the adventitial inflammatory infiltrate. RCMV infection was linked with an early, prominent expression of both PDGF-BB mRNA at 7 days (P<.05) and at 1 month (P<.025) and of transforming growth factor-β1 mRNA at 7 days (P<.025) and at 1 month (P<.025) after transplantation. A less-prominent mRNA up-regulation of acidic fibroblast growth factor (P<.05) was associated with RCMV infection at 7 days and at 1 month, as well as of epidermal growth factor at 1 month after transplantation, when compared with noninfected allografts, although the mRNA expression in both groups was below the levels of nontransplanted DA aortas. RCMV infection almost doubled basic fibroblast growth factor mRNA expression (P=NS) in the allograft vascular wall at 7 days and at 1 month. RCMV infection had no additional impact on insulin-like growth factor-1 mRNA expression when compared with noninfected allografts. Our results suggest that RCMV infection-induced early inflammatory response in the vascular wall is linked with an early activation of the inflammatory cells and enhanced mRNA expression of PDGF-BB and transforming growth factor-β1. We suggest that these biochemical responses may play a role in the generation of RCMV-enhanced allograft arteriosclerosis. (Arterioscler Thromb. 1994;14:2043-2052.)

Key Words  • cytomegalovirus  • allograft arteriosclerosis  • PDGF  • TGF-β1  • PCR

Cardiac allograft arteriosclerosis, ie, chronic rejection, has emerged as a major factor limiting the long-term survival of heart transplant recipients. In angiograms, accelerated arteriosclerosis is characterized by diffuse eccentric narrowing of the coronary vessels. Microscopically, the basic underlying lesion is diffuse, eccentric intimal thickening, mainly consisting of proliferating smooth muscle cells (SMCs) and macrophages. In addition, intramural and peri-
growth factor-1 (IGF-1), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF)) are prominently expressed both on the peptide and mRNA levels in the vascular wall.9,10 The levels of eicosanoids, with the exception of thromboxane B2, are less altered.9,10

In this study we describe the structure of rat CMV (RCMV) infection-associated inflammatory and immunologic responses in the allograft conduit. Furthermore, we demonstrate that RCMV infection-induced inflammatory and immunologic responses are linked with an enhanced mRNA expression of PDGF-BB and transforming growth factor (TGF)-β in the allograft vascular wall.

**Methods**

**Rats**

Inbred DA (AG-B4, RT1b) and WF (AG-B2, RT1b) rat strains were used as donors and recipients, respectively. The rats were purchased from the Zentralinstitut für Versuchstierzucht (Hannover, FRG). They were 2 to 3 months of age and weighed 200 to 300 g. The rats were fed with regular rat food (altromin, Standard Diet, Chr. Petersen A/S) and tap water ad libitum. All animals were maintained on a 12-hour light/dark cycle.

**Aortic Allografts**

The strain combination of DA to WF was used for allogeneic transplantation. An ~3-cm-long segment of descending aorta was removed, thoroughly perfused with phosphate-buffered saline (PBS), and used as transplant.11 End-to-end anastomosis was performed using 9-0 continuous nylon suture. The graft was transplanted into heterotopic position below renal arteries and above bifurcation, forming a loop in the recipient abdominal cavity. Total ischemic time varied from 45 minutes to 60 minutes, during which time the graft was kept in an ice bath at +4°C for 15 minutes. The noninfected control rats were kept in separate colonies with otherwise similar diet and conditions. All animals received humane care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication no. 86-23, revised 1985).

**Quantitation of Infectious RCMV**

Quantitation of infectious RCMV was done from the frozen virus stock by plaque assays as described by Wentworth and French.16 Confluent DA rat embryo fibroblast monolayers were washed with Hank’s balanced salt solution (1x) (GIBCO), infected with appropriate virus dilution, and incubated at +37°C in a 5% CO2 incubator for 60 minutes. After incubation, the infected cells were overlaid with 1 mL Eagle’s basal medium (GIBCO) supplemented with 200 mmol/L L-glutamine (Northumbria Biologicals LTD), 10,000 IU/mL penicillin/1000 µg/mL streptomycin solution (GIBCO), and 5% fetal calf serum (Sera-Lab) with a final concentration of 0.25% agarose (Sigma). After the incubation of 7 days, the cells were fixed with 10% formalin overnight. The solid base layer was removed, and the cell monolayers were stained with 1% aqueous crystal violet (Merck), and the plaques were counted by light microscopy.

