Eye (1991) **5**, 170–180

Neovascular Growth Factors

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Summary

Neovascularisation is the biological process of forming new blood vessels. Many conditions can initiate neovascularisation including trauma or chronic ischaemia produced by diseases such as diabetes. Neovascularisation proceeds through a series of steps beginning with destruction of the basement membrane surrounding the microvascular endothelial cells, which allows endothelial cells to extend cytoplasmic buds in the direction of chemotactic factors. Migrating endothelial cells elongate, divide and eventually form tube structures which join to form mature new capillaries.

Results of *in vitro* experiments, *in vivo* experiments, and clinical studies suggest that peptide growth factors can play key regulatory roles in each step of neovascularisation through both direct and indirect actions. At sites of vascular injuries, degranulating platelets release PDGF, IGF-I, EGF, and TGF-β. Macrophages and neutrophiles drawn into the ischaemic or injured areas synthesise and release TGF-α, TGF-β, and PDGF, and wounded endothelial cells secrete FGF. These peptide growth factors can stimulate migration, mitosis and differentiation of endothelial cells in culture and can induce neovascularisation in animal models. Clinical correlations suggest that peptide growth factors in the vitreous such as IGF-I and bFGF may promote diabetic retinopathy. As the biological mechanisms of neovascular growth factors become better understood, it may be possible to develop therapeutic approaches to selectively inhibit the peptide growth factors which regulate neovascular diseases.

Neovascularisation is the biological process of forming new blood vessels. It occurs in nearly every tissue and in a variety of normal and pathological conditions which can develop acutely, such as following trauma, or chronically, as a complication of diseases such as diabetes. Ischaemia is an important condition which contributes to initiating neovascularisation in many situations. Microscopically, neovascularisation develops as a series of sequential steps involving several types of cells with the microvascular endothelial cell having the dominant role. As reviewed by Folkman and Klagsburn,[1] new capillaries originate mainly at sites of local degradation of the basement membrane which surrounds the microvascular endothelial cells (Figure 1). Endothelial cells then extend cytoplasmic buds through the gaps in the basement membrane, elongate, and align with one another in the direction of chemotactic factors to form an endothelial capillary sprout. Mitosis of endo-

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Supported in part by funds from NIH Grant EY05587, EY07739 and the Juvenile Diabetes Foundation.

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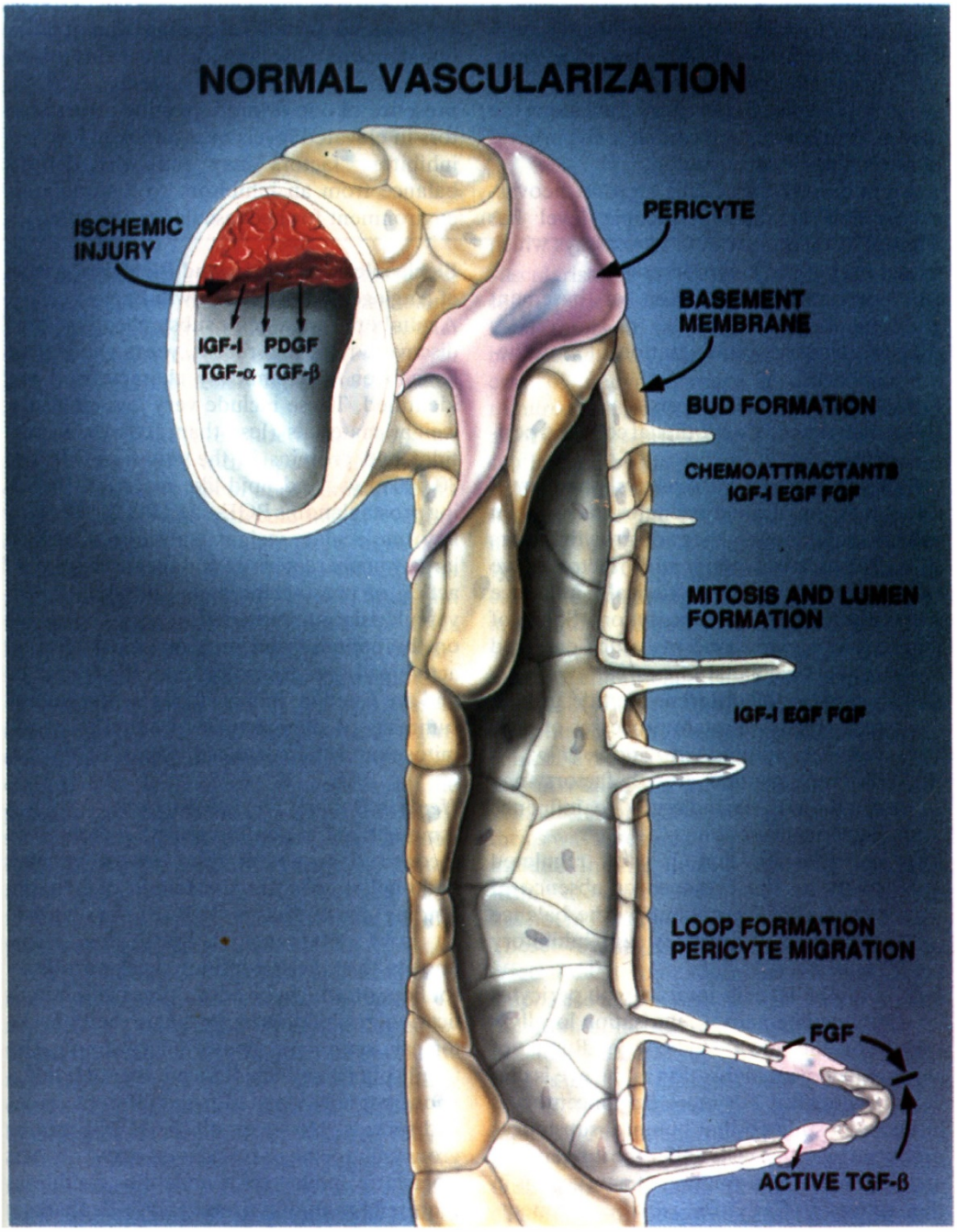

