REVIEW

Angiogenesis and rheumatoid arthritis: pathogenic and therapeutic implications

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Rheumatoid arthritis can be considered as one of the family of 'angiogenesis dependent diseases'. Angiogenesis in rheumatoid arthritis is controlled by a variety of factors found in the synovial fluid and pannus tissue. Modulation of the angiogenic component of the disease may alter the pathogenesis of the condition, and subsequent cartilage and joint destruction, by reducing the area of the endothelium in the pannus and restricting pannus growth. Current therapeutic strategies exert, to varying extents, an inhibitory effect on the angiogenic process. In particular, the mode of action of the slow acting antirheumatic drugs may be due to their effect on the angiogenic response. The development of novel angiostatic treatments for chronic inflammatory joint disease may lead to a new therapeutic approach in controlling disease progression.

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Angiogenesis, the development of new blood vessels, is an integral part of the body's physiology. It has a role in normal function—for example, in embryogenesis and in the menstrual cycle, but it is also important in pathological states. It may be beneficial as in wound healing, but may also contribute to the pathogenesis of some conditions—for example, tumour growth, neovascular glaucoma, and rheumatoid arthritis. Such diverse conditions may be grouped together as 'angiogenesis dependent diseases', and modulation of the angiogenic component in their pathogenesis may be used to control their progression.¹ One important aspect in the study of rheumatoid arthritis is the investigation of the potential role of angiogenesis in cartilage damage and the progressive joint destruction which characterises the disease. This review outlines the regulation of angiogenesis, associated aspects of endothelial cell function, the relation to the pathogenesis of rheumatoid arthritis, including cartilage damage, and the potential therapeutic implications.

The angiogenic response

New vascular networks are generated through a number of stages that follow a set programme, which is apparently independent of the source of the stimulus.² The process consists of seven distinct steps, which are summarised in fig 1.

Regulation of angiogenesis

Angiogenesis is controlled by a variety of factors, most of which are small proteins. They include a variety of growth factors, prostaglandins, and some low molecular weight compounds.

HEPARIN BINDING GROWTH FACTORS

As a result of studies on tumour angiogenesis it became clear that some growth factors could be identified by their affinity to heparin.³ A large number of angiogens have been isolated and studied as a result of this; they fall into two classes. Class I heparin binding growth factors have been shown to be anionic, 15–17 000 M_r, and found in high levels in neural tissue. They include acidic fibroblast growth factor, endothelial cell growth factor,⁴ retina derived growth factor,⁵ and eye derived growth factor II.⁶ Class II heparin binding growth factors are mainly cationic with an M_r of 16–18 500. They seem to be variants of basic fibroblast growth factor⁷ and have been isolated from the pituitary gland, brain, hypothalamus, eye, cartilage, bone, adrenal gland, kidney, macrophages, placenta, and tumour cells.⁸ All of these factors stimulate angiogenic processes in vitro and are angiogenic in vivo.

These growth factors have not been shown to possess signal sequences to allow their secretion and are held in their source cells.⁹ They are found bound in large quantities in the extracellular matrix.¹⁰ The regulation and control of their release into the extracellular environment from cells producing these factors is as yet unknown. It is thought that this extracellular matrix store is mobilised in the angiogenic response by the production of proteases, such as heparanase and plasmin, by endothelial cells.¹¹ Tissue type plasminogen activator is produced by endothelial cells¹² and will enhance their migration,¹³ and migrating endothelial cells will elaborate urokinase type plasminogen activator.¹⁴ Support for a role for plasminogen activator in the process is seen in the case of basic fibroblast growth factor, which causes the elaboration of both tissue type and urokinase type plasminogen activators.¹⁵ In some circumstances, such as in the rabbit cornea,¹⁶ urokinase type plasminogen activator induces neovascularisation, and in vitro it interacts with urokinase type plasminogen activator receptors on the endothelial cell surface to stimulate migration in a Boyden chamber assay.¹⁷

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Binding of these factors to cell and extracellular matrix macromolecules greatly enhances their stability and resistance to proteolytic destruction.¹⁸

ANGIOGENIN

Angiogenin was first isolated from the conditioned medium of a human adenocarcinoma cell line and stimulates angiogenesis at 0.5–290 ng in the chick embryo and at 50 ng in the rabbit cornea.¹⁹ It is a single chain, with an M_r of 14 400. It has been sequenced and cloned and shows 35% absolute homology to a family of ribonucleases.²⁰ It is generally inactive in this role but cleaves the RNA of ribosomes.²¹ Blockade of this activity inhibits the angiogenic response induced by this molecule.²² Angiogenin will not bind to heparin and unlike the heparin binding growth factors is secreted into surroundings but not bound by the extracellular matrix.²³ It is also non-mitogenic for endothelial cells and its mode of action is unknown.

Possibly, it acts by releasing angiogenic factors from host cells in tissue or immune cells such as the macrophage.

VASCULAR PERMEABILITY FACTOR

Vascular permeability factor has similarly been isolated from tumour cell lines in culture.²⁴ It has sequence homology to the β chain of platelet derived growth factor²⁵ and localises specifically to endothelial cells, unlike most other factors. It stimulates angiogenesis both in vivo and in vitro.²⁶

INTERFERON γ

Interferon γ is a dimer of M_r 50 000 produced by the cells of lymphoid origin. It inhibits proliferation of endothelial cells when given with endothelial cell growth factor,²⁷ endothelial cell migration, and in vitro angiogenesis.²⁸ It also induces a morphological change. The effects are reversible and seem to be effective through decreasing the number of binding sites for endothelial cell growth factor.²⁷ It will also downregulate epidermal growth factor²⁹ and other receptors.

