hibitor throughout the purification. Purified CDI inhibits capillary EC migration with an IC_{50} of 16 nM (Fig. 2B).

To determine whether CDI could inhibit neovascularization in vivo, we tested it in the chick chorioallantoic membrane assay (CAM) (*18*) as a third bioassay (Fig. 3). Over 100 CAMs were used. Purified CDI (4-μg samples) in methylcellulose discs caused significant inhibition of embryonic capillaries in the yolk sacs, resulting in large avascular zones. In contrast, control CAMs implanted with empty methylcellulose discs never developed avascular zones. Histological studies of CDI-treated CAMs revealed a mesoderm that was thinner than normal and avascular (Fig. 3D) relative to controls. We monitored the anti-angiogenic activity of CDI throughout its purification using this CAM assay, and dose dilution curves were generated for the inhibitor from each successive purification step through homogeneity. The increasing potency of the angiogenesis inhibitor with its increasing purification is shown in Fig. 3E. The cartilage-derived inhibitor is a potent inhibitor of angiogenesis, since inhibition was observed with as little as 4 μg. The lowest reported doses of described angiogenesis inhibitors tested alone in the CAM assay are 50 μg of protamine (*18*), 200 μg of bovine vitreous extract (*19*), and 10 μg of platelet factor–4 (*18, 20*). The lowest reported doses of angiogenesis inhibitors effective as combinations include heparin (50 μg) and hydrocortisone (60 μg) (*2*), and β-cyclodextrin tetradecasulfate (14 μg) and hydrocortisone (60 μg) (*2*).

Our data identify a single, tissue-derived macromolecule, CDI, as a potent inhibitor of angiogenesis. In vitro studies with this inhibitor indicate that it also negatively modulates two key components of the angiogenic process, the proliferation and migration of capillary endothelial cells. Because this angiogenesis inhibitor is also a collagenase inhibitor, additional interpretations of earlier studies may now be possible. For example, in 1978, platelet factor–4 was described as a collagenase inhibitor (*21*). Subsequently, several research groups have tested this factor for its ability to inhibit angiogenesis because of its high affinity for heparin and have found that it was anti-angiogenic (*18, 20*) and inhibited capillary EC proliferation (*13*). Our studies suggest that the collagenase inhibitory activity of platelet factor–4 may have a role in inhibiting neovascularization.

This study supports earlier work in which the importance of collagenase in the invasiveness of capillary endothelial cells during angiogenesis was demonstrated (*11, 22*). It may also help to explain why tissues such as cartilage are resistant to invasion and may contribute to a broader understanding of the control of vascular proliferation.

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Fibroblast Growth Factor Receptor Is a Portal of Cellular Entry for Herpes Simplex Virus Type 1

ROBERT J. KANER, ANDREW BAIRD, ALKA MANSUKHANI, CLAUDIO BASILICO, BARBARA D. SUMMERS, ROBERT Z. FLORKIEWICZ, DAVID P. HAJJAR*

Herpes simplex virus type 1 (HSV-1) is a ubiquitous pathogen responsible for considerable morbidity in the general population. The results presented herein establish the basic fibroblast growth factor (FGF) receptor as a means of entry of HSV-1 into vertebrate cells. Inhibitors of basic FGF binding to its receptor and competitive polypeptide antagonists of basic FGF prevented HSV-1 uptake. Chinese hamster ovary (CHO) cells that do not express FGF receptors are resistant to HSV-1 entry; however, HSV-1 uptake is dramatically increased in CHO cells transfected with a complementary DNA encoding a basic FGF receptor. The distribution of this integral membrane protein in vivo may explain the tissue and cell tropism of HSV-1.

THE PATHWAY FOR HERPES SIMPLEX virus type 1 (HSV-1) entry into vertebrate cells remains undefined. The putative sequence of events in herpes infection involves initial virion attachment to the cell surface through an interaction with heparin-like cell-associated glycosaminoglycans (GAGs) (*1*), fusion of the viral envelope with the plasma membrane (*2, 3*), removal of the envelope (*4*), and release of the viral nucleocapsid into the cytoplasm of the cell (*5*). The process of viral entry involves specific viral-associated glycoproteins as well as target cell binding sites and receptors (*3*); however, no specific receptor has been described for HSV-1. In contrast, several other viruses use binding sites for known physiological ligands. For example, rhinovirus, an

etiological vector responsible for the common cold, enters cells by means of the cell adhesion molecule ICAM-1 (*6*); human immunodeficiency virus (HIV), responsible for acquired immunodeficiency syndrome (AIDS), primarily uses the CD4 glycoprotein receptor (*7*); Epstein-Barr virus infects T lymphocytes by means of the C3d complement receptor (*8*); rabies virus infects cells through the acetylcholine receptor (*9*); reovirus enters through the β-adrenergic receptor (*10*); and, vaccinia virus can enter the cell by first interacting with the epidermal growth factor (EGF) receptor (*11*).