**Scoring of Intimal Cells**

A segment of graft was fixed in 10% buffered formalin, embedded in paraffin, and examined histologically after being sectioned and stained with Mayer’s hematoxylin-eosin. Cross sections were prepared for evaluation, and the number of intimal SMCs and intimal mononuclear inflammatory cells was quantified on cytological criteria according to standard morphometric principles17 and are expressed as point score units (PSU), ie, in mean number of points falling over a given anatomic area using straight, cross-sectional lines, and a 0.02-mm grid.

**Immunohistochemistry**

Immediately after graft removal, a piece of allograft was cut in optimal cutting temperature embedding (Tissue-Tek), snap-frozen in liquid nitrogen, and stored at −70°C. Frozen sections were air-dried onto poly-D-lysine-coated slides, fixed in acetone at −20°C for 20 minutes, and stored at −20°C until stained. Before immunostaining, the slides were refixed with chloroform for 30 minutes to remove unspecific background staining and air-dried. Cross sections 3 to 4 µm thick of aortic allografts were stained with three-layer indirect immunoperoxidase technique, using mouse monoclonal antibodies (MABs) to rat antigens.18 Primary antibodies (see below) were used at appropriate dilutions (usually 1:100; MHC class II 1:200) in tris(hydroxymethyl)aminomethane (TRIS) solution with 1% BSA. After a 30-minute incubation at room temperature, the sections were washed in TRIS buffer and incubated for 30 minutes with peroxidase-conjugated rabbit anti-mouse IgG (P161; Dako-Immunoglobulins A/S). After the washing in TRIS, the sections were incubated with goat anti-rabbit Ig (Dako; Caltag Laboratory). The reaction was revealed by staining 3-amino-9-ethylcarbazole containing hydrogen peroxide. The specimens were counterstained with hematoxylin, and coverslips were aquamounted (Aquamount; BDH Ltd).

To determine the leukocyte subsets of the inflammatory infiltrate, the following mouse antibodies to rat antigens were used: (1) W3/25 (Sera-Lab), a mouse IgG, MAB to rat T helper cells (CD 4 equivalent); (2) OK8 (Sera-Lab), a mouse
were used to detect the PCR products. The intensity of hybridization signals was measured by densitometry (Electro- 
 resumed. The samples were electrophoresed in PCR buffer (500 mmol/L Tris, pH 8.8, 15 mmol/L MgCl2, 150 mmol/L (NH4)2SO4, 1% sodium dodecyl sulfate) for 2 to 3 hours in an ice bath. The resuspension was put into microcentrifuge tubes (Beckman Instruments), and the guanine-lysed samples were added on top. The samples were centrifuged at 32,000 rpm for SW 50.1 at +20°C for 21 hours. After centrifugation, the supernatant was removed, and RNA was resuspended with TES buffer (10 mmol/L Tris, pH 7.4, 5 mmol/L EDTA, pH 7.4, 1% sodium dodecyl sulfate) for 2 to 3 hours in an ice bath. The resuspended RNA was put into microcentrifuge tubes, and the same volume of phenol was added. The samples were mixed and spun down to separate phases. The upper phase was removed to a new tube, and phenol purification was repeated twice. After that, phenol-chloroform (1:1) and, in the last step, only chloroform was added to samples to yield an acute infection with systemic virus dissemination earlier, RCMV 1 day after heterotopic aortic transplantation or RCMV infection in allograft recipients.
epidemic and proliferation of inflammatory cells in the allograft adventitia (perivasculitis) 7 days after transplantation. Second, acute RCMV infection induces concomitantly a subendothelial inflammation (endothelitis) of T helper cells (W3/25) and cells of the monocyte/macrophages lineage (OX42) in rat aortic allografts lasting for 1 month after transplantation. And third, SMC proliferation and intimal thickening are doubled by RCMV infection. In syngeneic transplants or in allografts infected with RCMV 60 days after transplantation, no intimal thickening occurs as a consequence of RCMV infection.