TRANSFORMING GROWTH FACTORS

Transforming growth factors were originally isolated from virally transformed rodent cells and induce this transformation in unaffected cells.³⁰ They exist in two classes: transforming growth factors α and β . Transforming growth factor α is a 50 amino acid polypeptide synthesised by transformed cells. It is about 35% homologous to epidermal growth factor and binds to the receptor for this.³¹ It stimulates endothelial cell proliferation at 1–5 ng/ml in culture as does epidermal growth factor,³² though its angiogenic potential is much greater. It seems, however, to be less active than heparin binding growth factors and angiogenin.

Transforming growth factor β is a dimer of two identical 112 amino acid chains found in tumours and nearly all normal cells, but in particularly high levels in macrophages and platelets.³³ It induces an increase in macrophage number at the site of injection, fibroblast recruitment, collagen production, and vascularisation.³⁴ It is angiogenically active in nanogram quantities in the rabbit cornea.³⁵ The effect of transforming growth factor β on angiogenesis in vitro is analogous to that of heparin in that it inhibits proliferation in endothelial cell cultures.³⁶ It antagonises the effects of other growth factors which stimulate endothelial proliferation. Its angiogenic activity in vivo in various models may reflect an indirect action on other cell types, principally macrophages.³⁵ At low concentrations, in the chorioallantoic membrane model, transforming growth factor β causes an inflammatory reaction leading indirectly to an angiogenic response. At higher concentrations it inhibits an angiogenic response in response to growth factors. Its physiological role may be in switching off the angiogenic process and resolution of wound and granulomatous tissue.³⁷

Figure 1 A synopsis of the angiogenic response—seven critical steps.

TUMOUR NECROSIS FACTOR α

Tumour necrosis factor α is secreted by macrophages when these are activated by inflammatory stimuli.³⁸ It is a potent angiogenic polypeptide *in vivo* and can also inhibit endothelial cell mitogenesis *in vitro*.³⁹ Its mode of presentation also affects its action: if injected intravenously tumour necrosis factor α causes procoagulant activity of endothelial cells, thrombosis, haemorrhage, and small vessel disruption,⁴⁰ which might explain its necrotising action in tumours. Extravascularly it acts as a potent antigen by promoting an inflammatory reaction. It stimulates endothelial cell production of granulocyte monocyte-colony stimulating factor, interleukin 1, and also stimulates prostaglandin E₂ and collagenase activity, which may in turn be angiogenic. Its physiological role, however, is probably as an inhibitor of the angiogenic response.²⁸

INTERLEUKINS

Interleukins 1 α and 1 β inhibit endothelial cell growth factor induced proliferation of endothelial cells.⁴¹ In this study there was a slight increase in tritiated thymidine uptake but no increase in cell numbers. Bovine acidic fibroblast growth factor is 30% homologous to human interleukins 1 α and 1 β and is related to endothelial cell growth factor, which suggests that there might be competition for binding sites between the two.⁴²

PLATELET DERIVED ENDOTHELIAL CELL GROWTH FACTOR AND PLATELET DERIVED GROWTH FACTOR
Platelet derived endothelial cell growth factor is a platelet factor with an M_r of 45 000, which does not bind to heparin. It shows no homology to other proteins so far sequenced and cloned.⁴³ Platelet derived growth factor is a separate polypeptide molecule of M_r 32 000. It can modulate expression of other factors, such as insulin-like growth factor 1.⁴⁴ It is synthesised by a variety of tissues as well as in endothelial cells and stimulates mitogenesis and chemotaxis in these cells.⁴⁵

PROSTAGLANDINS

Prostaglandins of the E series are angiogenic *in vivo* and stimulate angiogenesis at 0.1 μ g for E₁ in the cornea⁴⁶ and 0.2–20 ng in the chorio-allantoic membrane.⁴⁷ The mechanism is unclear, but prostaglandins are not in themselves mitogenic for endothelial cells.⁴⁸ Prostaglandin concentrations in tumours,⁴⁹ produced by activated macrophages in wounds,⁵⁰ and in inflammatory exudates⁵¹ are raised. They may act partly by attracting macrophages which secrete angiogenic components, but they have also been shown to be chemoattractive for endothelial cells *in vitro*.⁵²

FIBRINOGEN

Fibrinogen is chemotactic for endothelial cells⁵³ and also chemokinetic. Digestion of fibrinogen by plasmin and trypsin abolishes this response. One such fragment was shown to be able to

inhibit migration. Binding to endothelial cells was shown to be a specific interaction. Fibrin in itself is also chemotactic, suggesting that the activation of the clotting cascade in disease can also induce neovascularisation as part of the reparative process.⁵⁴

EFFECTS OF THE EXTRACELLULAR MATRIX ON ANGIOGENESIS

In the blood vessel the endothelial cell is attached to the vessel wall by interacting with the components of the basement membrane which interposes between the two. The basement membrane contains various collagens, glycosaminoglycans, elastin, microfilaments, laminin, fibronectins, and thrombospondin, all of which are synthesised by the endothelial cell.⁵⁵ Collagens I and III induce proliferation as was shown by experiments in which endothelial cells were cultured on the stromal and basement membrane surface of an acellular placental membrane; on the stromal surface proliferation was enhanced and vice versa where collagens IV and V predominate.⁵⁶ It is also known that cell to cell contact decreases proliferation, and in the blood vessel pericyte and smooth muscle interaction is thought to exert control.⁵⁷

As with mitogenesis, endothelial cell migration is stimulated by collagens I and III but inhibited by IV and V. These results were confirmed in acellular amniotic membranes, which have a basement membrane surface and a stromal surface of interstitial collagens. The endothelial cells grown on these migrated only on the stromal surface.⁵⁶ The influence of collagen is therefore profound and in haemorrhagic areas and areas of basement membrane destruction they may represent one stimulus for repair. Fibronectin has also been shown to be chemoattractive.⁵⁸ Fibronectin receptor blocking analogues can inhibit angiogenesis *in vivo*.⁵⁹ As fibronectin is synthesised by migrating cells it might be important in maintaining the proximity of endothelial cells to each other before their coalescence into capillary sprouts.