We have observed that proliferating arterial smooth muscle cells incubated with basic FGF for 2 hours have a reduced ability to be infected with HSV-1 (Fig. 1A). Coincubation of basic FGF with HSV-1 produced a similar inhibitory effect, whereas basic FGF added to cells after infection could not block viral replication (*12*). The effects of basic FGF on viral uptake and replication appeared to be specific. Nerve growth factor (NGF, 10 μg/ml), colony-stimulating factor type 1 (CSF-1, 10 μg/ml), and glucagon (10 μg/ml) (a polypeptide unrelated to cell growth) were ineffective in preventing viral uptake (*12*). Although poly-L-lysine and platelet factor 4 have been shown to reduce HSV-1 adsorption and infectivity (at microgram per milliliter concentrations) (*1*), other heparin-binding growth factors and proteins including transforming growth factor–β (TGF-β, 100 ng/ml) (*13, 14*), platelet-derived growth factor (PDGF, 20 ng/ml) (*13, 14*), thrombospondin (TSP, 10 μg/ml) (*13, 14*) or histidine-rich glycoprotein (HRGP, 10 μg/ml) (*13, 14*) were ineffective in preventing HSV-1 uptake and infectivity (*12*). Basic FGF had no effect on the uptake of adenovirus type 2 (Ad-2), an unrelated DNA virus (*12*). Compounds that inhibit the ability of basic FGF to bind to cells were also tested. The nonspecific inhibitors wheat germ agglutinin (*15*), protamine (*15, 16*), and suramin (*16*) inhibit HSV-1 uptake by smooth muscle cells (*12*).

Since the initial adsorption of HSV-1 to cells is mediated by attachment of virions to cell surface heparan sulfate (*1*), we attempted to distinguish whether basic FGF was competing with HSV-1 for binding to the high-affinity basic FGF receptors or to the low-affinity binding site, heparan sulfate (*14, 17*). We incubated cells with a polypeptide fragment of basic FGF, consisting of amino acids 103 to 120, which binds to the basic FGF receptor (*18*). This polypeptide inhibits binding of ^{125}I-labeled basic FGF to the high-affinity FGF receptor and blocks the biologic effects of basic FGF (*18, 19*). Basic FGF residues 103 to 120 [FGF(103–120)] inhibited HSV-1 uptake (Fig. 1B). However, polypeptide fragments that do not bind to the high-affinity receptor [that is, FGF(1–24) or FGF(121–146)] had no effect on HSV-1 uptake (the legend to Fig. 1B). These results support the concept that the virion uses the high-affinity receptor for basic FGF to penetrate cells after its adsorption to cells by means of cell-associated GAGs.

A high-affinity basic FGF receptor has been identified (*15, 20*), and a full-length cDNA clone has been isolated and sequenced (*21*). CHO cells contain fewer than 1000 FGF receptors per cell (background levels), do not respond mitogenically to FGF (*12, 22*), and are unable to take up HSV-1 (Fig. 2A); we therefore tested the hypothesis that CHO cells transfected with the FGF receptor gene would become targets for HSV-1 uptake. CHO cells were transfected with the expression vector 91023b (*23*), containing the cDNA of the mouse homolog of the chicken basic FGF receptor cDNA (*flg*) described by Lee *et al.* (*21*). Two stable transfectant lines were isolated, 3-3 and 4-1, and were found to express 31,000 and 100,000 high-affinity FGF receptors per cell, respectively (*22*). In addition, CHO cells were transfected with an antisense construct representing *flg*. The resulting cell line, A-1, did not express functional FGF receptors.