Fig. 1. *Growth factors and normal neovascularisation. Following an initial event such as ischaemic injury vascular endothelial cells extend buds through breaks in the basement membrane in the direction of chemotactic factors. The buds extend by mitosis which is stimulated by peptide growth factors, the new endothelial cells form lumens, and merge at their tips to form new capillary loops which are stabilised by inhibitory growth factors produced by pericytes when they are in contact with endothelial cells.*

thelial cells further extends the sprout and a small lumen forms by curvature within each endothelial cell. Two hollow sprouts eventually join at their tips to form a loop after which blood flow begins. Pericytes migrate along the new loop structure, and complete the process of normal neovascularisation.

An understanding of the steps of neovascularisation at the microscopic level has allowed development of *in vitro* assays which model four of the key processes:

(i) enzymatic degradation of basement membrane;
(ii) chemotaxis and migration of endothelial cells;
(iii) endothelial cell mitosis;
(iv) interaction of endothelial cells and pericytes.

Utilising these *in vitro* assays, samples of fluids and tissue homogenates have been examined, and several peptide growth factors as well as several nonpeptide factors have been identified which dramatically influence the process of neovascularisation. Some of the most important concepts which emerged from these molecular studies are:

(i) a number of different peptide growth factors can stimulate processes of neovascularisation *in vitro* and *in vivo*;
(ii) different peptide growth factors may act directly or indirectly to influence neovascularisation;
(iii) neovascularisation is not regulated solely by the presence or absence of stimulatory factors but by the balance of various stimulatory and inhibitory factors;
(iv) endothelial cells interact with pericytes to regulate neovascularisation locally.

One of the most studied conditions of abnormal neovascularisation in the eye is diabetic retinopathy. For more than a century it has been postulated that humoral substances produced by retina cells under the influence of anoxia lead to the formation of new vessels in the retina.[2,3] Early *in vitro* experiments demonstrated that aliquots of vitrectomy fluid from human eyes with neovascularisation stimulated blood vessel growth on the chick chorioallantonic membrane and stimulated endothelial cell division.[4] As vitreous samples from normal eyes were studied further, both stimulatory and inhibitory angiogenic activities were detected suggesting that it is an imbalance of these activities that leads to neovascularisation in diabetic patients. Thus, the presence of one or more peptide growth factors in elevated levels or insufficient levels of inhibitory factors may shift the delicate balance from an inhibitory to a stimulatory environment for neovascularisation.

Biochemical Properties and Biological Action of Angiogenic Peptide Growth Factors

A number of angiogenic substances have been discovered during recent years, and some have been biochemically characterised and identified. These include very low molecular weight materials (less than 100 m.w.), peptides and proteins in the range of 2,000 to 30,000 m.w., and lipid-like materials including prostaglandins PGE_1, and PGE_2. The very low molecular weight substances remain largely uncharacterised and the mechanism of action of prostaglandins in stimulating neovascularisiation remains unclear. Focussing on the peptides and proteins, most of these activities have been biochemically identified and are listed in Table I, along with a general summary of their action on endothelial cell mitoses and chemotosis *in vitro*.

Heparin Binding Growth Factors

Among the first peptide growth factors to be recognised as potent mitogens of vascular endothelial cells are the family of heparin-binding growth factors (HBGF). As reviewed by Lobb, Harper and Fett,[5] their name implies that these peptides share an affinity for heparin which, besides aiding in their purification, probably is important in their physiological action in neovascularisation. This family of factors includes a number of homogenous peptides isolated from different tissues and species. However, all the HBGF can be grouped into one of two classes based on their isoelectric point (pI) and the conditions required for elution from heparin-Sepharose.

Class 1 HBGF elute with 1 M sodium chloride at pH 7 and have pIs of approximately five. The parent molecule has 140 amino acids and a molecule weight of about 15,000. Class 1 HBGFs have been found almost exclusively in neural tissue such as brain, hypothalamus

Table I. *Properties of angiogenic proteins*

Protein	Angiogenesis *in vivo*	M.W.	Vascular Endothelial Cell Mitosis	Vascular Endothelial Cell Chemotaxis
Acidic FGF	Yes	15,000	Yes	Yes
Basic FGF	Yes	16,000	Yes	Yes
IGF-I	Yes	7,000	Yes	Yes
TGF-α/EGF	Yes	6,000	Yes	Yes
TGF-β	Yes	28,000	Inhibition	?
Angiogenin	Yes	14,400	No	?

and retina. During their isolation, these factors were given different names including acidic Fibroblast growth factor (FGF), retinal derived growth factor, eye derived growth factor and endothelial cell growth factor among others.

Class 2 HBGF are eluted from heparin-Sepharose at pH 7 with about 1.6 M sodium chloride and have pIs of approximately eight to ten. The parent molecule has 153 amino acids and a molecular weight of about 16,000. The class 2 HBGF have been isolated from pituitary, cartilage, granulosa cells, brain and vascular endothelial cells. These proteins have been designated as basic FGF or cartilage derived growth factor among others.

