Review

Angiogenesis and arthritis

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Abstract

Indices of angiogenesis are increased in synovia from patients with arthritis, and vascular proliferation may contribute to the pathogenesis of synovitis, pannus growth, bone and cartilage destruction, and osteophyte formation. Pharmacological inhibition of angiogenesis therefore has potential as a therapeutic strategy in human arthritis. However, vascular growth is also essential for normal development, female reproduction and tissue repair. Selective inhibition of undesirable angiogenesis requires an understanding of the different regulatory mechanisms in pathological and physiological angiogenesis. This review outlines the evidence that the rate of angiogenesis is increased in the inflamed human synovium, and possible approaches to, and consequences of, the modulation of vascular growth.

Key words: Angiogenesis, Cell proliferation, Apoptosis, Hypoxia, Synovitis, Ossification, Wound healing, Rheumatoid arthritis, Osteoarthritis.

Angiogenesis is a complex process through which new blood vessels grow from a pre-existing vasculature. It is an essential component of the normal female reproductive cycle, embryogenesis, growth and successful tissue repair. In other circumstances, however, angiogenesis is damaging to the organism, contributing to blindness in diabetic retinopathy, and facilitating growth and metastasis of tumours. Rheumatoid arthritis (RA) has been thought of as an angiogenesis-dependent disease, raising hopes that pharmacological inhibitors of angiogenesis may provide novel approaches to disease suppression [1, 2].

Angiogenesis is a process, rather than a single event. Endothelial cell proliferation and migration are followed by capillary tube formation, deposition of basement membrane, and proliferation and migration of pericytes and smooth muscle cells. Anastomoses are created and blood flow is established. There then follows a protracted period of vascular reorganization. Redundant vessels regress, a process which requires endothelial cell apoptosis [3, 4]. Appropriate vessels develop vasoregulatory systems through the expression of vasoactive peptides and their receptors [5, 6]. The desirable end product is a microvascular bed whose function matches, and is responsive to, changes in local metabolic requirements.

Each stage in this complex process can be subject to positive and negative regulation by a wide range of factors, many of which are present in normal or inflamed synovia (Table 1). Modulators of angiogenesis continue to be discovered. For example, angiopoietins-1 and -2 have recently been described as modulators of angiogenesis that act through Tie-2 receptors on endothelial cells [51, 52]. A role for angiopoietins in arthritis has not been defined yet. This large number of factors may indicate redundancy in angiogenic pathways. Alternatively, different factors may regulate angiogenesis during different phases of synovitis, in different tissues, and in different physiological and pathological conditions. This diversity of regulatory pathways may permit the targeted manipulation of pathological angiogenesis. This review outlines the evidence that the rate of angiogenesis is increased in human arthritis, and possible approaches to, and consequences of, the modulation of vascular growth.

Vascular turnover is increased in human synovitis

Endothelial cell proliferation is required for sustained angiogenesis [53]. Markers of endothelial cell proliferation are upregulated in rheumatoid synovitis, consistent with increased vascular growth. Endothelial cells within chronically inflamed synovia express early activation genes such as c-myc and c-fos [54]. They express proliferation markers such as Ki67 antigen, proliferating cell nuclear antigen and nuclear organizer regions [55–60]. [3H]Thymidine is incorporated into endothelial cells in synovial explants from patients with RA, and in experimental synovitis in animals [61–63]. Indices of endothelial cell proliferation are increased in inflamed human synovium to levels observed in tumours and healing wounds.

Microvessels in inflamed synovia resemble newly
formed blood vessels. They frequently lack ancillary cells such as pericytes and smooth muscle cells, and focally express increased amounts of matrix metalloproteinases, E-selectin and integrin $\alpha_v\beta_3$ [59, 64, 65]. These molecules not only characterize immature endothelia, but may also be required for angiogenesis to proceed [45, 66, 67]. Like proliferating endothelial cells, microvessels in inflamed synovia often express low levels of angiotensin converting enzyme [11, 68]. Newly formed vessels are associated with incomplete vasoregulatory systems [5]. Chronically inflamed synovia contain blood vessels that lack nerves and receptors for vasoregulatory neuropeptides such as substance P [9, 69].