Fig 1 demonstrates the effect of RCMV infection on the intimal cell composition during the development of arteriosclerotic changes.

Effect of RCMV Infection on Phenotypic Distribution of Leukocyte Subsets in the Allograft Adventitia

To investigate the effect of RCMV infection on the phenotypic distribution of leukocyte subsets in the allograft adventitia, aortic frozen cross sections were stained with three-layer immunoperoxidase technique using mouse MAb to rat T helper (W3/25), T cytotoxic (OX8), NK (3.2.3) cells, and monocyte/macrophages (OX42). The results are depicted in Fig 2, and representative illustrations are shown in Fig 3.

Frozen cross sections of nontransplanted DA aortas did not contain any T helper, T cytotoxic, NK cells, or cells of the monocyte/macrophage lineage (not shown). In noninfected allografts, T helper cells and monocyte/macrophages were present in small numbers and reached a clear peak 1 month after transplantation. Few T cytotoxic and NK cells were also found (Fig 2). RCMV infection significantly enhanced (P<.05) T helper, T cytotoxic, and NK cells in the adventitial inflammatory infiltrate 7 days after transplantation when compared with noninfected allografts. RCMV infection also elevated the presence of monocyte/macrophages, but the difference was not statistically significant. Later, the differences disappeared (Fig 2).

Effect of RCMV Infection on the Level of Immune Activation in the Allograft Adventitia

To investigate the effect of RCMV infection on immune activation of adventitial inflammatory infiltrate, frozen aortic cross sections were stained with three-layer indirect immunoperoxidase technique using mouse MAb to rat IL-2 receptor (CD25), MHC class II (OX6), ICAM-1 (CD54; 1A29), and LFA-1 α-chain (CD11a; WT.1). The results are given in the Table.

In the adventitia of nontransplanted DA aortas there was no expression of IL-2 receptor, ICAM-1, or LFA-1. 

Fig 2. Semilogarithmic plots showing the effect of rat cytomegalovirus (RCMV) infection on the phenotypic distribution of leukocyte subsets in the adventitia of aortic allografts as demonstrated from frozen aortic cross sections by monoclonal antibodies using indirect three-layer immunoperoxidase technique. Positive staining was scored semiquantitatively from 0 to 5 in a blind review, and values between rats are given as mean±SEM. The responses were determined from ≥3 rats per group per time point. NK indicates natural killer cells; M0, monocyte/macrophages. Significances by Mann-Whitney U test; *P<.05.
FIG 3. Photomicrographs showing immunohistochemical analysis of inflammatory cells in the adventitia of aortic allografts 7 days after transplantation. Left panels: noninfected aortic allografts; right panels: rat cytomegalovirus-infected aortic allografts. Positive staining for W3/25 for T helper cells (A, B), OX8 for T cytotoxic cells (C, D), 3.2.3. for natural killer cells (E, F), and OX42 for monocyte/macrophages (G, H). Original magnification ×400. The outer layer of media is separated from the adventitia by external elastic laminae, and this is indicated by an arrow.
Effect of Rat Cytomegalovirus (RCMV) Infection on the Level of Immune Activation in the Allograft Adventitia