The parent molecules of the two classes appear to be proteolytically processed to generate the different members of the two classes. The amino acid sequences of the parent molecules of the two classes of HBGF share about 50% absolute sequence homology. Recently, the putative receptor for both acidic and basic FGFs was identified and found to be a single chain, transmembrane protein with tyrasine kinase activity.[6]

FGF is a potent mitogen for cultures of retinal derived vascular endothelial cells.[7] In addition, FGF is a chemoattractant for endothelial cells and will stimulate neovascularisation in several *in vivo* assays including corneal implant assay.[8] The production of bFGF by bovine capillary endothelial cells in culture implicates FGF in the regulation of neovascularisation.[9] In addition, FGF has been localised in the basement membrane of capillaries where it is presumed to be bound in an inactive state to heparin.[10] This observation raises the interesting possibility that, following an injury to the capillaries, latent FGF in the basement membrane is released and rapidly stimulates the initiation of neovascularisation by its action on endothelial cells, pericytes and fibroblasts. Thus, this local reservoir of latent FGF is activated immediately following injury to begin the process of neovascularisation and does not require several hours before neovascular growth factor mRNA and protein could be synthesised by viable cells in the wound site.

More direct *in vivo* experiments recently demonstrated the effect of FGF and ischaemia on retinal capillary endothelial cell DNA synthesis.[11] FGF was injected into the vitreous cavity of cats along with tritiated thymidine and mitosis of retinal vascular endothelial cells was measured by counting labelled nuclei of endothelial cells. Endothelial cells in retinas of eyes treated with FGF contained large numbers of labelled nuclei compared to control eyes. In a second experiment, local retinal ischaemia was produced by branch vein occlusion and spontaneous DNA synthesis was assessed by uptake of tritiated thymidine. Intense nuclear labelling was seen within the distribution of the occluded vein but not in the areas outside the occluded vein. These results demonstrated the powerful effects of FGF on endothelial cell mitosis and emphasise the importance of ischaemia on the initiation of localised neovascularisation.

Clinical studies also have suggested that FGF is involved in retinal neovascularisation. Levels of bFGF were recently reported in vitreous of 36 patients undergoing vitrectomy for a variety of retinal conditions including proliferative diabetic retinopathy, macular degeneration, and retinal detachment with and without proliferative vitreoretinopathy.[12] Approximately half of the 17 patients with proliferative diabetic retinopathy had elevated (>30 ng/ml) levels of bFGF in the vitreous, and six of the eight patients with active proliferative retinopathy had elevated FGF

levels compared to two of seven with inactive disease. These elevated levels of bFGF are sufficient to stimulate DNA synthesis of endothelial cells *in vitro*. Thus, FGF appears to play key roles in regulating several steps of neovascularisation.

Insulin-Like Growth Factors

Insulin-like growth factor I (IGF-I) is a single chain protein of 70 amino acids which is a member of a family of peptides that share substantial sequence homology to proinsulin. Other members of the family include insulin-like growth factor II (IGF-II) and relaxin. IGF-I, known previously as somatomedin C, is now recognised to stimulate many of the cellular effects originally attributed to the action of growth hormone.[13] Evdience has led to a dual effector theory of IGF-I and growth hormone action which proposes that growth hormone first causes cell differentiation then IGF-I acts as the mitotic signal to stimulate division of these newly differentiated cells. Thus, IGF-I may act in concert with pituitary growth hormone to influence mitosis of cells.

Serum levels of IGF-I primarily reflect hepatic synthesis which is regulated predominantly by the action of growth hormone derived from the pituitary. Unlike other peptide growth factors, IGF-I is bound in blood and other physiologic fluids to specific binding proteins which prolong the lifetime of IGF-I in blood and modulate the biological activity of IGF-I.[14–16] In blood over 80% of IGF-I is bound to a 150 kD protein whose synthesis is coordinately regulated predominately by growth hormone and 20 to 30% is bound to a 35–45 kD binding protein whose synthesis is not regulated by growth hormone.[17] While the 150 kD binding protein is confined to the circulation, IGF-I in body fluids other than plasma are associated with the 35–45 kD binding protein.[18] The presence of the 35 kD binding protein may regulate the biological activity of IGF-I.[19]

It is now recognised that most tissues of the body are capable of synthesising IGF-I mRNA and protein.[20] It is likely that locally synthesised IGF-I may be more important in regulating neovascularisation by autocrine and paracrine mechanisms rather than hepatic synthesised, systemic (serum) IGF-I.

Results from several *in vitro* studies support a role for IGF-I in retinal neovascularisation. IGF-I receptors are present on retinal microvascular cells which respond to IGF-I with a 5-fold increase in DNA synthesis.[21] IGF-I significantly promotes chemotaxis of human and bovine retinal endothelial cells and fetal bovine aorta endothelial cells in a dose dependent manner.[22] IGF-I stimulated the release of plasminogen activator of human retinal endothelial cells derived from diabetic patients but not from retinal cells derived from nondiabetic individuals.[23] Thus, IGF-I stimulates several key processes of neovascularisation including endothelial cell proliferation, migration, and secretion of proteases.

Additional evidence supporting a role of IGF-I in retinal neovascularisation is provided by clinical studies. In an initial study, Merrimee and colleagues[24] reported elevated levels of IGF-I in serum of patients with rapidly accelerating diabetic retinopathy. Subsequent studies by Kohner and colleagues[25] refined this concept by showing that patients with preproliferative retinopathy who later went on to develop neovascularisation had significantly increased serum levels of IGF-I at the time of the first appearance of new retinal vessels when compared to their serum IGF-I levels three months prior to the onset of their retinal vessel proliferation. Since vitreal levels of IGF-I may reflect the local environment of the retina more closely than serum levels, Grant and colleagues[26] measured levels of IGF-I by RIA in vitreous samples of diabetic and nondiabetic patients. They reported 2-fold higher levels of IGF-I in vitreous from 23 patients with proliferative diabetic retinopathy compared to age matched control subjects (Fig. 2). IGF-II concentrations in vitreous of diabetics and control subjects were not significantly different. Thus, clinical results provide strong support for a role of vitreal IGF-I in development of retinal neovascularisation.