The effects of other components of the basement membrane have not been studied so extensively. Glycosaminoglycans are present both in the matrix and the membrane. The latter is composed predominantly of heparan sulphate and may be important in the sequestration of growth factors.⁶⁰ Heparan sulphate, dermatan sulphate, and chondroitin sulphate are present extracellularly. In particular, heparan sulphate has significant anticoagulant activity and activates the antithrombin III protein.⁶¹ It is also involved in cellular attachment. Laminin is similarly produced and secreted most when subconfluent; it is thought to promote proliferation and decrease differentiation.⁶² The endothelial cell can also modify the basement membrane and release collagenases capable of digesting types I, II, III, IV, and V.^{63 64} This activity is tightly controlled by the cosecretion of inhibitors.⁶⁵ This degradation is presumably also carried out by those cells which can cross the endothelial cell barrier, as has been shown in neutrophils⁶⁶ and in macrophages.⁶⁷

The endothelium and angiogenesis in rheumatoid arthritis

In extensive histological studies Fassbender *et al* concluded that one of the earliest and most striking changes in the rheumatoid synovium occurred in the microvasculature.⁶⁸ The development of the pannus tissue was seen to consist of three stages: synovial lining hyperplasia, pannus formation accompanied by the ingrowth of a new vascular network, and further consolidation of the pannus leading to a relatively avascular, fibrotic tissue. Surveys of capillary density in pannus specimens have shown an apparent decrease, but this may reflect variation in the stage of development of the tissue studied.⁶⁹ Other authors have remarked on the intense angiogenic activity which correlates with clinical score, synovial hyperplasia, and infiltration of inflammatory cells.⁷⁰ The question of which is the primary event, vascular change or synovial activation, is as yet undecided. The lack of vasculature in the leading edge of the encroaching pannus may be due in part to the presence of an inhibitor within cartilage which will block angiogenesis *in vivo* induced by other angiogenic factors.⁷¹ It has been suggested that the presence of this factor is responsible for the resistance of cartilage to tumour invasion. Also of note is the presence of mast cells in pannus tissue.⁷² These can produce heparin, which is important in the mobilisation and presentation of heparin binding growth factors to endothelial cells.

Angiogenic factors in rheumatoid arthritis

SYNOVIAL FLUID

Synovial fluid contains quantities of inflammatory mediators, such as interleukin 1, interferon α , and transforming growth factor β , which can exert influence on the angiogenic process.⁷³ It has also been shown that 15% of fluids contain an endothelial cell specific growth factor—'endothelial-cell stimulating angiogenic factor'—which is similar to tumour angiogenic factor.⁷⁴ This specific growth factor is of further interest as it can cause dissociation of protease-inhibitor complexes, such as tissue inhibitor of metalloproteinase complexed with collagenase.⁷⁵ This is the only known naturally occurring factor which can achieve this dissociation. It will further activate procollagenase, progelatinase, and prostromelysin.⁷⁶

PANNUS TISSUE

Angiogenesis in rheumatoid pannus is thought to be primarily under the control of cytokines, particularly from the monocyte-macrophage lineage—for example, by production of tumour necrosis factor α . Macrophages from synovial biopsy specimens have been shown to stimulate angiogenesis in rabbit and rat cornea and to stimulate endothelial cell migration *in vitro*.⁷⁷ The factor responsible is as yet uncharacterised. Epidermal and platelet derived growth factor has been localised by immunohistochemistry and is associated to some extent with areas of new vessel growth.⁷⁸ Induction of platelet derived growth factor receptors on cells in pannus tissue is also seen in chronically inflamed synovium with accompanying vessel growth.⁷⁹ Similarly, acidic and basic fibroblast growth factors have been demonstrated in endothelial cells in rheumatoid pannus⁸⁰ and streptococcus induced arthritis in rats.⁸¹

Figure 2 Angiogenesis and cartilage destruction.

Angiogenesis and cartilage destruction

Aside from the gross functions of blood vessels in tissue maintenance, it is now known that the endothelial cell lining also has an important role in other processes involved in the inflammatory response (fig 2). By the control of adhesion molecule expression, endothelial cells modulate inflammatory cell influx into tissues.⁸² Endothelial cells produce a variety of cytokines such as interleukins 1,⁸³ 6,⁸⁴ and 8,⁸⁵ and can present antigen.⁸⁶ They produce a number of growth factors, such as platelet derived growth factor-like activity,⁸⁷ and much interest is now being expressed in their ability to produce vasoactive substances and mediators of vascular permeability, such as the endothelins, endothelium derived relaxing factor, and prostacyclin.⁸⁸ Endothelin may also be of importance in the control of cell proliferation in combination with other growth factors.⁸⁹ Endothelial cells can modulate platelet activation and coagulation⁵⁴ and can produce reactive oxygen species using the enzyme xanthine oxidase, which is thought to be important in the pathogenesis of rheumatoid disease.⁹⁰ These functions suggest that endothelial cells in the pannus tissue may actively contribute to, and help to maintain, the chronically inflamed state.