None of these observations alone is conclusive of synovial angiogenesis. Endothelial cell proliferation is also required to replace damaged endothelial cells, loss of pericytes is a feature of vascular regression, and denervation may be a consequence of neurotoxic factors within the synovium. Together, however, these findings suggest that synovial angiogenesis is increased in longstanding RA. It perhaps should seem surprising, therefore, that rheumatoid synovitis may persist for many years with little overall change in synovial volume or vascular density (Fig. 1) [70, 71].

The normal synovium is highly vascular, both in man and in animals, satisfying the metabolic demands of the avascular cartilage [70, 72]. In particular, the synovial lining region is associated with a dense microvascular network. Detailed morphometric studies have indicated that rheumatoid synovitis may result in a reduced vascular density within 50 $\mu$m of the synovial surface [70, 71]. There is a redistribution of blood vessels and increased vascular density in the deeper synovium [70, 73]. These changes in microvascular architecture, as well as defects in vasoregulation, may contribute to pockets of hypoxia and increased anaerobic metabolism in chronically inflamed synovia [74, 75].

A solution to the apparent paradox of increased angiogenesis and decreased vascular density came from the finding that endothelial cell death, a necessary process during vascular regression, is increased in rheumatoid synovitis [59]. Samples of synovia which displayed increased endothelial cell expression of proliferation markers also displayed increased labelling of DNA fragments in endothelial cells by terminal uridyldeoxynucleotide nick-end labelling (TUNEL). Evidence of angiogenesis and vascular regression was found in different microscopic foci of the same synovial samples. Increased rates of vascular turnover in rheumatoid synovitis may exceed the capacity of vasoregulatory systems to develop, resulting in a qualitatively abnormal microvasculature, without large increases in vascular density.

Angiogenesis in the inflamed joint represents a balance between the effects of angiogenic and anti-angiogenic factors (Table 1). Although angiogenic and anti-
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Angiogenic factors are each expressed, they are localized to separate microscopic foci within chronically inflamed human synovia [59]. Microvessels that display vascular endothelial growth factor (VEGF) and integrin αvβ3 immunoreactivities also express markers of cellular proliferation, whereas other non-proliferating vessels express the anti-angiogenic factor thrombospondin. Angiogenic and anti-angiogenic factors, endothelial cell survival factors and stimulators of endothelial cell death each regulate vascular density during chronic inflammation.

Sites of angiogenesis in arthritis

The above discussion has focused on the synovium as a site of angiogenesis in RA. Vascular densities are not uniform throughout the normal synovium [72]. The number of blood vessels is typically greatest near to the synovial surface. Other sites are also highly vascular, such as the surfaces of tendons and ligaments, and the junctions between synovium, capsule, articular cartilage and bone (entheses). The highly vascular interface between articular cartilage and synovium may be particularly important in the angiogenesis associated with the formation of pannus. New synovial tissue grows over the articular surface and erodes into the cartilage and underlying bone, contributing to joint destruction.

The articular cartilage itself is avascular in the normal, mature joint [76]. Vascularization of articular cartilage would compromise its biomechanical properties and mechanical loading would be expected to compromise vascular function. Cartilage generates a variety of angiogenic factors that can inhibit its vascularization [77, 78]. During osteoarthritis and RA, new vessels invade the cartilage from the underlying bone, probably in response to perturbations in the balance between angiogenic and anti-angiogenic factors [79, 80]. Angiogenesis within cartilage is also an important component of enchondral bone formation, observed during the development of osteophytes at the margins of osteoarthritic joints [81]. The growth of blood vessels into damaged intervertebral discs may be comparable to the vascularization of abnormal cartilage [82]. Sensory nerves are associated with the neovasculature in human discs, raising the possibility that angiogenesis may create pathways through which so-called ‘degenerative’ diseases can be painful [83].

Roles of angiogenesis during synovitis

Angiogenesis may play many different roles during health and disease. Of possible direct benefit in arthritis are positive effects on tissue repair and the relief of hypoxia. On the other hand, angiogenesis may exacerbate inflammation, synovial proliferation and invasion, and osteophyte formation. Of additional concern in the development of angiogenesis inhibitors for the treatment of arthritis are potentially detrimental effects on female reproductive capacity, fetal growth and tissue repair.