Epidermal Growth Factor and Transforming Growth Factor alpha

Epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) are members of another family of angiogenic growth

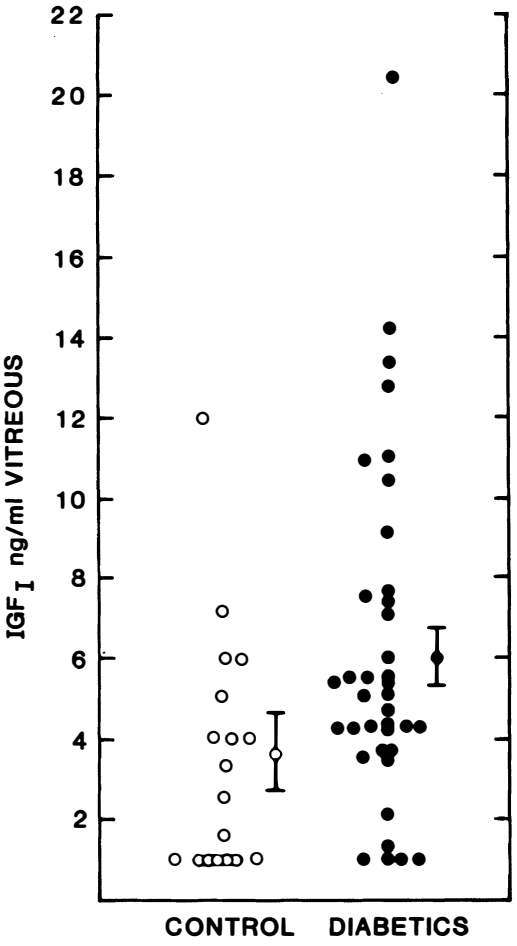

Fig. 2. *Vitreal levels of IGI-I in diabetic and nondiabetic patients. Levels of immunoreactive IGF-I were measured in vitreal samples from diabetic and age-matched diabetic patients. Reproduced from Diabetes* 1986, **35:** 416–420.

factors which also includes the proteins vaccinia growth factor and amphiregulin.[27–29] All these peptides possess a similar core structure of approximately 50 amino acids which contain three intrachain disulfide loops. The amino acid sequence homology within this core structure is significantly conserved with approximately 35% direct sequence homology between the members. All the factors can bind to and activate a 170,000 molecular weight, transmembrane, glycoprotein receptor which possesses growth factor-activated tyrosine kinase activity in the cytoplasmic domain. Site directed mutations of the ATP binding site in the kinase domain have shown that all the major biological actions of the EGF receptor depend on the tyrosine kinase activity of the receptor.[30]

Several physiological roles for EGF and TGF-α have been proposed. Both are potent mitogens for a variety of different types of cells in culture including keratinocytes and fibroblasts. TGF-α mRNA and protein, as well as the EGF/TGF-α receptor, are present in the basal layer of epidermal cells of the skin which suggests that TGF-α is the primary factor responsible for normal growth of the epidermis.[31] EGF and TGF-α are potent inhibitors of gastric acid secretion and stimulate mitosis of intestinal epithelium.

EGF and TGF-α also have angiogenic actions. EGF stimulated neovascularisation in rabbit corneas when formulated in slow release polymers and placed in corneal pockets,[32] and EGF stimulated neovascularisation of embryonic chick membranes.[33] Both EGF and TGF-α stimulated neovascularisation in the hamster cheek pouch assay,[34] and were potent chemoattractants for rat heart endothelial cells in culture.[35] Murine lung microvascular endothelial cells and bovine pulmonary artery endothelial cells bound EGF and TGF-α specifically, and increased DNA synthesis two to six fold in response to EGF and TGF-α.[34] EGF/TGF-α receptors also were detected in bovine retinal vessels by autoradiography of ^{125}I-EGF binding, and TGF-α protein and mRNA were detected in bovine retina (Fig. 3).[36] Thus, EGF and TGF-α stimulate neovascularisation using *in vitro* and *in vivo* models. However, additional research needs to be performed to establish what role, if any, they play in vascular proliferative diseases such as diabetic retinopathies.

Transforming Growth Factor beta

The newest family of neovascular peptide growth factors is the transforming growth factor beta family.[37,38] TGF-β1 was originally purified from platelets and found to be a dimeric peptide composed of identical 112-amino acid subunits which are linked by disulfide bonds. Each monomer of the 25 kDA dimer is synthesised as a larger 390 amino acid pre-pro-TGF molecule that is proteolytically processed to a 361 amino acid pro-TGF-β