<table>
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<tr>
<th></th>
<th>IL-2R (CD25)</th>
<th>MHC Class II (OX8)</th>
<th>ICAM-1 (CD54, 1A29)</th>
<th>LFA-1 α-Chain (CD11a, WT.1)</th>
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<tr>
<td>DA-&gt;WF</td>
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<tr>
<td>7 d</td>
<td>10±6</td>
<td>1.3±0.3</td>
<td>1.2±0.5</td>
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<td>1 mo</td>
<td>255±52</td>
<td>4.7±0.3</td>
<td>3.0±0.3</td>
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<td>3 mo</td>
<td>46±19</td>
<td>2.8±0.3</td>
<td>1.3±0.3</td>
<td>0.0±0.0</td>
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<tr>
<td>DA-&gt;WF+RCMV</td>
<td></td>
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<tr>
<td>7 d</td>
<td>29±9</td>
<td>3.2±0.2*</td>
<td>2.5±0.3</td>
<td>2.0±0.5</td>
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<tr>
<td>1 mo</td>
<td>299±46</td>
<td>4.5±0.3</td>
<td>3.6±0.2</td>
<td>2.2±0.1</td>
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<tr>
<td>3 mo</td>
<td>77±14</td>
<td>3.2±0.4</td>
<td>2.8±0.3</td>
<td>1.3±0.8</td>
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MHC indicates major histocompatibility complex; ICAM-1, intercellular adhesion molecule-1; and LFA-1, lymphocyte function-associated antigen-1.

To evaluate the level of immune activation in the allograft adventitia, frozen sections of aortic allografts were stained with mouse monoclonal antibodies to rat antigens by a three-layer indirect immunoperoxidase technique. Positive staining for interleukin (IL)-2 receptor was scored as the number of positive cells per cross section and is given as mean±SEM. The expression of other markers was scored semiquantitatively from 0 to 5 in a blind review, and values represent mean±SEM. The responses were determined in ≥3 rats per group per time point.

*P<.05 by nonparametric Mann-Whitney U test.

α-chain. Very few cells stained positively for antibody for class II molecules (not shown). In noninfected allografts, immune activation on the adventitial inflammatory infiltrate, as demonstrated by the expression of IL-2 receptor, MHC class II, ICAM-1, and LFA-1 α-chain, reached a peak 1 month after transplantation and declined thereafter (Table). RCMV infection induced an early activation on the adventitial inflammatory cells, detectable already 7 days after transplantation with a significant upregulation (P<.05) of MHC class II molecule and elevations (P=NS) of IL-2 receptor, ICAM-1, and LFA-1 α-chain.

Effect of RCMV Infection on Growth Factor mRNA Expression in the Allograft Vascular Wall

To investigate the effect of RCMV infection on SMC growth factor mRNA expression in the allograft vascular wall, total RNA was extracted and used for RT-PCR. The results are given as the ratio of intensity of growth factor mRNA expression in the allograft vascular wall both at 7 days and at 1 month, to 259±31 at 7 days and 121±42 at 1 month, but increased to 535±178 at 3 months. Compared with noninfected allografts, the expression of EGF mRNA in RCMV-infected allografts was significantly upregulated at 1 month to 551±112 (P<.05). Note, however, that EGF mRNA expression in both groups was below the level of nontransplanted DA aortas during the whole experiment (Fig 4b).

PDGF-BB. The level of PDGF-BB mRNA in nontransplanted DA aortas was 634±46. In noninfected allografts, PDGF-BB mRNA expression was increased when compared with nontransplanted aortas, reaching 1478±217 at 7 days, 1366±522 at 1 month, and 2480±435 at 3 months. RCMV infection induced a significant upregulation in PDGF-BB mRNA expression to 3170±378 at 7 days (P<.05) and to 3276±381 at 1 month (P<.025), when compared with noninfected allografts. Thereafter, the response declined (Fig 4c, 5a).

TGF-β. The level of TGF-β1 mRNA in nontransplanted DA aortas was 270±30. In noninfected allografts, the expression of TGF-β1 was 955±343 at 7 days, 615±107 at 1 month, and reached a peak of 2299±687 at 3 months. RCMV infection significantly enhanced TGF-β1 mRNA expression to 4556±461 at 7 days (P<.025) and to 2307±131 at 1 month (P<.025), when compared with noninfected allografts but declined to 50% of that observed in noninfected allografts at 3 months (Fig 4d, 5b).

