cell under conditions of high-level expression.

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Site-Directed Neovessel Formation in Vivo

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Angiogenesis is an important component of organogenesis and wound repair and occurs during the pathology of oncogenesis, atherogenesis, and other disease processes. Thus, it is important to understand the physiological mechanisms that control neovascularization, especially with methods that permit the molecular dissection of the phenomenon in vivo. Heparin-binding growth factor–1 was shown to bind to collagen type I and type IV. When complexed with gelatin, heparin-binding growth factor–1 can induce neovascularization at polypeptide concentrations that are consistent with the biological activity of the mitogen in vitro. The adsorption strategy induces rapid blood vessel formation at and between organ- and tissue-specific sites and permits recovery of the site-specific implant for examination and manipulation by molecular methods.

ANGIOGENESIS IS THE FORMATION of blood vessels in situ and involves the orderly migration, proliferation, and differentiation of vascular cells (*1, 2*). The initiation of angiogenesis by the direct stimulation of endothelial cell proliferation is the presumed responsibility of two polypeptide mitogens (*1, 2*): the class I heparin-binding growth factor (HBGF-I), also known as acidic fibroblast growth factor, and class II heparin-binding growth factor (HBGF-II) or basic fibroblast growth factor. These polypeptides are mitogens for endothelial cells in vitro (*1, 3, 4*) and angiogenesis signals in vivo (*4, 5*); they exert their biological response in vitro through high-affinity cell surface receptors (*6–8*). HBGF-I and HBGF-II are similar in structure (*9, 10*), and both are synthesized as polypeptides lacking a consensus signal peptide sequence (*11, 12*). Cells that express HBGF-I do not secrete the polypeptide in vitro (*13*). Furthermore, HBGF-II is associated with the extracellular matrix (*14*), and heparin protects HBGF-I from proteolytic inactivation (*15*). To define further the angiogenic action of HBGF-I in vivo, we used the affinity of HBGF-I for polypeptide components of the extracellular matrix in a manner that permits the construction of site-specific neovessels in vivo.

Gelatin-Sepharose and collagen type IV–Sepharose adsorb radiolabeled HBGF-I (Fig. 1). Most (approximately 80%) of the growth factor binds to immobilized gelatin and to collagen type IV and can be eluted with 1.5*M* NaCl (Fig. 1, C and G). HBGF-I labeled with ^{125}I can also be eluted with 0.5*M* NaCl (*16*) and with heparin (Fig. 1, A and E). Approximately 20% of the growth factor, which remains bound after heparin elution, can be eluted with 1.5*M* NaCl (Fig. 1, A and E). Pretreatment of the gelatin and collagen type IV matrix with heparin (50 U) significantly reduces the ability of either matrix to adsorb ^{125}I–HBGF-I (Fig. 1, B and F). Regeneration of either matrix by washing with 1.5*M* NaCl permits ^{125}I–HBGF-I adsorption (Fig. 1, B and F). Denaturation of ^{125}I–HBGF-I by heat (90°C for 1 min) significantly reduces the ability of the polypeptide to bind to immobilized gelatin and collagen type IV (Fig. 1, C and G). Furthermore, bovine serum albumin (1 mg/ml) and human fibronectin (1 mg/ml) do not elute significant quantities of ^{125}I–HBGF-I adsorbed to either matrix (Fig. 1, D and H).

Since HBGF-I binds to immobilized gelatin and collagen type IV, we evaluated the possibility that direct implantation of commercial gelatin sponges [Gelfoam (Upjohn)] treated with HBGF-I could be used to induce angiogenesis in situ. HBGF-I–treated Gelfoam was independently placed in the neck (Fig. 2, A and B) and peritoneal cavities (Fig. 2, C to F) of the rat. We observed a significant angiogenic response in situ 1 week after surgery with HBGF-I (1