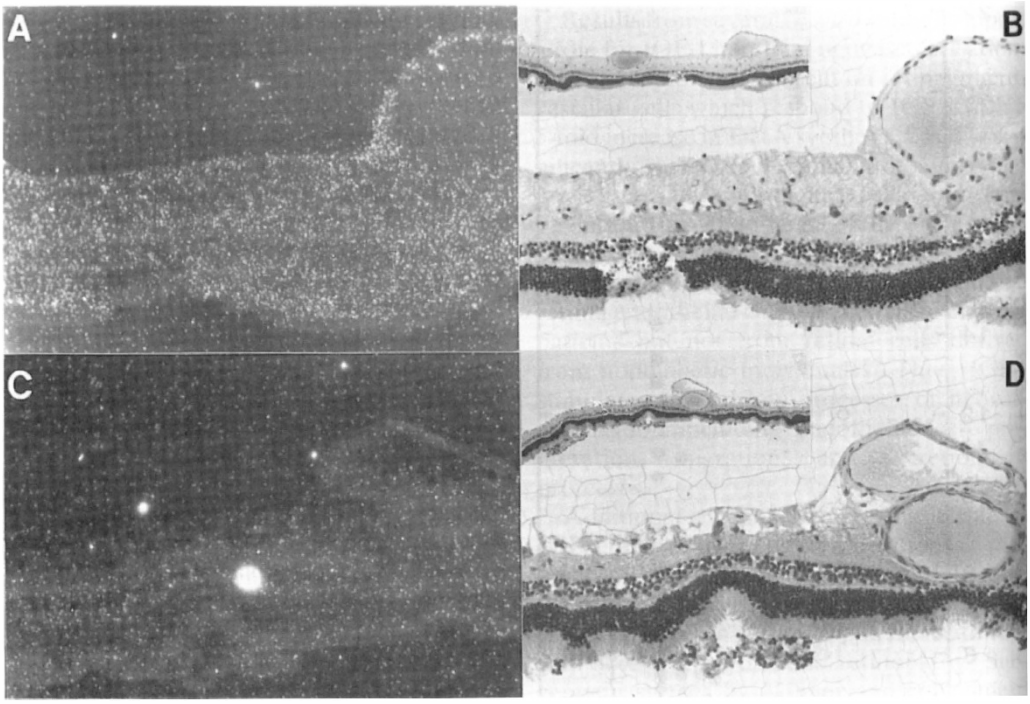

Fig. 3. *Autoradiography of ^{125}I-EGF binding to bovine retina. Specific binding of ^{125}I-EGF to EGF/TGF α receptors is represented by the difference in grains of total binding specimens (Panels A and B) and nonspecific binding specimens (Panels C and D). Dark field photographs (Panels A and C) and bright field photographs (Panels B and D) show autoradiographic grains located over retinal vascular endothelial cells cells in neural retina. Reproduced from Invest Ophthalmol Vis Sci* 1989, **30:** 1916–22.

form that dimerises to form a latent TGF-β dimer. Activation of the latent TGF-β dimer *in vivo* may be accomplished by proteolytic nicking by plasmin within the amino-terminal segment of the pro-TGF-β monomers which causes a disruption of tertiary structure and noncovalent bonds that releases the active 25 kD TGF-β.[39] It is now recognised that TGF-β1 belongs to a larger family of closely related genes that includes two closely related proteins in humans designated TGF-β2 and TGF-β3. Evaluations of recombinant TGF-β's indicate that the proteins have very similar although not identical biological actions.

TGF-β1 can stimulate neovascularisation *in vitro*. Injection of TGF-β1 under the skin of mice led to fibrosis and neovascularisation at the site of injection which was characterised by an increase in macrophages, fibroblasts, and collagen formation.[40] In contrast to these *in vivo* results, TGF-β1 strongly inhibited bFGF-induced proliferation and motility of fetal bovine heart endothelial cells in culture.[41] It is possible that the paradoxical effects of TGF-β1 *in vivo* are a result of an indirect effect of TGF-β1 on inflammatory cells *in vivo*. TGF-β1 is an extremely potent chemoattractant (0.1 to 10 pg/ml) for peripheral blood monocytes at levels of TGF-β1 which are approximately 1,000 fold lower than the concentration reported to reduce DNA synthesis of heart endothelial cells *in vitro*.[42] Macrophages have been shown to synthesise and secrete angiogenic peptide growth factors such as TGF-α when activated.[43] Thus, it is possible that TGF-β1 initially attracted macrophages into the site of injection while also inhibiting endothelial cell division. Subsequently, as the level of TGF-β decreases due to diffusion and degradation, the growth factors secreted by the macrophages stimulated neovascularisation.

The local effects of TGF-β on neovascularisation was also studied using an *in vitro* coculture system to mimic the interactions of