Implications for existing treatments in rheumatoid arthritis

The non-steroidal anti-inflammatory drug indomethacin has been shown to promote endothelial cell migration in culture.⁹¹ It inhibits angiogenesis, however, in the rat cornea in response to the implantation of adipose tissue⁹² or silver/potassium nitrate,⁹³ and in the murine chronic granulomatous air pouch.⁹⁴ It is suggested that this inhibition in the cornea may be due to suppression of prostaglandin E₂ production, which has been shown to be angiogenic in the chorioallantoic membrane model. Sulphasalazine can inhibit endothelial cell proliferation.⁹⁵ Dexamethasone does not show angiostatic activity on neovascularisation during the repair of brain trauma.⁹⁶ This is consistent with its reported activity in the chick chorioallantoic membrane model,⁹⁷ which analyses angiogenesis in the absence of inflammation. It does, however, reduce the clearance of radioactive xenon gas from implanted sponges in rats, which gives a measure of the surface area of the vasculature within a tissue (Hori Y, personal communication). Prednisolone is used as an adjunct to chemotherapy in the treatment of cancer. It has been suggested that its effectiveness may be due to an effect on angiogenesis.⁹⁸ The immunosuppressants methotrexate, azathioprine, and cyclophosphamide are also used as antineoplastic agents where angiogenesis is a major feature of tumourigenesis. They exert a direct inhibitory action on any proliferating cell, and have a direct action on endothelial cell turnover as well as other cell types. Of these three, methotrexate has been shown to be angiostatic *in vivo* and *in vitro*.⁹⁹ It has been shown that the slow acting antirheumatic drugs (sodium aurothiomalate, gold chloride, auranofin) inhibit endothelial cell proliferation *in vitro*.¹⁰⁰ This direct action on endothelial cells may be due to free radical generation.¹⁰¹ D-Penicillamine has similar activity *in vitro* and is also angiostatic in the corneal model of angiogenesis *in vivo*.¹⁰² Possibly, the suppression of the rheumatoid synovitis seen with these slow acting drugs may be due to a reduction in the number of new blood vessels within the developing pannus tissue.¹⁰⁰ Chloroquine has also been reported to inhibit *in vitro* parameters of angiogenesis (endothelial cell migration, proliferation, and protease production)¹⁰³ and to reduce angiogenesis in the healing of traumatised brain tissue.⁹⁶ These drugs, with the exception of sulphasalazine, have been tested in a chronic granulomatous model of pannus tissue and shown to inhibit vascular growth (paper in preparation). The inhibition of the disease seen with these drugs may therefore be due, at least in part, to a direct effect of endothelial cells and the angiogenic process.

Potential new therapeutic strategies in rheumatoid arthritis

ANGIOSTATIC STEROIDS

Use of the chorioallantoic membrane to study angiogenesis led to the discovery of the angiostatic steroids.⁹⁷ These are steroids which in the presence of heparin, heparin fragments, or

analogues (which are inactive as anticoagulants) can inhibit the angiogenic response. This activity is independent of the mineralocorticoid or glucocorticoid activity of these molecules. Indeed some of these steroids—for example, tetrahydrocortisol, were thought to be biologically inactive before the discovery of this activity. They are thought to act by the modulation of the production or breakdown of the basement membrane around the vessels involved in the angiogenic process,¹⁰⁴ though a direct action on endothelial migration *in vitro* has also been reported for tetrahydrocortisol and a heparin analogue.¹⁰⁵ This therapeutic approach has been used to inhibit tumour growth in mice, and in some cases led to complete regression.¹⁰⁶ It has similarly been used to inhibit the development of chronic granulomatous tissue and the destruction of cartilage by such tissue in a murine model of pannus mediated cartilage destruction.¹⁰⁷ It is important to note that heparin alone tends to be angiogenic in action by enhancing growth factor availability, and that the angiostatic potential of heparins from different sources may vary considerably when combined with these steroids.¹ The use of the synthetic analogue β -cyclodextrin tetradecyl sulphate, which is biologically inert and a 1000-fold more potent than heparin in this regard, may be important in the development of this therapeutic approach.¹⁰⁸

PROTEASE INHIBITORS

Interest is now being expressed in the use of protease inhibitors in controlling the angiogenic process.¹⁰⁹ Inhibition of the degradation and the maintenance of the basement membrane would provide a barrier to endothelial cell migration and also inhibit endothelial cell activity because of the effects that the components of the membrane, such as collagens IV and V, exert on the cells. Little work has been published, but tissue inhibitors of metalloproteinases have been shown to be angiostatic in the chick chorioallantoic membrane model.¹¹⁰

ANGIOSTATIC ANTIBIOTICS

A bacterial product, fumagillin, has been described, which was first shown to be a potent angiostatic *in vitro*.¹¹¹ Although this compound is toxic *in vivo*, a synthetic derivative has been produced, AGM1470, which has been used *in vivo* in the treatment of collagen II arthritis,¹¹² leading to an almost total obliteration of the disease (28/30 developed disease in the control group; only 1/19 developed arthritis in the treated group). This is so far the most dramatic demonstration of the potential effectiveness of such a therapeutic strategy.