There was a greater than tenfold increase in the uptake of [^{3}H]HSV-1 by the transfected cells, compared to either the parental cell line or cells transfected with the antisense cDNA (Fig. 2A). The FGF peptide

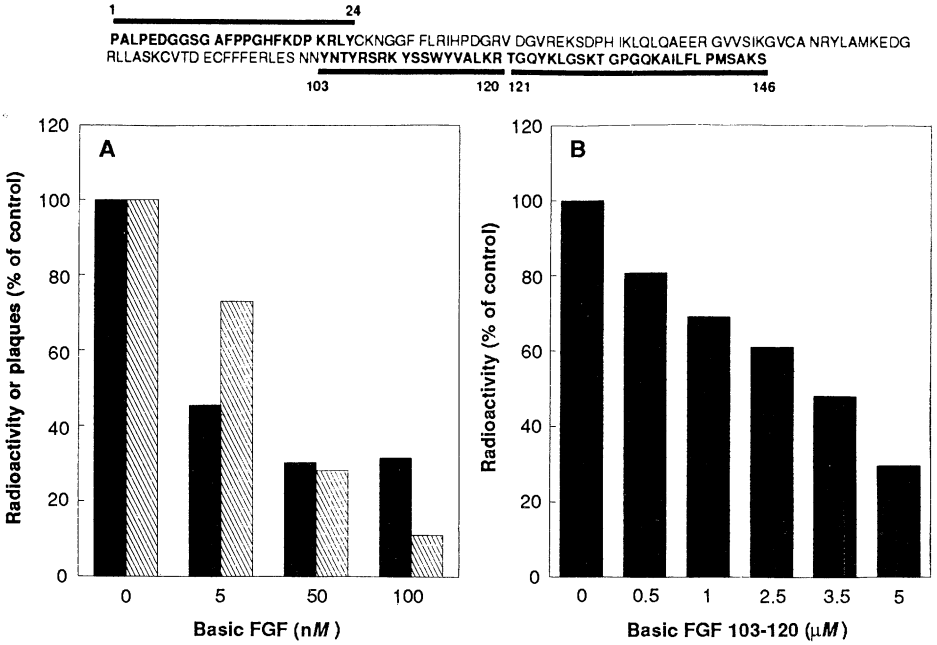

Fig. 1. Inhibition of HSV-1 uptake and infectivity by FGF and competitive polypeptide antagonists. (**A**) Bovine arterial smooth muscle cells (subpassaged three to five times) were incubated for 2 hours with purified recombinant human basic FGF and then inoculated with [^{3}H]thymidine-labeled virus at 37°C for 2 hours. To determine the uptake of virus (solid bars), we washed the cells twice with phosphate-buffered saline (PBS), and the amount of radioactivity that was cell-associated was determined by scintillation counting (*28*). Each bar represents the mean of triplicate determinations normalized to control incorporation (17,000 dpm per well). The infectivity of the virus (hatched bars) was determined by a plaque inhibition assay. Triplicate wells of bovine smooth muscle cells plated in 12-well plates were incubated with basic FGF and inoculated with HSV-1 for 2 hours, followed by three washes with PBS before the addition of an agarose overlay (*29*). Plaques were counted after incubation at 37°C for 72 hours. The results were normalized to the number of control plaques [79 ± 6 (SEM)]. The mean uptake of labeled HSV-1 and mean number of plaques of each of the basic FGF–treated groups was significantly different from the control group ($P < 0.05$) by analysis of variance. (**B**) The dose-dependent effects of basic FGF(103–120) were examined on the uptake of HSV-1. Various concentrations of basic FGF(103–120), added to the wells with labeled virus were tested for their ability to inhibit uptake of HSV-1 in a dose-dependent manner. The mean uptake of FGF(103–120)–treated and control groups were significantly different ($P < 0.05$) by analysis of variance. Basic FGF(1–24) and basic FGF(121–146) had no significant effect on HSV-1 uptake [basic FGF(1–24): 116% of control; basic FGF(121–146): 114% of control]. For comparison, the sequence of basic FGF(1–146) is presented above the panels with the sequences of interest highlighted.

R. J. Kaner, Pulmonary Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
A. Baird and R. Z. Florkiewicz, Department of Molecular and Cellular Growth Biology, The Whittier Institute for Diabetes and Endocrinology, La Jolla, CA 92037.
A. Mansukhani and C. Basilico, Department of Pathology, New York University School of Medicine, New York, NY 10016.
B. D. Summers and D. P. Hajjar, Departments of Biochemistry and Pathology, Cornell University Medical College, New York, NY 10021.