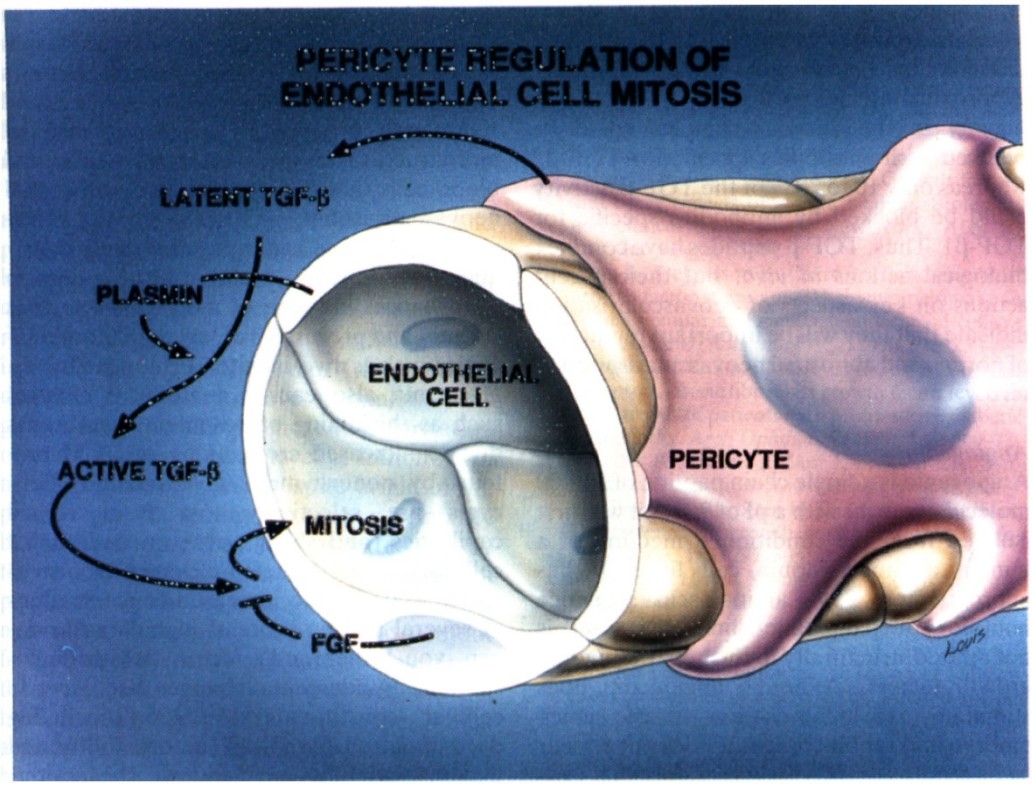

Fig. 4. *Pericyte regulation of endothelial cell mitosis. Pericytes synthesise and secrete latent TGF-β which is activated by endothelial cells when they are in contact. Active TGF-β blocks FGF-induced mitosis of endothelial cells.*

endothelial cells and cells of the vessel wall.[44,45] Conditioned medium from cultures of pericytes, smooth muscle cells, or endothelial cells which were grown independently or in co-cultures wells that prevented cell contact did not inhibit endothelial cell growth. In contrast, conditioned medium from co-cultures of endothelial cells and pericytes grown in contact was strongly inhibitory to endothelial cell growth. Acid activation of conditioned medium from cultures of pericytes strongly inhibited endothelial cell growth indicating the presence of a latent inhibitor. Furthermore, either immunoadsorption of TGF-β from conditioned medium of co-cultures or addition of neutralising anti-TGF-β antibody to co-cultures completely prevented pericyte-mediated inhibition of endothelial cell growth. These results suggest that growth of endothelial cells *in vivo* may be regulated by contact between pericytes and endothelial cells (Fig. 4). Under normal conditions, latent TGF-β produced by pericytes appears to be activated by endothelial cells and acts to limit endothelial cell mitosis. If close contact between endothelial cells and pericytes is lost, activation of latent TGF-β produced by pericytes is lost, and growth factors such as bFGF which are produced by endothelial cells act to stimulate endothelial cell mitosis and migration. It is possible that inhibitory factors like TGF-β are released from cells following laser treatment of neovascularised regions of the retina which contribute to the anti-angiogenic effect of laser treatments for retinal neovascularisation.

Although TGF-β is perhaps best known for its ability to inhibit growth of many types of cells, TFG-β also stimulates *in vitro* growth of fibroblasts and synthesis of collagen.[40,46] These actions have suggested that TGF-β may play a role in proliferative vitreoretinopathy (PVR). Vitreous aspirates from eyes with intraocular fibrosis associated with PVR had

three times the average level of TGF-β than vitreous aspirates from eyes with uncomplicated retinal detachments without fibrosis.[47] Approximately 80 to 100% of the TGF-β activity in the vitreous samples could be blocked with antibodies specific for TGF-β2 whereas only 10 to 20% of the TGF-β activity could be blocked by antibodies specific for TGF-β1. Thus, TGF-β peptides have complex biological actions *in vivo,* but their potent actions on key aspects of neovascularisation indicate that they will be important regulators of normal and abnormal neovascularisation *in vivo.*

Angiogenin

Angiogenin is a single chain peptide of 14,400 molecular weight with a pI of 9.5 that was first isolated from the conditioned medium of a human adenocarcinoma cell line.[1] It has approximately 35% amino acid sequence homology to pancreatic ribonuclease and is not related structurally to the heparin binding growth factors. Although angiogenin does stimulate neovascularisation in the chick embryo and rabbit cornea, it does not appear to be a mitogen for vascular endothelial cells *in vitro.* Since the target cell for angiogenin is not known, its mechanism of action has not yet been established and it remains to be determined whether angiogenin stimulates neovascularisation directly or indirectly by stimulating other cells to release angiogenic factors.

Pathological Neovascularisation and Peptide Growth Factors

Understanding the roles various peptide growth factors play in regulating normal neovascularisation is far from complete, but substantial progress has been made in establishing a molecular basis for many of the steps of neovascularisation which were previously understood only at a microscopic level. The ultimate clinical goal, however, is to prevent pathological neovascularisation from occurring. To accomplish this goal, it will be necessary to understand how the normal actions of peptide growth factor have been perturbed in the environment of a disease to ultimately cause pathological neovascularisation.

Focussing on diabetic retinopathy as an example of pathological neovascularisation, it is clear that fundamental biochemical changes begin early in the course of the diabetes and relentlessly progress to generate functional and structural changes in retinal tissue and blood cells that eventually initiate abnormal neovascularisation. As reviewed by Merrimee,[48] prolonged hyperglcaemia causes alterations in glucose metabolism leading to disturbances in levels of metabolites such as sorbitol and precursors of key regulatory molecules such as myo-inositol. Prolonged hyperglycaemia also causes anatomical changes such as thickening of basement membranes due to increased crosslinking of ECM proteins by nonenzymatic glycosylation. Pericytes are selectively lost from retinal capillaries, and sheer stress on endothelial cells is increased due to elevated blood viscosity secondary to increased concentrations of several plasma proteins including fibrinogen, von Willebrand's factor, and thrombospondin. Fundamental changes also occur in cellular constituents of blood including decreased red blood cell deformability and hypercoagulability of platelets. These alterations in extracellular matrix, blood cells, pericytes and endothelial cells combine to create an environment of low perfusion and increased capillary permeability which eventually result in anoxia, injury to the retina, and leakage of blood proteins into the retina and finally into the vitreous. The retina appears to respond to this anoxia and injury by attempting to form new capillaries and reestablish normal blood perfusion.