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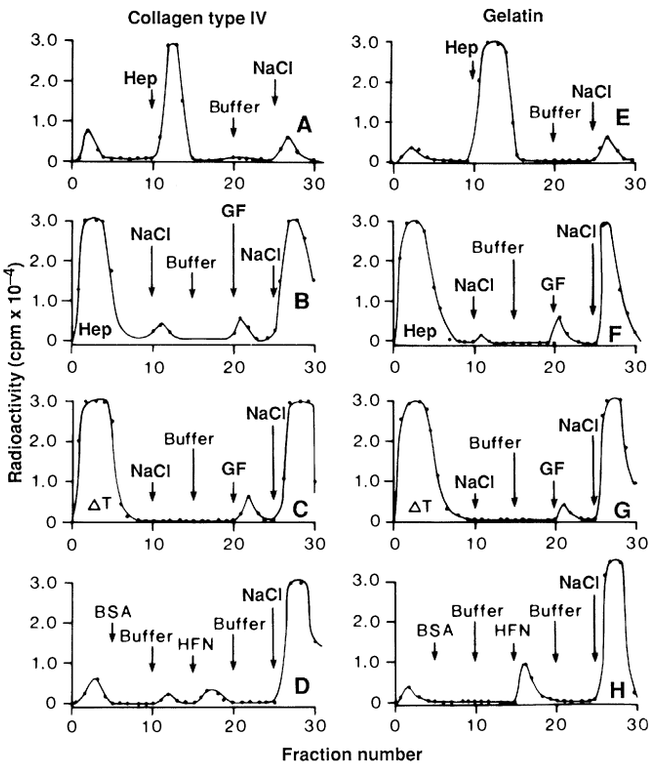

Fig. 1. HBGF-I binds to gelatin and collagen type IV. The matrix affinity procedures were performed at room temperature. Collagen type IV–Sepharose (A to D) or gelatin-Sepharose (E to H) were packed in a column (1 ml) and washed with 5 ml of 2*M* NaCl in 50 m*M* tris-HCl, *p*H 7.4, followed by exhaustively washing with 50 m*M* tris-HCl, *p*H 7.4 [adsorption buffer (AB)]. Gelatin-Sepharose was from Pharmacia, and ^{125}I–HBGF-I was prepared as described (*3*). (**A** and **E**) ^{125}I–HBGF-I (approximately 5×10^5 cpm for all experiments) in AB was added to the matrix columns (in a volume of approximately 0.1 ml) and the column was washed with AB. Elution of column-associated ^{125}I–HBGF-I (shown as GF in all profiles) was achieved with 1.5*M* NaCl in AB or 50 U of heparin (Hep) (Upjohn) in AB. The NaCl-eluted column was regenerated by washing with AB, and the heparin-eluted column was regenerated by multiple washings with 1.5*M* NaCl in AB, followed by washing with AB. (**B** and **F**) The matrix columns were treated with 100 U of heparin in AB and washed with AB. ^{125}I–HBGF-I was added to the matrix columns and washed with AB; the growth factor was eluted with 1.5*M* NaCl in AB. A large breakthrough peak was observed. The columns were washed with AB and ^{125}I–HBGF-I was added, washed with AB, and eluted with 1.5*M* NaCl in AB to demonstrate that the matrix affinity columns can be regenerated. (**C** and **G**) ^{125}I–HBGF-I was denatured (90°C for 1 min) (ΔT) and added to each column. The columns were washed with AB and eluted with 1.5*M* NaCl in AB. A large breakthrough peak was seen. The columns were regenerated as described for (B) and (F). (**D** and **H**) Columns were adsorbed with ^{125}I–HBGF-I as described for (A) and (E) and washed with AB. The columns were washed consecutively with bovine serum albumin (1 mg/ml), AB, human fibronectin (HFN) (1 mg/ml), AB, and 1.5*M* NaCl in AB.

