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. 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. 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. 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 M0, and found in high levels in neural tissue. They include acidic fibroblast growth factor, endothelial cell growth factor, retina derived growth factor, and eye derived growth factor II. Class II heparin binding growth factors are mainly cationic with an M0 of 16–18 500. They seem to be variants of basic fibroblast growth factor and have been isolated from the pituitary gland, brain, hypothalamus, eye, cartilage, bone, adrenal gland, kidney, macrophages, placenta, and tumour cells. 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. They are found bound in large quantities in the extracellular matrix. 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. Tissue type plasminogen activator is produced by endothelial cells and will enhance their migration, and migrating endothelial cells will elaborate urokinase type plasminogen activator. 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. In some circumstances, such as in the rabbit cornea, 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.
Binding of these factors to cell and extracellular matrix macromolecules greatly enhances their stability and resistance to proteolytic destruction.  

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.\(^\text{19}\) It is a single chain, with an M, of 14 400. It has been sequenced and cloned and shows 35% absolute homology to a family of ribonucleases.\(^\text{20}\) It is generally inactive in this role but cleaves the RNA of ribosomes.\(^\text{21}\) Blockade of this activity inhibits the angiogenic response induced by this molecule.\(^\text{22}\) Angiogenin will not bind to heparin and unlike the heparin binding growth factors is secreted into surroundings but not bound by the extracellular matrix.\(^\text{23}\) It is also non-mitogenic for endothelial cells and its mode of action is unknown.

VASCULAR PERMEABILITY FACTOR

Vascular permeability factor has similarly been isolated from tumour cell lines in culture.\(^\text{24}\) It has sequence homology to the β chain of platelet derived growth factor\(^\text{25}\) and localises specifically to endothelial cells, unlike most other factors. It stimulates angiogenesis both in vivo and in vitro.\(^\text{26}\)

INTERFERON γ

Interferon γ is a dimer of M, 50 000 produced by the cells of lymphoid origin. It inhibits proliferation of endothelial cells when given with endothelial cell growth factor,\(^\text{27}\) endothelial cell migration, and in vitro angiogenesis.\(^\text{28}\) 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.\(^\text{27}\) It will also downregulate epidermal growth factor\(^\text{29}\) and other receptors.

TRANSFORMING GROWTH FACTORS

Transforming growth factors were originally isolated from virally transformed rodent cells and induce this transformation in unaffected cells.\(^\text{30}\) 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.\(^\text{31}\) It stimulates endothelial cell proliferation at 1–5 ng/ml in culture as does epidermal growth factor,\(^\text{32}\) 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.\(^\text{33}\) It induces an increase in macrophage number at the site of injection, fibroblast recruitment, collagen production, and vascularisation.\(^\text{34}\) It is angiogenically active in nanogram quantities in the rabbit cornea.\(^\text{35}\) 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.\(^\text{36}\) 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.\(^\text{35}\) 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.\(^\text{37}\)

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**Figure 1** A synopsis of the angiogenic response—seven critical steps.
Angiogenesis and rheumatoid arthritis

921

TUMOUR NECROSIS FACTOR α
Tumour necrosis factor α is secreted by macrophages when these are activated by inflammatory stimuli. It is a potent angiogenic polypeptide in vivo and can also inhibit endothelial cell mitogenesis in vitro. 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, which might explain its necrotising action in tumours. Extravascularly it acts as a potent angiogenen 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.

INTERLEUKINS
Interleukins 1α and 1β inhibit endothelial cell growth factor induced proliferation of endothelial cells. 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 1α and 1β and is related to endothelial cell growth factor, which suggests that there might be competition for binding sites between the two.

PLATELET DERIVED ENDOTHELIAL CELL GROWTH FACTOR AND PLATELET DERIVED GROWTH FACTOR
Platelet derived endothelial cell growth factor is a platelet factor with an Mr of 45 000, which does not bind to heparin. It shows no homology to other proteins so far sequenced and cloned. Platelet derived growth factor is a separate polypeptide molecule of Mr 32 000. It can modulate expression of other factors, such as insulin-like growth factor 1. It is synthesised by a variety of tissues as well as in endothelial cells and stimulates mitogenesis and chemotaxis in these cells.

PROSTAGLANDINS
Prostaglandins of the E series are angiogenic in vivo and stimulate angiogenesis at 0·1 μg for E1 in the cornea and 0·2–20 ng in the choroidal lamina.