Peptide growth factors probably function late in the temporal sequence of the development of diabetic retinopathy. It seems clear that peptide growth factors do not initiate the pathology which eventually leads to the anoxia and retinal injury. It seems equally clear, however, that peptide growth factors, which may be produced in the injured areas of the retina, act to stimulate the local neovascularisation as they do in response to anoxia or injury under normal conditions. However, these new vessels have abberrant structure which results in abnormal function due to the lack of functional tight junction complexes, absence of pericytes, and scant basement

membrane. The abberrant capillary tufts are very susceptible to leakage and rupture with minimal provocation resulting in vitreous haemorrhage. This induces more stimulation by growth factors and more abberrant neovascularisation.

As we begin to understand more about the role of peptide growth factors in normal and pathological neovascularisation it may become possible actively to intervene and control the process. Several key questions remain unanswered such as whether the retinal neovascularisation that accompanies diabetes results from over stimulation by growth factors which promote vascularisation or due to an insufficient level of inhibitors of neovascularisation. What is the origin of the peptide growth factors? Are they primarily blood born or are they synthesised locally by the retinal vascular endothelial cells, retinal pigmented epithelial cells, or glial cells? Can neovascularisation be accomplished without formation of defective capillaries? Answers to these and other questions may become the basis for new therapeutic approaches which are now possible because of the availability of biochemically well-characterised neovascular peptide growth factors.

Key words: Angiogenesis, growth factors, ischemia, neovascularisation.

References

[1] Folkman J and Klagsbrun M: Angiogenic factors. *Science* 1987, **235:** 442–7.

[2] Ashton N: Neovascularisation in ocular disease. *Trans Ophthalmol Soc* 1961, **81:** 145–61.

[3] Michaelson IC: The mode of development of retina vessels. *Trans Ophthalmol Soc* 1968, **68:** 137–80.

[4] Raymond L and Jacobsen B: Isolation and identification of stimulatory and inhibitory cell growth factors in bovine vitreous. *Exp Eye Res* 1982, **34:** 267–86.

[5] Lobb RR, Harper JW, Fett JW: Purification of heparin-binding growth factors. *Analyt Biochem* 1986, **154:** 1–14.

[6] Neufeld G and Gospodarowicz D: The identification and partial characterisation of the fibroblast growth factor receptor of baby hamster kidney cells. *J Biolog Chem* 1985, **260:** 13860–8.

[7] Gospodarowicz D, Massoglia S, Cheng J, Fujii DK: Effect of retina-derived basic and acidic fibroblast growth factor and lipoproteins on the proliferation of retina-derived capillary endothelial cells. *Exp Eye Res* 1986, **43:** 450–76.

[8] Gospodarowicz D, Bialecki H, Thakral T: The angiogenic activity of the fibroblast and epidermal growth factor. *Exp Eye Res* 1979, **28:** 501–14.

[9] McNeil PL, Muthukrishnan L, Warder E, D'Amore PA: Growth factors are released by mechanically wounded endothelial cells. *J Cell Biol* 1989, **109:** 811–22.

[10] Klagsburn M and Vlodavsky I: Biosynthesis and storage of basic fibroblast growth factor (bFGF) by endothelial cells: Implication for the mechanism of action of angiogenesis. Growth factors and other aspects of wound healing: Biological and clinical implications. New York, Alan R. Liss, Inc., 1988, 55–61.

[11] de Juan E, Stefansson E, Ohiro A: Basic fibroblast growth factor stimulates 3H-thymidine uptake in retinal venular and capillary endothelial cells *in vivo*. *Invest Ophthalmol Vis Sci* 1990, **31:** 1238–44.

[12] Sivalingam A, Kenney J, Brown GC, Benson WE, Donoso L: Basic fibroblast growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. *Arch Ophthalmol* 1990, **108:** 869–72.

[13] Schwarder JC, Hauri C, Zapf J, Froesch ER: Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: Dependence on growth hormone status. *Endocrinol* 1983, **113:** 297–305.

[14] Daughaday WH and Kipnis DM: The growth-promoting and anti-insulin actions of somatotropin. *Recent Prog Horm Res* 1966, **22:** 49–93.

[15] Zapf J, Schoenle E, Jagars G, Sand I, Grundwald J, Froesch ER: Inhibition of the action of nonsuppressible insulin-like activity on isolated rat fat cells by binding to its carriers protein. *J Clin Invest* 1979, **63:** 1077–84.

[16] Elgin RG, Busby WH, Clemmons DR: An insulin-like growth factor (IGF) binding protein enhances the biological reponse to IGF 1. *Proc Natl Acad Sci USA* 1986, **84:** 3254–8.

[17] Hintz RL and Liu F: Somatomedin plasma binding proteins. In: Pecile A, Muller EE, eds. Growth hormone and other biologically active peptides. Amsterdam: Excerpta Medica, 1980, 133–143.

[18] Cohen KL and Nissley SP: The serum half-life of Somatomedin activity: Evidence for growth hormone dependency. *Acta Endocrinol* 1979, **83:** 243–58.

[19] Povoa G, Hall K, Collins VP: Studies on Somatomedin binding proteins: In: Muller EE, Cocch D. Advances in growth hormone and growth factor research, Berlin-Heidelberg Springer Verlag. 1989, 121–132.

[20] D'Ercole AJ, Stiles AD, Underwood LE: Tissue concentrations of Somatomedin C: Further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci USA* 1984, **81:** 935–9.

[21] King GL, Goodman AD, Bosnex S, Moses A, Kahn CR: Receptors and growth promoting effects of insulin and IGF 1 on cells from bovine retinal capillaries and aorta. *J Clin Invest* 1985, **75:** 1028.

[22] Grant M, Jerdan J, Merimee TJ: Insulin-like growth factor-I modulates endothelial cell chemotaxis. *J Clin Endocrin Metab* 1987, **65:** 370–1.