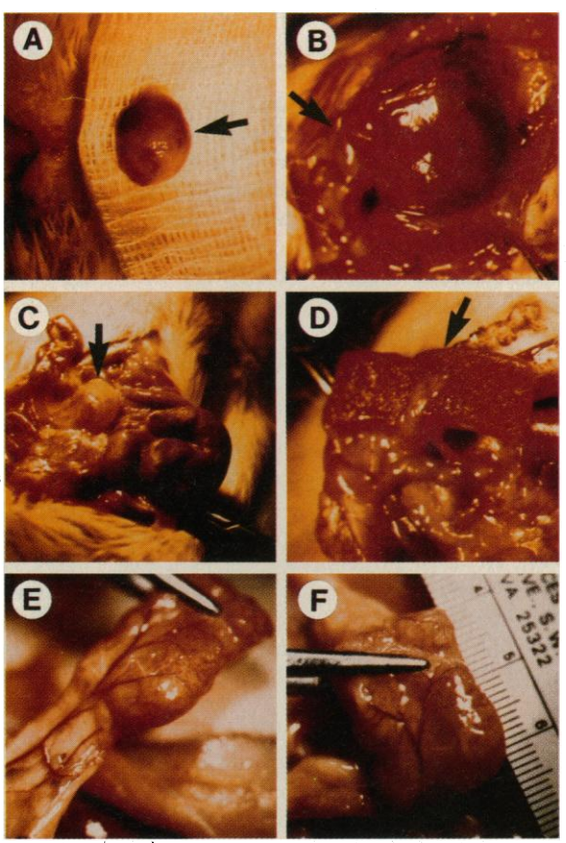

Fig. 2. The angiogenic activity of HBGF-I Gelfoam implants in vivo. The abdomen of anesthesized Lewis male rats (200 g) was washed with 70% ethanol, and an incision was made into the abdominal cavity wall to expose the abdominal cavity. Similar surgical procedures were used to access the neck cavity. Gelfoam (Upjohn) containing various amounts of HBGF-I per cubic millimeter of sponge was cut into strips (approximately 5 by 20 by 7 mm) and implanted in the neck or peritoneum. The surgical opening was closed with a staple gun, and the animals were fed a normal diet. After 1 or 2 weeks, the incision was opened and the sponge was photographed. (**A**) After 2-week implantation without HBGF-I in the neck cavity; (**B**) after 1-week implantation with HBGF-I (1 ng/mm^3) in the neck cavity; (**C**) after 2-week implantation in the peritoneum without HBGF-I; (**D**) after 2-week implantation in the peritoneum with HBGF-I (1 ng/mm^3); (**E**) after being cemented for 2 weeks to omentum with *n*-butylcyanoacrylate (*n*-BCA) with HBGF-I (1 ng/mm^3) dorsal view; and (**F**) same as (E), lateral side of implant cemented with *n*-BCA. The implant sponges were also surgically extracted, grossly examined for blood vessel formation, and prepared for histological examination.

ng/mm^3) (Fig. 2, B and D). Blood vessels, which have migrated away from the tissue site of implantation, could be observed macroscopically within the gelatin sponge (Fig. 2, E and F). Control sponges without HBGF-I (Fig. 2, A and C) and sponges treated with heparin and then with HBGF-I did not induce neovascularization after 1 or 2 weeks in vivo (*16*). The latter result is consistent with the ability of heparin to prevent HBGF-I adsorption to immobilized gelatin and collagen type IV (Fig. 1, B and F). Titration with various concentrations of HBGF-I was also performed by use of this procedure, and similar results were observed with HBGF-I concentrations of 1 to 100 ng per cubic millimeter of sponge. The concentration of HBGF-I that induces an angiogenic response in the Gelfoam implant model is significantly less than that required to induce neovascularization in other in vivo systems (*1*). Histological examination of the sponge after 1 week (Fig. 3B) and 2 weeks (Fig. 3C) in situ revealed that new blood vessel growth within the HBGF-I–treated sponge (1 ng/mm^3) was accompanied by an infiltration of lymphoid cells. Examination of control sponges 2 weeks after implant in the absence of HBGF-I did not reveal histological staining of inflammatory or vascular cells (Fig. 3A).