Conclusions

Angiogenesis is an integral part of the pathogenesis of rheumatoid arthritis. The increased area of endothelium thus generated may play an important part in the development and maintenance of the chronically inflamed state. Current antirheumatic drug treatment has, at

least in part, an angiostatic component, of particular note in the case of the disease modifying drugs. Angiostatic treatment has the potential to be an effective treatment in the control of pannus development and subsequent cartilage degradation. This treatment may use compounds with relatively few, if any, side effects from the compounds used.

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- Folkman J. How is blood vessel growth regulated in normal and neoplastic tissue. *Cancer Res* 1986; **46**: 467-73.
- Ausprunk D, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumour angiogenesis. *Microvasc Res* 1977; **14**: 53-65.
- Shing Y, Folkman J, Sullivan R, Butterfield C, Murray J, Klagsbrun M. Heparin affinity and purification of a tumour derived capillary endothelial cell growth factor. *Science* 1984; **223**: 1296-8.
- Lobb R R, Fett J W. Purification of two distinct growth factors from bovine neural tissue by heparin affinity chromatography. *Biochemistry* 1984; **23**: 6295-9.
- Baird A, Esch F, Gospodarowicz D, Guillemin R. Retina- and eye-derived endothelial cell growth factors: partial molecular characterisation and identity with acidic and basic fibroblast growth factors. *Biochemistry* 1985; **24**: 7855-60.
- Courty J, Chevallier B, Moenner M, et al. Evidence for FGF-like growth factor in adult bovine retina: analogies with EDGF I. *Biochem Biophys Res Commun* 1985; **136**: 102-8.
- Gospodarowicz D. Purification of a fibroblast growth factor from bovine pituitary. *J Biol Chem* 1975; **250**: 2515-20.
- Baird A, Bohlen P. Fibroblast growth factors. In: Sporn M B, Roberts A B, eds. *Handbook of experimental pharmacology*. Vol 95. Berlin: Springer, 1990: 369-418.
- Rifkin D B, Moscatelli D. Recent developments in the cell biology of basic fibroblast growth factor. *J Cell Biol* 1989; **109**: 1-6.
- Vlodavsky I, Folkman J, Sullivan R, et al. Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into the subendothelial extracellular matrix. *Proc Natl Acad Sci USA* 1987; **84**: 2292-6.
- Saksela O, Rifkin D B. Release of basic fibroblast growth factor-heparan sulphate complexes from endothelial cells by plasminogen activator-mediated proteolytic activity. *J Cell Biol* 1990; **110**: 767-75.
- Loskutov D J, Edgington T S. Synthesis of a fibrinolytic activity and inhibitor by endothelial cells. *Proc Natl Acad Sci USA* 1977; **74**: 3903-7.
- Inyang A L, Tobelem G. Tissue-plasminogen activator stimulates endothelial cell migration in wound assays. *Biochem Biophys Res Commun* 1990; **171**: 1326-32.
- Pepper M S, Vassalli J D, Montesano R, Orci L. Urokinase-type plasminogen activator is induced in migrating capillary endothelial cells. *J Cell Biol* 1987; **105**: 2535-41.
- Mignatti P, Mazziere R, Rifkin D B. Expression of the urokinase receptor in vascular endothelial cells is stimulated by basic fibroblast growth factor. *J Cell Biol* 1991; **113**: 1193-201.
- Berman M, Winthrop S, Ausprunk D, Rose J, Langer R, Gage J. Plasminogen activator (urokinase) causes vascularisation of the cornea. *Invest Ophthalmol Vis Sci* 1982; **22**: 191-9.
- Fibbi G, Ziche M, Morbidelli L, Magnelli L, Del Rosso M. Interaction of urokinase with specific receptors stimulates mobilisation of bovine adrenal capillary endothelial cells. *Exp Cell Res* 1988; **179**: 385-95.
- Vlodavsky I, Korner G, Ishai-Michaeli P, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix resident growth factors and enzymes: possible involvement in tumour metastasis and angiogenesis. *Cancer Metastasis Rev* 1990; **9**: 203-26.
- Fett J W, Strydom D J, Lobb R R, et al. Isolation and characterisation of angiogenin, an angiogenic protein from human carcinoma cells. *Biochemistry* 1985; **24**: 5480-6.
- Strydom D J, Fett J W, Lobb R R, et al. Amino acid sequence of human tumour derived angiogenin. *Biochemistry* 1985; **24**: 5486-94.
- Shapiro R, Riordan J, Vallee B L. Characteristic ribonucleolytic activity of human angiogenin. *Biochemistry* 1986; **25**: 3527-32.
- Shapiro R, Vallee B L. Human placental ribonuclease inhibitor abolishes both angiogenic and ribonucleolytic activities of angiogenin. *Proc Natl Acad Sci USA* 1987; **84**: 2238-41.
- Anonymous. Angiogenin [editorial]. *Lancet* 1985; **ii**: 1340-1.
- Connolly D T, Olander J V, Heuvelman D, et al. Human vascular permeability factor. Isolation from U937 cells. *J Biol Chem* 1989; **264**: 20017-24.
- Keck P J, Hauser S D, Krivi G, et al. Vascular permeability factor, an endothelial cell mitogen related to PDGF. *Science* 1989; **246**: 1309-12.