*To whom correspondence should be addressed.

FGF(103–120) that binds to the high-affinity FGF receptor, blocked this increase. Despite increased uptake of HSV-1, the transfected cells did not develop cytopathologic effects (*12*), indicating that there may be another block to viral replication. Furthermore, binding experiments at 4°C showed that 60% of HSV binding to the FGF receptor–expressing cells was sensitive to displacement by prolonged (30-min) washing with dextran sulfate (1 mg/ml) (Fig. 2B). The residual bound radioactivity was increased by greater than tenfold (disintegrations per minute) in the FGF receptor–expressing cell lines relative to controls, indicating that the virus was bound to the receptor. These data also suggest that heparan sulfate may help to stabilize the binding of virions to the receptor. By contrast, binding studies with the FGF receptor–expressing cell lines showed no differences in low-affinity (heparan sulfate) binding of ^{125}I-labeled basic FGF relative to the wild-type cell lines (*22*). These findings further support our hypothesis that HSV-1 is bound and internalized by the high-affinity receptor.

Although it is difficult to establish whether the basic FGF receptor is the only portal of entry, it clearly contributes as much as 70% (Fig. 1B) of HSV-1 uptake into vascular cells. These results are consistent with the findings of other investigators (*8, 10, 11*), who have attributed 20 to 30% of viral penetration as being "non-specific." Cross-linking studies have identified at least two forms of the basic FGF receptor having different molecular weights (*20*), and it is possible that either form can serve as a portal of cellular entry. It is also possible that HSV-1 could use other currently unidentified FGF receptors (*24*) that are related to but distinct from the *flg* protein.

The basic FGF receptors are expressed on the surface of many cells in culture (*15, 20, 24*), a finding that is consistent with the in vitro tropism of HSV-1. However, little is known regarding the distribution and expression of the basic FGF receptor in vivo that would correlate HSV-1 with its known tissue tropism in vivo. Nevertheless, one of the richest sources of basic FGF receptor is the nervous system (*25*), a well-described locus of HSV-1 infection and latency.

The concept that HSV-1 utilizes the basic FGF receptor is an extension of the recent demonstration (*1*) that HSV-1 binds heparan sulfate on the cell surface as the "initial" interaction for viral adsorption to the cell surface. This mechanism is thus almost identical to that proposed for basic FGF interactions with target cells (*25*). In the case of HSV-1, the loss by cells of infectivity after heparinase treatment (*1*) most likely reflects the decreased efficiency of virion delivery and binding to the basic FGF high-affinity receptor.

The mechanism by which the HSV-1 virion recognizes the basic FGF receptor is undefined. HSV may bind basic FGF on its surface during assembly of the virion or bind FGF released into the extracellular environment during normal cell turnover and thereby use FGF as a vehicle for binding to the receptor. In support of this concept, density gradient–purified HSV-1 tests strongly positive for basic FGF in a protein immunoblot (*19*). As has been proposed for human cytomegalovirus (*26*), this would be a mechanism where a virus interacts with a natural ligand to facilitate receptor-mediated uptake. This mechanism would also explain why heparan sulfate on the cell surface facilitates the adsorption of HSV, since FGF is a strong heparin-binding protein. Alternatively, a direct interaction between the HSV envelope glycoprotein and the FGF receptor may also contribute to the ability of HSV to penetrate cells by the FGF receptor (*27*). The availability of specific FGF receptor antagonists that can prevent HSV-1 uptake may be helpful in the development of therapeutic strategies to control HSV-1 infection and its related pathology.