aFGF. The intensity of aFGF mRNA expression in nontransplanted DA aortas was 1883±297. In noninfected allografts, aFGF mRNA expression declined to 259±31 at 7 days and to 581±158 at 1 month but peaked to 2734±800 at 3 months. RCMV infection significantly elevated (P<.025) aFGF mRNA expression in the allograft vascular wall both at 7 days and at 1 month, when compared with noninfected allografts (Fig 4e). Note, however, that aFGF mRNA expression in both groups was below the levels of nontransplanted DA aortas at 7 days and 1 month.

bFGF. The expression of bFGF mRNA was 473±45 in nontransplanted DA aortas. In noninfected allografts, the intensity of bFGF mRNA increased to 751±280 at 7 days, to 756±210 at 1 month, and to 1283±487 at 3 months. RCMV infection almost dou-
bled the bFGF mRNA expression in the allograft vascular wall to 1547±262 at 7 days and to 1342±215 at 1 month, but the difference was not statistically significant. At 3 months, the bFGF mRNA expression was similar to noninfected allografts (Fig 4f).

Discussion

Cardiac allograft arteriosclerosis is a major obstacle in long-term survival after heart transplantation.1,2 The detailed mechanism of this process is unknown, but clinical studies have identified certain risk factors predisposing the graft to chronic rejection. Such risk factors include humoral and cellular immune response,22 hyperlipidemia,24-25 and CMV infection.4-6 Our recent studies show that RCMV infection enhances inflammatory cell proliferation in the allograft adventitia (perivasculitis) and induces a T helper cell- and monocyte/macrophage-containing subendothelial inflammation (endothelialitis) beneath the endothelium.8 Concomitantly with the inflammatory response, RCMV infection doubles SMC proliferation and intimal thickening of rat aortic allografts.7

In this report we provide immunologic and molecular evidence on how RCMV infection enhances the generation of allograft arteriosclerosis. First, RCMV infection was associated with an early, significant upregulation of T helper, T cytotoxic, and NK cells in the
adventitia. Second, these cells displayed enhanced levels of immune activation, i.e., the expression of MHC class II, and possibly IL-2 receptor, ICAM-1, and LFA-1 α-chain. Third, and most notably, RCMV infection was linked with an early, prominent PDGF-BB and TGF-β1 mRNA expression in the allograft vascular wall. The expression of other growth factors (EGF, aFGF, bFGF) was also elevated, but the differences were not statistically significant, and/or the level of response was below nontransplanted controls.

Allograft arteriosclerosis is generally considered to result from the proliferation and migration of SMCs from the media into the intima, in association with increased deposits of extracellular matrix material and lipids. Our current results suggest some pathways whereby the process is regulated in RCMV-enhanced allograft arteriosclerosis.

So far we have not been able to demonstrate any trace of RCMV in the allograft. Only occasionally have some inflammatory cells and some endothelial cells of the allografts been positive in immunostaining for early and late antigens of RCMV (unpublished results). Thus, the regulation by intact virus is unlikely. Another possible explanation is that viral infection transforms cells by incorporating into the cell genome. This is also unlikely, as we have been unable to demonstrate any viral genome in the proliferating cells by in situ hybridization (unpublished results). In other words, we have not been able to provide any evidence for a direct regulatory effect of RCMV on allograft arteriosclerosis.

On the other hand, we have demonstrated that RCMV infection enhances the early inflammatory response and the activation level of inflammatory cells in the allograft adventitia and may thus have an indirect impact on SMC proliferation via acute and/or chronic inflammation. Immune activation in RCMV-infected allografts and the enhancement of peptide growth factor mRNA expression, coincided with the accelerated and enhanced SMC proliferative response seen in RCMV-infected allografts. Thus, we consider that the RCMV-enhanced intimal changes may reflect the elevated inflammatory and immunologic responses, linked with upregulated transcription of SMC mitogens, especially of PDGF-BB and TGF-β1.

Several other studies also suggest the pro-inflammatory role of CMV infection. Evidence exists that human CMV infection upregulates IL-1β gene expression, leading to increased production of IL-1 by monocyte/macrophages and to enhancement of inflammatory responses. Studies in AIDS patients with CMV-induced severe colitis have shown that CMV induces the production of TNF-α by monocytes and mucosal macrophages. The immediate early gene of human CMV is able to code for a protein that has sequence homology and immunologic cross-reactivity with HLA-DR β-chain. In addition, human CMV encodes a glycoprotein that is homologous to MHC class I antigens. In vitro studies have demonstrated that the immediate early genes of human CMV upregulate the expression of IL-2 and IL-2-receptor genes. These observations are in accordance with our observations and with our interpretation.