- Connolly D T, Heuvelman D M, Nelson R, et al. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. *J Clin Invest* 1989; **84**: 1470-8.
- Friesel R, Komoriya A, Maciag T. Inhibition of endothelial cell proliferation by gamma interferon. *J Cell Biol* 1987; **104**: 689-96.
- Sato N, Nariuchi H, Tsuruoka N, et al. Actions of TNF and IFN- γ on angiogenesis in vitro. *J Invest Dermatol* 1990; **95** (suppl): 85S-9S.
- Zoon K C, Karasaki Y, Zur Nedden D L, Hu R, Arnheiter H. Modulation of epidermal growth factor receptors by human α interferon. *Proc Natl Acad Sci USA* 1986; **83**: 8226-30.
- De Larco J E, Todaro G J. Growth factors from murine sarcoma virus-transformed cells. *Proc Natl Acad Sci USA* 1978; **75**: 4001-5.
- Marquardt H, Hunkapiller M W, Hood L E, et al. Transforming growth factors produced by retrovirus-transformed rodent fibroblasts and human melanoma cells: amino acid sequence homology with epidermal growth factor. *Proc Natl Acad Sci USA* 1983; **80**: 4684-8.
- Schreiber A B, Winkler M E, Derynck R. TGF α : a more potent angiogenic mediator than epidermal growth factor. *Science* 1986; **232**: 1250-3.
- Barnard J A, Lyons R M, Moses H L. The cell biology of transforming growth factor β . *Biochem Biophys Acta* 1990; **1032**: 79-87.
- Roberts A B, Sporn M B, Assoian R K, et al. Transforming growth factor-beta: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA* 1986; **83**: 4167-71.
- Fiegel V D, Knighton D R. Transforming growth factor-beta causes indirect angiogenesis by recruiting monocytes. *FASEB J* 1988; **2**: A1601.
- Frater-Schroder M, Muller G, Birchmeier W, Bohlen P. Transforming growth factor-beta inhibits endothelial cell proliferation. *Biochem Biophys Res Commun* 1986; **137**: 295-302.
- Yang E Y, Moses H L. Transforming growth factor beta 1-induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. *J Cell Biol* 1990; **111**: 731-41.
- Beutler B, Cerami A. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* 1986; **320**: 584-8.
- Frater-Schroder M, Risau W, Hallmann R, Gautschi P, Bohlen P. TNF α , a potent inhibitor of cell growth in vitro, is angiogenic in vivo. *Proc Natl Acad Sci USA* 1987; **84**: 5277-81.
- Carswell E A, Old L J, Kassel R L, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumours. *Proc Natl Acad Sci USA* 1975; **72**: 3666-70.
- Norioka K, Hara M, Kitani A, et al. Inhibitory effects of human recombinant interleukin-1 α and β on growth of human vascular endothelial cells. *Biochem Biophys Res Commun* 1987; **145**: 969-75.
- Gimenez-Gallo G, Rodkey J, Bennett C, Rios-Candelore M, Disalvo J, Thomas K. Brain derived acidic fibroblast growth factor: complete amino acid sequence and homologies. *Science* 1985; **230**: 1385-8.
- Ishikawa F, Miyazono K, Hellman U, et al. Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor. *Nature* 1989; **338**: 557-62.
- Bar R S, Boes M, Booth B A, Dake B L, Henley S, Hart M N. The effects of platelet-derived growth factor in cultured microvessel endothelial cells. *Endocrinology* 1989; **124**: 1841-8.
- Knighton D R, Hunt T K, Thakral K K, Goodson W H. Role of platelets and fibrin in the healing sequence: an in vivo study of angiogenesis and collagen synthesis. *Ann Surg* 1982; **196**: 379-88.
- Silverman K J, Lund D P, Zetter B R, et al. Angiogenic activity of adipose tissue. *Biochem Biophys Res Commun* 1988; **153**: 347-52.
- Form D M, Auerbach R. PGE $_2$ and angiogenesis. *Proc Soc Exp Biol Med* 1983; **172**: 214-8.
- Bagavandoss P, Wilks J W. Isolation and characterization of microvascular endothelial cells from developing corpus luteum. *Biol Reprod* 1991; **44**: 1132-9.
- Owen K, Gomolka D, Droller M J. Production of prostaglandin E $_2$ by tumour cells in vitro. *Cancer Res* 1980; **40**: 3167-71.
- Stenson W I, Parker C W. Prostaglandins, macrophages and immunity. *J Immunol* 1980; **125**: 1-5.
- Vane J R. Prostaglandins as mediators of inflammation. *Adv Prostaglandin Thromboxane Res* 1976; **2**: 791-801.
- Joyce N C, Meklik B, Neufeld A H. In vitro pharmacologic separation of corneal endothelial migration and spreading responses. *Invest Ophthalmol Vis Sci* 1990; **31**: 1816-26.
- Dejana E, Languino L, Polentarutti N, et al. Interaction between fibrinogen and cultured endothelial cells: induction of migration and specific binding. *J Clin Invest* 1985; **75**: 11-18.
- Thompson W D, Harvey J A, Kazmi M A, Stout A J. Fibrinolysis and angiogenesis in wound healing. *J Pathol* 1991; **165**: 311-8.
- Jaffe E A. Endothelial cells. In: Gallin J I, Goldstein I M, Snyderman R, eds. *Inflammation: basic principles and clinical correlates*. New York: Raven Press, 1988: 559-76.
- Madri J A, Williams K S. Capillary endothelial cells cultures: phenotypic modulation by matrix components. *J Cell Biol* 1983; **97**: 153-65.