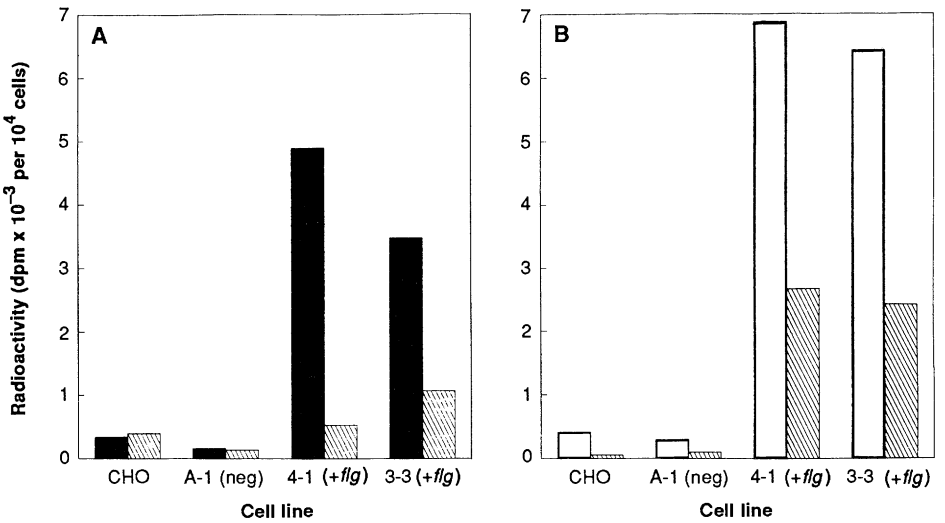

Fig. 2. Enhanced uptake and binding of radiolabeled HSV-1 by CHO cells transfected with a cDNA encoding a basic FGF receptor protein (*flg*). The A-1 cell line is composed of CHO cells transfected with the same cDNA in a reverse 3′ → 5′ orientation. The ratio of input virus to cells was far below saturation for the number of FGF receptors. Each bar represents the mean of quadruplicate samples. The results are expressed as mean disintegrations per minute (thousands) per 10^4 cells. (**A**) Viral uptake studies were performed at 37°C as described in Fig. 1 [black bars, HSV-1 alone; hatched bars, HSV plus FGF (103–120)]. Differences between cell lines expressing the FGF receptor and controls were significant ($P < 0.001$) by analysis of variance. A variety of washing procedures [30 min of shaking with dextran sulfate (1 mg/ml) at 4°C, prolonged trypsinization, or 1-min wash with acid glycine, pH 3.0 (*30*)] failed to reduce the amount of cell-associated radioactivity. These procedures were done to prove that the labeled virus was internalized and not merely adsorbed to the cell surface. (**B**) Enhanced binding of radiolabeled HSV-1 by CHO cells transfected with a cDNA encoding a basic FGF receptor protein (*flg*). Binding studies were carried out at 4°C as above, except that twice the input amount of labeled HSV-1 was used. After a 2-hour incubation with labeled HSV-1, the cells were washed for another 30 min with PBS alone (open bars) or PBS containing dextran sulfate at 1 mg/ml (hatched bars). Differences between cells expressing the FGF receptor and controls were significant ($P < 0.001$) by analysis of variance.

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27. There is a structural homology between basic FGF (91–120) and HSV envelope glycoprotein D (117–146): ECSYNKSLGACPIRTQPRWNYYDSFSAVSE, although this represents only six identical noncontiguous amino acids. This sequence of FGF contains the receptor binding domain and HSV entry blocking activity. This does not rule out other homologies at the secondary and tertiary structural level. Homologies were identified by means of the FASTA program search of HSV-1 envelope glycoproteins [W. R. Pearson and D. J. Lipman, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2444 (1988)]. The amino acid numbers for glycoprotein D are based on the sequence reported by R. J. Eisenberg, D. Long, R. Hogue-Angelletti, and G. H. Cohen [*J. Virol.* **49**, 265 (1984)].
28. The HSV-1 used in these studies was the type 1 strain F from facial vesicle and obtained from American Tissue Type Collection. For preparation of radiolabeled virus for the studies in Fig. 1, HSV-1 was propagated in human vascular endothelial cells or VERO cells (MOI, 5.0) in the presence of [^{3}H]thymidine (20 μCi/ml). Then cell lysates were chromatographed on a T10 dextran column, the void volume was centrifuged at 1000*g* for 10 min to remove cellular debris, and the viral preparation was further purified through a 0.2-mm filter. Viral preparations typically contained 10^6 dpm/ml and 10^7 plaque-forming units (PFU) per milliliter. HSV preparations purified by dextran gradient centrifugation [E. N. Cassai, M. Sarmiento, P. G. Spear, *J. Virol.* **16**, 1327 (1975)] were used in Fig. 2. The gradient-purified virus preparations typically contained 10^7 dpm/ml and 10^8 PFU/ml. Greater than 90% of the radiolabel was incorporated into DNA as assessed by trichloroacetic acid precipitation. Viral uptake experiments were performed as a modification of the procedure described (*1*), the differences being a 2-hour incubation period of virus with cells at 37°C, followed by sequential washes with phosphate-buffered saline. At this time, virtually all of the bound virus is internalized as shown by the inability of acid glycine (pH 3) (*30*) to remove any additional radioactivity. Cells were then dissolved in 0.2*N* NaOH containing 0.1% SDS and the uptake of radiolabeled virus was determined by scintillation counting. Bovine arterial smooth muscle cells were cultured as described in D. P. Hajjar *et al.* [*J. Clin. Invest.* **70**, 469 (1982)].
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Correction of CD18-Deficient Lymphocytes by Retrovirus-Mediated Gene Transfer