Angiogenesis and inflammation

Much has been written about the possible causal links between angiogenesis and chronic inflammation [1, 84]. Although chronic inflammation is almost always accompanied by angiogenesis, angiogenesis can occur in the absence of inflammation. Animal models of angiogenesis in the rabbit cornea and chick chorioallantoic membrane have been developed in order to study angiogenesis in the absence of inflammation. These models can be used to distinguish factors that directly increase angiogenesis from those that do so by inducing the generation of angiogenic agents by infiltrating inflammatory cells. Data from animal models clearly demonstrate that angiogenesis by itself does not necessarily cause inflammation. Indeed, it may be argued that successful angiogenesis during wound healing contributes to the resolution of inflammatory.

Under some circumstances, angiogenesis may potentially or perpetuate inflammation, rather than cause it. Newly formed blood vessels have increased permeability to macromolecules, facilitating oedema formation [85]. The neovasculature also expresses increased levels of some adhesion molecules such as E-selectin that can permit the migration of inflammatory cells [65, 86, 87]. Neural and peptide regulatory factors modulate angiogenesis in the mature vasculature, and deficiencies in these regulatory pathways in the neovascular bed could result in inappropriate inflammation [5]. The close association between angiogenesis and inflammation is highlighted by the often dual functionality of angiogenic factors. VEGF was originally described as the potent inflammmogen vascular permeability factor before its vascular function. Cartilage generates a variety of anti-angiogenic factors that can inhibit its vascularization [77, 78]. During osteoarthritis and RA, new vessels invade the cartilage from the underlying bone, probably in response to perturbations in the balance between angiogenic and anti-angiogenic factors [79, 80]. Angiogenesis within cartilage is also an important component of enchondral bone formation, observed during the development of osteophytes at the margins of osteoarthritic joints [81]. The growth of blood vessels into damaged intervertebral discs may be comparable to the vascularization of abnormal cartilage [82]. Sensory nerves are associated with the neovasculature in human discs, raising the possibility that angiogenesis may create pathways through which so-called ‘degenerative’ diseases can be painful [83].

Angiogenesis and tissue repair

Repair of injured tissues typically requires angiogenesis. Inhibitors of angiogenesis reduce the healing rates of cutaneous wounds and gastrointestinal ulcers [89, 90]. Conversely, stimulators of angiogenesis may facilitate tissue healing [91, 92]. Hypoxia delays wound healing, and relief of hypoxia may be one mechanism by which angiogenesis permits tissue repair. The early stages of angiogenesis resulting in anastomosed new vessels may not be sufficient for successful repair. Sensory denervation also impairs healing, indicating that intact vasoregulatory systems may be important for wound repair [93]. In contrast to its role in tissue repair, angiogenesis has also been implicated in tissue injury. Migrating endothelial cells generate proteases that are capable of degrading articular structures such as cartilage [94]. However, the direct contribution of blood vessels to tissue destruction may be small compared with that of fibroblasts and inflammatory cells. Angiogenesis may facilitate growth of the invasive synovial pannus, rather than directly leading to that invasive process [1]. Pannus has been compared with a neoplastic growth [95].
Angiogenesis is associated with increased tumour growth, and reduced tumour cell apoptosis [96]. Similarly, angiogenesis was localized to regions of inflamed synovia that contained many proliferating and few dying synovial cells [59].

**Angiogenesis and tissue hypoxia**

Hypoxia is a potent stimulator of angiogenesis, upregulating genes that are associated with hypoxic response elements, such as that encoding VEGF [97]. The synovium in the inflamed joint is hypoxic and intra-articular pressure during exercise can exceed capillary perfusion pressure, further exacerbating local hypoxia [98, 99]. The generation of angiogenic factors by the inflamed synovium may be viewed as an attempt to relieve excessive hypoxia within the joint.

Vascular densities may be increased and metabolism is consequently anaerobic within the inflamed synovium, despite the generation of angiogenic factors and evidence of rapid endothelial cell proliferation [70, 71, 75]. Synovial inflammation differs in this respect from tissue repair, where angiogenesis is associated with relief of hypoxia and successful wound healing. Persistent synovitis can be viewed as injured synovium which fails to repair, and abnormalities of vascular growth may contribute to this persistence. Factors which may permit sustained hypoxia despite enhanced angiogenesis include the generation of agents that are toxic to microvessels, inherent inability of vascular densities to increase, and qualitative changes in the synovial microvasculature.