Since HBGF-I–treated Gelfoam was an efficient inducer of angiogenesis from the serosa, we assessed the ability of immobilized HBGF-I–treated implants to induce and sustain the process of neovascularization within the peritoneal cavity. Separate surgical implants were cemented as strips of Gelfoam to peritoneal omentum in the rat. After 2 weeks in vivo, the implants were examined for the extent of neovascularization. We observed the formation of new intact blood vessels along the HBGF-I–treated gelatin sponge cemented to the liver,

spleen, or aorta (Fig. 3, D to F). Similar results were observed with implants cemented to either the kidney, spleen, or abdominal wall. Histological examination of these implants suggested that the neovascular response was composed of cells recruited from the host organ.

The ability of HBGF-I–treated gelatin to sustain neovessel formation in the peritoneum also permitted bidirectional neovessel formation in vivo. Surgical implants cemented as strips of HBGF-I–treated gelatin between two organs or sites created a vascular bridge on the implant. Bidirectional neovessel formation was induced between the liver and the spleen 2 weeks after implantation of the HBGF-I–treated Gelfoam bridge (Fig. 3G). Similar bidirectional neovessels were observed with implants cemented from the abdominal aorta to the kidney, spleen, or abdominal wall.

HBGF-I–induced neovascularization within the peritoneal cavity was also able to sustain the survival and proliferative potential of a rat hepatocyte cell line (RL-PR-C) (*17*) simultaneously implanted with the HBGF-I–treated Gelfoam. RL-PR-C cells were transduced with a murine leukemia–based retroviral vector, N2, which contains the neomycin resistance gene (Neo^R), which codes for neomycin phosphotransferase (NPT) (*18, 19*). RL-PR-C (Neo^R) clones were identified by their ability to sustain growth in the presence of a neomycin analog, G418, by their expression of NPT, and by Neo^R gene DNA blot analysis (*20*). The NPT gene served as a marker for identifying and selecting implanted cells. RL-PR-C (Neo^R) cells were seeded on Gelfoam sponges treated with HBGF-I (1 ng/mm^3). Control sponges did not contain adsorbed HBGF-I. Separate surgical implants were cemented as a bridge between the liver and the spleen and allowed to remain in vivo for 4 to 6 weeks. The implants were removed and digested with either trypsin or collagenase to recover implanted cells. The explanted cells from the HBGF-I–treated sponges were successfully grown in vitro in the presence of G418. In contrast, few cells were recovered from control sponges and these cells displayed an abnormal phenotype and a loss of proliferative potential in vitro in the presence of G418. Histological examination of sponges containing RL-PR-C (Neo^R) cells revealed that HBGF-I induced a similar inflammatory response in these implants. These data suggest that HBGF-I–induced site-specific neovessel formation in vivo may find utility as a host and vector for the establishment of a vascular bridge between organs and genetically manipulated cells. Further, this strategy may be pertinent to issues of tissue graft and replacement and may provide a means to derive site-specific vascular cells for in vitro manipulation.

These data also demonstrate that the HBGF-I–treated gelatin implant induces neovascularization independent of the site of attachment in situ. Apparently, HBGF-I is capable of signaling the squamous mesothelial cells of the serosa and the proximal cells of the tunica adventitia to initiate angiogenesis. This is consistent with the observations that HBGF-I is a mitogen for epithelial cells, fibroblasts, and endothelial cells in vitro (*7, 8, 21*). The rat contains approximately 3×10^3 high-affinity receptors [dissociation constant (K_d) ~1.6 n*M*] on the surface of the abdominal aorta in situ (*22*). Further, like other growth factors (*23*), HBGF-I requires only partial receptor occupancy to initiate DNA synthesis in vitro (*7*). These are data consistent with the ability of HBGF-I–treated Gelfoam to induce mesothelial or endothelial cell division (or both) in vivo at HBGF-I concentrations near the K_d for the HBGF-I receptor. In addition, the potency of the HBGF-I gelatin implant in vivo may also be relevant to the issue of HBGF-I as a structure without a consensus signal peptide sequence (*11*), because low concentrations of HBGF-I expressed in an inappropriate situation as an extracellular polypeptide could initiate a premature and inopportune angiogenic response in vivo.