How do the likely effector molecules, the growth factors, operate in the atherosclerotic process? PDGF is a potent SMC chemoattractant and mitogen. It is present in platelets and can be produced by endothelial cells, SMCs, macrophages, and a large number of cells transformed by DNA or retroviruses. Observations on subcultured rat aortic SMCs have demonstrated that PDGF-BB is the most potent growth factor for SMCs, followed by FGF, EGF, and to lesser extent by PDGF-AA, IGF-1, and TGF-β1. Furthermore, recent experiments strongly suggest that SMC migration is a key step for the growth of intimal lesions and is possibly mediated by PDGF. Increased expression of PDGF-β receptor in the media of arteries and in a number of myocardial cells in association with T cells and monocyte/macrophage infiltration has been observed in rat cardiac allografts.

Transforming growth factors were first identified as products of virus-transformed cells. TGF-β1 is present in α-granules of platelets and is secreted by monocyte/macrophages, activated T cells, endothelial cells, and SMCs. TGF-β1 is secreted in vivo in inactive form, and the target specificity of TGF-β action may be determined by the ability of a cell to activate the latent complex. TGF-β controls the production of extracellular matrix proteins and inhibits the degradation of newly formed matrix proteins and may thus contribute to the development of fibroproliferative disorder. Furthermore, a recent study using rat cardiac allografts shows that TGF-β bioactivity dramatically increases in the transplanted allografts during chronic rejection.

TGF-β is also a potent inhibitor of the proliferation of many cell types in vitro. TGF-β inhibits the proliferative response of quiescent SMCs exposed to serum or EGF; however, when the same cells are grown in soft agar in 10% serum, TGF-β, combined with EGF or PDGF, stimulates SMC proliferation. TGF-β stimulates the growth of certain fibroblasts in vitro in the presence of PDGF but inhibits it if EGF is present. Furthermore, evidence exists showing that TGF-β is able to modulate immune responses and inflammation, augmenting certain immune responses while suppressing others.

Most of the cell types involved in allograft arteriosclerosis are capable of expressing IGF-1, IGF-1 receptors, and IGF-binding proteins, or combinations of these proteins, but IGF-1 alone appears in most cases to be only a weak mitogen for vascular SMCs. Autocrine or paracrine production of IGF-1 is believed to be necessary for PDGF to exert its mitogenic effects on SMCs and fibroblasts in vitro. Several studies strongly suggest that bFGF may be a key mitogen for the initiation of SMC replication after vascular injury but is not important for the continuation of intimal SMC replication. A recent study in human cardiac allografts using quantitative PCR and intracoronary ultrasound suggests a trend toward a correlation between the quantity of aFGF mRNA present in the allograft myocardium and the degree of coronary intimal thickening.

Principal growth regulatory molecules participating in the development of arteriosclerotic lesions, both in ordinary coronary artery disease and in transplantation-associated arteriosclerosis, include PDGF, TGF-β, and IGF-1. Our studies with rat aortic allografts demonstrate that during chronic rejection, in the absence of RCMV infection, the messages of IGF-1, PDGF-BB, and TGF-β1 are prominently expressed at 3
months after transplantation. RCMV infection in the allograft recipients induces an early, enhanced inflammatory response both in the adventitia and intima, i.e., perivascularis and endothelialitis, which is followed by enhanced SMC replication and enhanced intimal thickening, as well as by concomitant upregulation of PDGF-BB and TGF-β1 mRNA. Thus, the present study suggests that these growth factors may be responsible for the RCMV-enhanced proliferative response and intimal thickening by increasing the migration of SMCs into the subendothelial space and their proliferation there. In addition, TGF-β1 may enhance extracellular matrix formation as well as modulate inflammatory responses.

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References
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