Mechanism is unclear, but prostaglandins are not in themselves mitogenic for endothelial cells. Prostaglandin concentrations in tumours produced by activated macrophages in wounds, and in inflammatory exudates 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.

FIBRINOGEN
Fibrinogen is chemotactic for endothelial cells 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.

EFFECTS OF THE EXTRACELLULAR MATRIX ON ANGIGENESIS
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. 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. 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. 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. 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 chemo-attractive. Fibronectin receptor blocking analogues can inhibit angiogenesis in vivo. 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. Heparan sulphate, dermanan sulphate, and chondroitin sulphate are present extracellularly. In particular, heparan sulphate has significant antiangiogenetic activity and acts as an antithrombin IIII protein. 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. The endothelial cell can also modify the basement membrane and release collagens capable of digesting types I, II, III, IV, and V. This activity is tightly controlled by the cosecretion of inhibitors. This degradation is presumably also carried out by those cells which can cross the endothelial cell barrier, as has been shown in neutrophils and in macrophages.
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. 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. Other authors have remarked on the intense angiogenic activity which correlates with clinical score, synovial hyperplasia, and infiltration of inflammatory cells. 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. 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. 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. 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. 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. This is the only known naturally occurring factor which can achieve this dissociation. It will further activate procollagenase, progelatinase, and prostromelysin.

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. 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. Induction of platelet derived growth factor receptors on cells in pannus tissue is also seen in chronically inflamed synovium with accompanying vessel growth. Similarly, acidic and basic fibroblast growth factors have been demonstrated in endothelial cells in rheumatoid pannus and streptococcus induced arthritis in rats.

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. Endothelial cells produce a variety of cytokines such as interleukins 1, 6, and 8 and present antigen. They produce a number of growth factors, such as platelet derived growth factor-like activity, and much interest is now being expressed in their ability to produce vasoactive substances and mediators of vascular permeability, such as the endothelins, endothelin derived relaxing factor, and prostacyclin. Endothelin may also be of importance in the control of cell proliferation in combination with other growth factors. Endothelial cells can modulate platelet activation and coagulation and can produce reactive oxygen species using the enzyme xanthine oxidase, which is thought to be important in the pathogenesis of rheumatoid disease. These functions suggest that endothelial cells in the pannus tissue may actively contribute to, and help to maintain, the chronically inflamed state.

Figure 2 Angiogenesis and cartilage destruction.
Implications for existing treatments in rheumatoid arthritis

The non-steroidal anti-inflammatory drug indomethacin has been shown to promote endothelial cell migration in culture. It inhibits angiogenesis, however, in the rat cornea in response to the implantation of adipose tissue or silver/potassium nitrate, and in the murine chronic granulomatous air pouch. 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. Dexamethasone does not show angiostatic activity on neovascularisation during the repair of brain trauma. This is consistent with its reported activity in the chick chorioallantoic membrane model, which analyses angiogenesis in the absence of inflammation. It does, however, reduce the clearance of radiolabelled 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. 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. It has been shown that the slow acting antirheumatic drugs (sodium aurothiomalate, gold chloride, auranofin) inhibit endothelial cell proliferation in vitro. This direct action on endothelial cells may be due to free radical generation.

D-Penicillamine has similar activity in vitro and is also angiostatic in the corneal model of angiogenesis in vivo. 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. Chloroquine has also been reported to inhibit in vitro parameters of angiogenesis (endothelial cell migration, proliferation, and protease production) and to reduce angiogenesis in the healing of traumatised brain tissue. 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. 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, though a direct action on endothelial migration in vitro has also been reported for tetrahydrocortisol and a heparin analogue. This therapeutic approach has been used to inhibit tumour growth in mice, and in some cases led to complete regression. It has similarly been used to inhibit the development of chronic granulomatous tissue and the destruction of cartilage by such tissues in a murine model of pannus-mediated cartilage destruction. It is important to note that heparin alone tends to be angiogenic in action by enhancing growth factor availability, and thus the angiostatic potential of heparins from different sources may vary considerably when combined with these steroids. The use of the synthetic analogue β-cyclohextrin tetracycl 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.

PROTEASE INHIBITORS

Interest is now being expressed in the use of protease inhibitors in controlling the angiogenic process. 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.

ANGIOSTATIC ANTIBIOTICS

A bacterial product, rifamycin, has been described, which was first shown to be a potent angiostatic in vitro. 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, leading to an almost total obliteration of the disease. 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, 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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Angiogenesis and rheumatoid arthritis


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Angiogenesis and rheumatoid arthritis: pathogenic and therapeutic implications.

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