- 57 Orlidge A, D'Amore P. Inhibition of endothelial cell growth by pericytes and smooth muscle cells. *J Cell Biol* 1987; 105: 1455-62.
- 58 Clark R A. Potential roles of fibronectin in cutaneous wound repair. *Arch Dermatol* 1988; 124: 201-6.
- 59 Saiki I, Murata J, Makabe T, Nishi N, Tokura S, Azuma I. Inhibition of tumor angiogenesis by a synthetic cell-adhesive polypeptide containing the Arg-Gly-Asp (RGD) sequence of fibronectin, poly(RGD). *Jpn J Cancer Res* 1990; 81: 668-75.
- 60 Baird A, Ling N. Fibroblast growth factors are present in the extracellular matrix produced by endothelial cells in vitro. Implications for a role of heparinase-like enzymes in the neovascular response. *Biochem Biophys Res Commun* 1987; 142: 428-35.
- 61 Marcum J A, McKinney J B, Rosenberg R D. Acceleration of thrombin-antithrombin complex formation in rat hindquarters via heparinlike molecules bound to the endothelium. *J Clin Invest* 1984; 74: 341-50.
- 62 Grant D S, Kleinman H K, Martin G R. The role of basement membranes in vascular development. *Ann N Y Acad Sci* 1990; 588: 61-72.
- 63 Gross J L, Moscatelli D, Jaffe E A, Rifkin D B. Plasminogen activator and collagenase production by cultured capillary endothelial cells. *J Cell Biol* 1982; 95: 974-81.
- 64 Kalebic T, Garbisa S, Glaser B, Liotta L A. Basement membrane collagen: degradation by migrating endothelial cells. *Science* 1983; 221: 281-3.
- 65 Herron G S, Banda M J, Clark E J, Gavrilovic J, Werb Z. Secretion of metalloproteinases by stimulated capillary endothelial cells. II. Expression of collagenase and stromelysin activities is regulated by endogenous inhibitors. *J Biol Chem* 1986; 261: 2814-8.
- 66 Uitto V J, Schwartz D, Veis A. Degradation of basement membrane collagen by neutral proteases from human leukocytes. *Eur J Biochem* 1980; 105: 409-17.
- 67 Werb Z, Banda M J, Jones P A. Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. *J Exp Med* 1980; 152: 1340-57.
- 68 Fassbender H G, Simmling-Annefeld A. The potential aggressiveness of synovial tissue in rheumatoid arthritis. *J Pathol* 1983; 139: 399-406.
- 69 Stevens C R, Blake D R, Merry P, Revell P A, Levick J R. A comparative study by morphometry of the microvasculature in normal and rheumatoid synovium. *Arthritis Rheum* 1991; 34: 1508-13.
- 70 Rooney M, Condell D, Quinlan W, et al. Analysis of the histologic variation in rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 956-63.
- 71 Moses M A, Sudhalter I, Langer R. Identification of an inhibitor of neovascularisation from cartilage. *Science* 1990; 248: 1408-10.
- 72 Mican J M, Metcalfe D D. Arthritis and mast cell activation. *J Allergy Clin Immunol* 1990; 86: 677-83.
- 73 Arend W P, Dayer J M. Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum* 1990; 33: 305-15.
- 74 Brown R A, Tomlinson I W, Hill C R, Weiss J B, Phillips P, Kumar S. Relationship of angiogenesis factor in synovial fluid to various joint diseases. *Ann Rheum Dis* 1983; 42: 301-7.
- 75 McLaughlin B, Cawston T, Weiss J B. Activation of matrix metalloproteinase inhibitor by low molecular weight angiogenic factor. *Biochem Biophys Acta* 1991; 1073: 295-8.
- 76 Brown R A, Weiss J B. Neovascularisation and its role in the osteoarthritic process. *Ann Rheum Dis* 1988; 47: 881-5.
- 77 Koch A, Polverini P J, Leibovitch S J. Stimulation of neovascularisation by human rheumatoid synovial tissue macrophages. *Arthritis Rheum* 1986; 29: 471-9.
- 78 Shiozawa S, Shiozawa K, Tanaka Y, et al. Human epidermal growth factor for the stratification of synovial lining layer and neovascularisation in rheumatoid arthritis. *Ann Rheum Dis* 1989; 48: 820-8.
- 79 Rubin K, Terracio L, Ronnstrand L, Heldin C H, Klagskog L. Expression of platelet-derived growth factor receptors is induced on connective tissue cells during chronic synovial inflammation. *Scand J Immunol* 1988; 27: 285-94.
- 80 Sano H, Forough R, Maier J A M, et al. Detection of high levels of heparin binding growth factor 1 (acidic fibroblast growth factor) in inflammatory arthritic joints. *J Cell Biol* 1990; 110: 1417-26.
- 81 Ou Z, Planck R, Hart C E, Rosenbaum J T. Distribution pattern of basic fibroblast growth factor in synovial tissue from patients with rheumatoid arthritis. *Arthritis Rheum* 1990; 33 (suppl): 75.
- 82 Springer T A. Adhesion receptors of the immune system. *Nature* 1990; 346: 425-31.
- 83 Windt M R, Rosenwasser L J. Human vascular endothelial cells produce interleukin-1. *Lymphokine Res* 1984; 3: 281a.
- 84 Jirik F R, Podor T J, Hirano T, et al. Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. *J Immunol* 1989; 1, 144-7.
- 85 Mantovani A, Dejana E. Cytokines as communication signals between leukocytes and endothelial cells. *Immunol Today* 1989; 10: 370-5.