[23] Grant MB and Gary C: Plasminogen activator production by Human retina endothelial cells of non-diabetic and diabetic origin. *Invest Ophthalmol Vis Sci* 1991, **32:** 53–63.

[24] Merimee TJ, Zapf J, Froesch ER: Insulin-line growth factors: Studies in diabetics with and without retinopathy. *N Engl J Med* 1983, **309:** 527–30.
[25] Hyer SL, Sharp PS, Brooks RA, Burrin JM, Kohner EM: A two-year follow-up study of serum insulin-like growth factor-I in diabetes with retinopathy. *Metabolism* 1989, **38:** 586–9.
[26] Grant M, Burrell B, Fitzgerald C, Merimee TJ: Insulin-like growth factors in vitreous: studies in control and diabetic subjects with neovascularisation. *Diabetes* 1986, **35:** 416–20.
[27] Burgess AW: Epidermal growth factor and transforming growth factor α. *Br Med Bull* 1989, **45:** 401–24.
[28] Brown JP, Twardzik Dr, Marquardt H, Todaro J: Vaccinia virus encodes a polypeptide homologous to epidermal growth factor and transforming growth factor. *Nature* 1985, **313:** 491–2.
[29] Shoyab M, Plowman GD, McDonald VL, Bradley JG, Todaro GJ: Structure and function of human amphiregulin: A member of the epidermal growth factor family. *Science* 1989, **243:** 1075–6.
[30] Chen WS, Lazar CS, Poenie M, Tsien RY, Gill GN, Rosenfeld MG: Requirement for intrinsic protein tyrosine kinase in the immediate and late actions of the EGF receptor. *Nature* 1987, **328:** 820–3.
[31] Coffey RJ, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, Pittelkow MR: Production and auto-induction of transforming growth factor α in human keratinocytes. *Nature* 1987, **328:** 817–20.
[32] Gospodarowicz D, Bialecki H, Thakral TK: The angiogenic activity of the fibroblast and epidermal growth factor. *Exp Eye Res* 1979, **28:** 501–14.
[33] Steward R, Nelson J, Wilson DJ: Epidermal growth factor promotes chick embryonic angiogenesis. *Cell Biol Inter Reports* 1989, **13:** 957–65.
[34] Schreiber AB, Winkler ME, Derynck R: Transforming growth factor-α: A more potent angiogenic mediator than epidermal growth factor. *Science* 1986, **232:** 1250–3.
[35] Grotendorst GR, Soma Y, Takehara K, Charett M: EGF and TGF-alpha are potent chemoattractants for endothelial cells and EGF-like peptides are present at sites of tissue regulation. *J Cell Physiol* 1989, **139:** 617–23.
[36] Fassio JB, Brockman EB, Jumblatt M, Greaton C, Henry JL, Geoghegan TE, Barr C, Schultz GS: Transforming growth factor alpha and its receptor in neural retina. *Invest Ophthal Vis Sci* 1989, **30:** 1916–22.
[37] Roberts AB and Sporn MB: The transforming growth factor-χs. In Piez KA and Sporn MB eds. Transforming growth factor-βs. New York: New York Academy of Sciences 1990, 1–6.
[38] Lyons RM and Moses HL: Transforming growth factors and the regulation of cell proliferation. *Eur J Biochem* 1990, **187:** 467–73.
[39] Lyons RM, Gentry LE, Purchio AF, Moses HL: Mechanism of activation of latent recombinant transforming growth factor β1 by plasmin. *J Cell Biol* 1990, **110:** 1361–7.
[40] Roberts AB, Sporn MB, Assoian RK, Smith JM, Roche NS, Wakefield LM, Heine UI, Liotta LA, Falanga V, Kehrl JH, Fauci AS: Transforming growth factor type B: Rapid induction of fibrosis and angiogenesis *in vivo* and stimulation of collagen formation *in vitro*. *Proc Natl Acad Sci* 1986, **83:** 4167–71.
[41] Muller G, Behrens J, Nussbaumer U, Bohlen P, Birchmeier W: Inhibitory action of transforming growth factor β on endothelial cells. *Proc Natl Acad Sci* 1987, **84:** 5600–04.
[42] Wahl SM, Hunt DA, Wakefield LM, McCartney-Francis N, Wahl LM, Roberts AB, Sporn MB: Transforming growth factor type β. *Proc natl Acad Sci* 1987, **84:** 5788–92.
[43] Rappolee DA, Mark D, Banda MJ, Webb Z. Wound macrophages express TGF-α and other growth factors *in vivo*: Analysis by mRNA phenotyping. *Science* 1988, **241:** 708–12.
[44] Orlidge-Antonelli A, Saunders KB, Smith SR, D'Amore PA: An activated form of transforming growth factor β is produced by co-cultures of endothelial cells and pericytes. *Proc Natl Acad Sci* 1989, **86:** 4544– 8.
[45] Orlidge-Antonelli A, Smith SR, D'Amore PA: Influence of pericytes on capillary endothelial cell growth. *Am Rev Respir Dis* 1989, **140:** 1129–31.
[46] Moses HL, Tucker RF, Leof EB, Coffey RJ, Halper J, Shipley GD: Type-β transforming growth factor is a growth stimulator and a growth inhibitor. *Cancer Cells* 1985, **3:** 65–71.
[47] Connor TB, Roberts AB, Sporn MB, Danielpour D, Dart LL, Michels RG, de Bustros S, Enger C, Kato H, Lansing M, Hayashi H, Glaser BM: Correlation of fibrosis and transforming growth factor-β type 2 levels in the eye. *J Clin Invest* 1989, **83:** 1661–6.
[48] Merimee TJ: Diabetic retinopathy a synthesis of perspectives. *N Eng J Med* 1990, **322:** 978–83.