The ability of the HBGF-I–treated gelatin implant to coordinate site-specific neovessel formation at low concentrations of the growth factor also suggests that HBGF-I may induce the expression of genes that might serve angiogenic helper functions or may signal the release of latent HBGF-I or HBGF-II from extracellular matrix sites in situ (*14, 15, 24*). The ability of HBGF-I to bind extracellular matrix components, the resistance of HBGF-I in the presence of heparin to proteolytic modification by trypsin and plasmin (*15*), the association of HBGF-II with the extracellular matrix (*14*), and the ability of HBGF-I and HBGF-II to induce the expression of plasminogen activa-

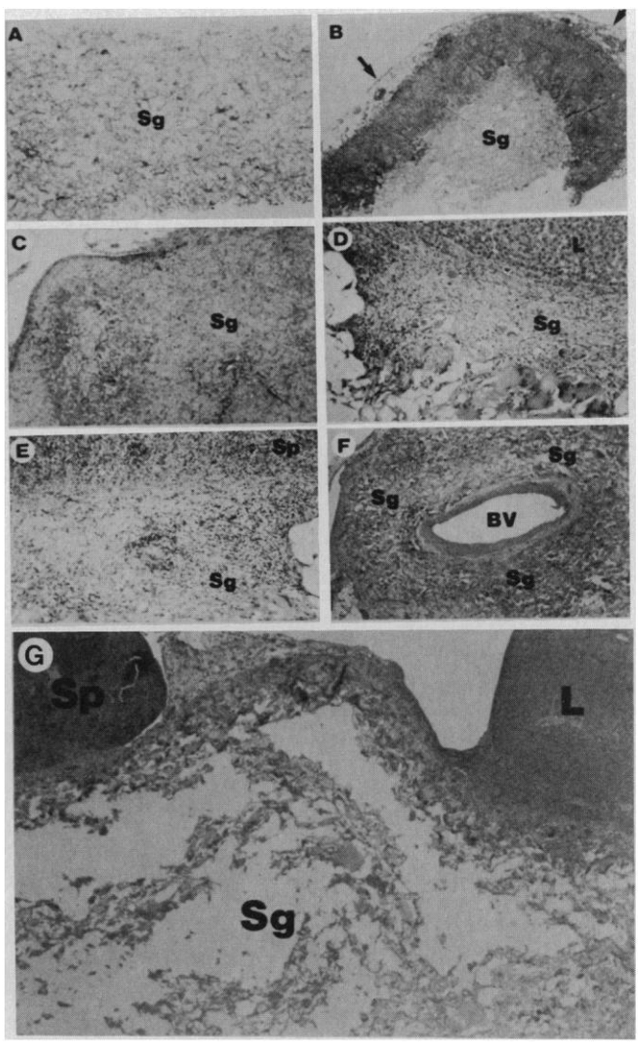

Fig. 3. Histological examination of HBGF-I–treated implants. All explants were surgically removed, fixed in 10% phosphate-buffered formalin, *p*H 7.0, and thin sections (micrometer thickness) were prepared. The sections were stained with hematoxylin and eosin and examined by light microscopy. (**A**) Control Gelfoam sponge (Sg) without HBGF-I after 2-week implantation in the peritoneal cavity (×15). No nucleated cells are seen. (**B**) HBGF-I–treated implant (Sg) after 1-week implantation in the peritoneal cavity with HBGF-I (1 ng/mm^3) (×15). Cells have invaded the sponge and there is an inflammatory response (arrows) around the periphery of the sponge. (**C**) Same as (B) but 2 weeks after implantation (×15). The entire sponge is permeated with cells. (**D**) HBGF-I–adsorbed implant (Sg) containing HBGF-I (approximately 1 ng/mm^3) after 2 weeks of being cemented to the liver (L) with *n*-BCA (×32). (**E**) Same as (D) but cemented to the spleen (Sp) (×32). (**F**) Same as in (D) and (E), but wrapped around the abdominal aorta (BV) (×15). The entire sponge is infiltrated with cells recruited from the organ. (**G**) Same as (C) but cemented as in (D) and (E) between the liver (L) and spleen (Sp) (×8). The vascular bridge is continuous between the organs.

tor and other proteases in vitro (*24*) support this possibility. In addition, the recent structural identification of the hst/KS oncogene from a human stomach tumor (*25*) and Kaposi sarcoma (*26*), and the *int*-2 oncogene from a human epithelioma (*27*) may be relevant to the issue of angiogenic helper genes because both oncogenes have significant sequence similarities to HBGF-I and HBGF-II (*2*).