JAMES M. WILSON,* APRIL JOY PING, JOHN C. KRAUSS, LAURA MAYO-BOND, CLARE E. ROGERS, DONALD C. ANDERSON, ROBERT F. TODD III

Leukocyte adhesion deficiency (LAD) is an inherited disorder of leukocyte function caused by derangements in CD18 expression. The genetic and functional abnormalities in a lymphocyte cell line from a patient with LAD have been corrected by retrovirus-mediated transduction of a functional CD18 gene. Lymphocytes from patients with LAD were exposed to CD18-expressing retrovirus and enriched for cells that express CD11a and CD18 (LFA-1) on the cell surface. Molecular and functional analyses of these cells revealed (i) one copy of proviral sequence per cell, (ii) viral-directed CD18 RNA that exceeded normal endogenous levels, (iii) normal quantities of CD11a and CD18 protein on the cell surface, and (iv) reconstitution of LFA-1–dependent adhesive function.

LEUKOCYTE ADHESION DEFICIENCY (LAD) is a rare autosomal recessive disorder that is characterized by profound abnormalities in leukocyte function and recurrent, life-threatening infections (*1, 2, 3*). Leukocytes from affected patients are deficient in three transmembrane glycoproteins that mediate many adhesion-dependent interactions in the immune system. Current management of LAD patients is aimed at treating infectious episodes with appropriate antibiotics. However, many patients die of sepsis in childhood despite aggressive supportive therapy. Partial correction of leukocyte dysfunction has been achieved in a few patients after allogeneic bone marrow transplantation from partially histocompatible normal siblings (*4*).

The glycoproteins deficient in LAD are normally heterodimers formed between one of three CD11 subunits and a common CD18 subunit; they are called LFA-1 (CD11a and CD18), Mo1 (CD11b and CD18), and p150,95 (CD11c and CD18) (*5*). Each type of subunit is encoded at a different genetic focus. The first clue concerning the specific molecular defect underlying LAD came from experiments on the biosynthesis of CD11 and CD18 subunit proteins in metabolically radiolabeled cells (*6, 7*). Leukocytes from LAD patients synthesized diminished or structurally abnormal CD18 subunit molecules suggesting that the primary defect is an abnormality in CD18 expression or function. Direct support of this hypothesis was provided by analyses of DNA and RNA from LAD patients, which identified mutations in the gene encoding CD18 (6 to 8). The observation that LAD patients are deficient in the expression of all three heterodimers suggests that CD11 processing and transport to the membrane is linked to expression of normal CD18 subunits.

An alternative approach to the treatment and potential cure of LAD is somatic gene therapy (*9*). Studies in somatic cell hybrids suggest that the introduction of a functional CD18 gene into LAD cells may reconstitute CD11 and CD18 expression on the cell surface (*10*). Clinical severity in LAD is correlated with residual CD11 and CD18 expression, suggesting that significant clinical improvement may be realized with less than complete gene reconstitution (*3*). The relative success of allogeneic bone marrow transplantation in treating LAD indicates

J. M. Wilson, Howard Hughes Medical Institute, Departments of Internal Medicine and Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109–0650.
A. J. Ping, Program in Cellular and Molecular Biology, University of Michigan Medical School, Ann Arbor, MI 48109–0650.
J. C. Krauss, L. Mayo-Bond, C. E. Rogers, R. F. Todd III, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109–0650.
D. C. Anderson, Departments of Pediatrics and Cell Biology, Baylor College of Medicine, and the Speros P. Martel Leukocyte Biology Laboratory, Texas Childrens Hospital, Houston, TX 77054.

*To whom reprint requests should be addressed.