- 86 Pober J S, Gimbrone M. Expression of Ia-like antigens by human vascular endothelial cells is inducible in vitro: demonstration by monoclonal antibody binding and immunoprecipitation. *Proc Natl Acad Sci USA* 1982; 79: 6641-5.
- 87 Dicorleto P E, Bowen-Pope D F. Cultured endothelial cells produce a platelet-derived growth factor-like protein. *Proc Natl Acad Sci USA* 1983; 80: 1919-23.
- 88 Gryglewski R G, Botting R M, Vane J R. Mediators produced by the endothelial cell. *Hypertension* 1988; 12: 530-48.
- 89 Brown K D, Littlewood C J. Endothelin stimulates DNA synthesis in Swiss 3T3 cells. *Biochem J* 1989; 263: 977-80.
- 90 Jarasch E-D, Bruder G, Heid H W. Significance of xanthine in capillary endothelial cells. *Acta Scand Physiol* 1986; 548 (suppl): 39-46.
- 91 Joyce N C, Matkin E D, Neufeld A H. Corneal endothelial wound closure in vitro. Effects of EGF and/or indomethacin. *Invest Ophthalmol Vis Sci* 1989; 30: 1548-59.
- 92 Silverman K J, Lund D P, Zetter B R, et al. Angiogenic activity of adipose tissue. *Biochem Biophys Res Commun* 1988; 153: 347-52.
- 93 Haynes W L, Proia A D, Klintworth G K. Effects of inhibitors of arachidonic acid metabolism on corneal neovascularisation in the rat. *Invest Ophthalmol Vis Sci* 1989; 30: 1588-93.
- 94 Amemiya K, Suzuki I, Kimura M. Mechanism of action of pronase on chronic proliferative inflammation including granuloma angiogenesis in mice. *Nippon Yakurigaku Zasshi* 1986; 88: 279-88.
- 95 Wijlath E, Madhok R, Gillespie J, Watson J, Capell H A. Inhibition of human endothelial cell proliferation by sulfasalazine and sulfapyridine. *Arthritis Rheum* 1989; 32 (suppl): 129.
- 96 Giulian D, Chen J, Ingeman J E, George J K, Noponen M. The role of mononuclear phagocytes in wound healing after traumatic injury to adult mammalian brain. *J Neurosci* 1989; 9: 4416-29.
- 97 Crum R, Szabo S, Folkman J. A new class of steroids inhibits angiogenesis in the presence of heparin or a heparin fragment. *Science* 1985; 230: 1375-8.
- 98 Beranek J T. Prednisolone enhancement of primary endocrine anticancer treatment: a possible anti-angiogenic effect [letter]. *Br J Cancer* 1989; 60: 149.
- 99 Hirata S, Matsubara T, Saura R, Tateishi H, Hirohata K. Inhibition of in vitro vascular endothelial cell proliferation and in vivo neovascularisation by low dose methotrexate. *Arthritis Rheum* 1989; 32: 1065-73.
- 100 Matsubara T, Ziff M. Inhibition of endothelial cell proliferation by gold compounds. *J Clin Invest* 1987; 79: 1440-6.
- 101 Grootveld M, Blake D R, Sahinoglu T, et al. Control of oxidative damage in rheumatoid arthritis by gold(I)-thiolate drugs. *Free Radic Res Commun* 1990; 10: 199-200.
- 102 Matsubara T, Saura R, Hirohata K, Ziff M. Inhibition of human endothelial cell proliferation in vitro and neovascularisation in vivo by D-penicillamine. *J Clin Invest* 1989; 83: 158-67.
- 103 Inyang A L, Bikfalvi L, Lu H, Tobelem G. Chloroquines modulation of endothelial cell activity induced with basic fibroblast growth factor and human serum: effect on mitogenesis, protease production and cell migration. *Cell Biol Int Rep* 1990; 14: 35-46.
- 104 Folkman J, Ingber D E. Angiostatic steroids: method of discovery and mechanism of action. *Ann Surg* 1987; 206: 374-82.
- 105 Stokes C L, Weisz P B, Williams S K, Lauffenburger D A. Inhibition of microvascular endothelial cell migration by beta-cyclodextrin tetradecasulfate and hydrocortisone. *Microvasc Res* 1990; 40: 279-84.
- 106 Folkman J, Langer R, Linhardt R J, Haudenschild C, Taylor S. Angiogenesis inhibition and tumour regression caused by heparin or a heparin fragment in the presence of cortisone. *Science* 1983; 221: 719-25.
- 107 Chander C L, Colville-Nash P R, Moore A R, Howat D W, Desa F M, Willoughby D A. The effects of heparin and cortisone on an experimental model of pannus. *Int J Tissue React* 1989; 11: 113-6.
- 108 Folkman J, Weisz P B, Joullie M M, Li W W, Ewing W R. Control of angiogenesis with synthetic heparin substitutes. *Science* 1989; 243: 1490-2.
- 109 Mignatti P, Tsuboi R, Robbins E, Rifkin D B. In vitro angiogenesis on a human amniotic membrane: requirement for basic fibroblast growth factor-induced proteinases. *J Cell Biol* 1989; 108: 671-82.
- 110 Takigawa M, Nishida Y, Suzuki F, Kishi J, Yamashita K, Hayakawa T. Induction of angiogenesis in chick yolk-sac membrane by polyamines and its inhibition by tissue inhibitors of metalloproteinases (TIMP and TIMP-2). *Biochem Biophys Res Commun* 1990; 171: 1264-71.
- 111 Ingber D, Fujita T, Kishimoto S, et al. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature* 1990; 348: 555-7.
- 112 Peacock D J, Baquerigo M L, Brahn E. (1991) Angiogenesis inhibition suppresses collagen arthritis. *Arthritis Rheum; Proceedings of the 55th annual meeting of the American College of Rheumatology*, 1991. In press.