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Autologous Red Cell Agglutination Assay for HIV-1 Antibodies: Simplified Test with Whole Blood

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An antibody detection procedure based on agglutination of autologous red cells has been developed for samples of whole blood. A nonagglutinating monoclonal antibody to human red blood cells conjugated to a synthetic peptide antigen (in this case residues 579 to 601 of the HIV-1 envelope precursor, Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys) permitted the detection of antibodies to the human immunodeficiency virus type 1 (HIV-1) in 10 microliters of whole blood within 2 minutes. Agglutination was specifically inhibited by addition of synthetic peptide antigen but not by unrelated peptides. The frequency of false positive results was 0.1% with HIV-1 seronegative blood donors (n = 874). The false negative results were approximately 1% (n = 81). The autologous red cell agglutination test is potentially suitable for simple, rapid, qualitative screening for antibodies to a variety of antigens of medical and veterinary diagnostic significance.

METHODS FOR DETECTION OF INdividuals infected with HIV-1 provide a vital key in attempts to curtail the spread of the virus, particularly by way of blood product contamination. In addition, the need to protect health care personnel has highlighted the demand for simple, rapid, inexpensive, and specific tests for the presence of HIV-1 antibodies.

The diagnostic tests already developed to detect HIV-1 antibodies in infected patients include a variety of enzyme-linked immunoassays (EIA), protein immunoblot (Western blot) procedures, and radioimmune precipitation assays (RIA) (*1*). Such procedures are relatively slow, requiring at least 3 hours to complete. Gelatin particle and latex agglutination tests have also been reported (*2*), and these are more rapid but cannot be used on whole blood. We sought to develop a simple qualitative procedure for use with small samples of whole blood that would take 2 min or less to complete. Historically, red cell agglutination has been a widely used technique in serology and immunology (*3*), but these procedures require the use of heterologous red cells coated with a variety of antibodies or antigens. In the test we describe here, the patient's own red cells are used to provide a detection system for antibodies. A nonagglutinating monoclonal antibody (mAb) to human red blood cells is chemically cross-linked to a synthetic peptide antigen. When this conjugate (30 μl) is added to 10 μl of the patient's blood, specific agglutination of the patient's red cells occurs in the presence of antibodies to this antigen. The conjugate reagent consisted of 0.2 mg/ml of conjugate and 2.5 mg/ml of blocking mAb in phosphate-buffered saline containing 10 m*M* sodium azide. The agglutination reaction was done on a plastic plate and scored visually after 2 min on a scale of 4 to 0, where 0 represents no agglutination.

The mAb was prepared by immunizing mice with washed human red blood cells (*4*) and selecting only those antibodies that bound to red blood cells but did not cause agglutination without the addition of antibody to mouse immunoglobulin G (IgG). In the presence of the rabbit antibody to mouse IgG, the mAb to red blood cells caused agglutination of all human red blood cells tested, including those from patients with red cell disorders, and was independent of blood group (*4*). It did not recognize red cells from other species. The synthetic pep-

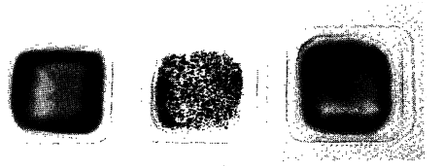

Fig. 1. Agglutination of a HIV-1 seropositive patient's red cells. The synthetic peptide–conjugated mAb to red blood cells causes agglutination (middle well). There is no agglutination in the presence of either unconjugated mAb (left) or with conjugated mAb in the presence of excess (100 μg/ml) free synthetic peptide antigen (right).

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