A wide variety of factors may induce death of vascular endothelial cells, including oxidative stress, tumour necrosis factor alpha, serine proteases and angiotensin II [100–104]. Evidence for the presence of each of these factors has been observed in inflamed joints, suggesting mechanisms by which vascular regression may be stimulated alongside vascular growth.

Vascular endothelium requires ancillary cells for its survival and normal function. High vascular densities are found adjacent to the normal synovial surface, and there may be limited scope to increase further the fraction of tissue volume that is occupied by vascular endothelium.

Tissue oxygenation may be affected by the quality, as well as the density, of microvessels. Increased vascular turnover is associated with deficient vasoregulatory systems [5]. Inability of the microvascular bed to match blood flow to metabolic requirements could result in persistent tissue hypoxia despite high vascular densities. This may be a particular feature of synovium, whose dense microvascular network serves the metabolic needs of the avascular articular cartilage. Perturbations in microvascular function could contribute to persistence of inflammation in the synovium, whereas similar changes would be compatible with repair in other tissues [59].

In contrast to the increased angiogenesis in structures within the inflamed joint, adjacent striated muscles typically atrophy. Continued, recurrent electrical activity is required to maintain the high vascular densities that are required to support aerobic metabolism in normal muscle, whereas decreased activity is associated with reduced vascular densities [105]. Muscles with low vascular densities perform poorly in sustained activities, and deficient muscle angiogenesis may contribute to the weakness and fatigability associated with the disability of arthritis.

**Angiogenesis and ossification**

During enchondral ossification, chondrocytes hypertrophy and new blood vessels grow from the perichondrial vascular network, invading the cartilage [106]. Osteoblastic activity then commences around the neo-vasculature. Hypertrophic chondrocytes and osteoblasts each generate angiogenic factors such as transferrin and VEGF [107–109], and exogenous angiogenic factors can stimulate enchondral ossification [110]. The angiogenesis inhibitor TNP-470 inhibits the ectopic bone formation that follows the implantation in mice of pellets containing recombinant human bone morphogenetic protein-2 [111]. In summary, angiogenesis is required for enchondral ossification to proceed normally.

Angiogenesis inhibitors may have a therapeutic role in conditions associated with excessive new bone formation, as well as inhibiting bone destruction by inflammatory tissues. Angiogenesis-stimulating activity is increased in plasma of patients with osteoarthritis or ankylosing spondylitis, suggesting that angiogenesis may play an important role in new bone formation in these diseases [112, 113]. The potential for anti-angiogenic agents to inhibit osteophyte formation or reduce disease progression in inflammatory spinal diseases deserves further study.

In summary, angiogenesis may play various roles in arthritis. In established, persistent synovitis, angiogenesis may contribute to inflammation, tissue damage, osteophyte formation and to altered mechanical characteristics of articular cartilage. Angiogenesis is also a feature of early synovitis in man [114]. In an animal model of synovitis, early increases in endothelial cell proliferation were predictive of subsequent persistence of inflammation, whereas acute synovitis in the absence of angiogenesis was followed by resolution of inflammation [115]. Furthermore, the anti-angiogenic agents TNP-470 and integrin αvβ3 antagonists each protected against the development of persistent synovitis in animal models [116–118]. We have speculated that angiogenesis during early synovitis may contribute to the switch from acute to persistent inflammation by disturbing the delicate balance between synovial perfusion and metabolic demand.

**The pharmacological modulation of angiogenesis**

The development of pharmacological agents that inhibit angiogenesis offers great potential for dissecting the roles of angiogenesis in synovitis, and hopes for treating human arthritis. To demonstrate the importance of angiogenesis in synovitis, agents are required whose sole
action is to inhibit angiogenesis. For clinical therapeutics, the agents should improve outcome with minimal adverse events. Much progress has been made towards the development of effective and safe pharmacological tools. Despite these advances, difficulties still exist in the measurement of appropriate outcomes and the specificity of agents as inhibitors of angiogenesis.

Outcomes of angiogenesis inhibition
Since angiogenesis is a process rather than a single event, it cannot be adequately described by a single quantity. Measures of the earliest stages of angiogenesis, e.g. endothelial cell proliferation indices, do not necessarily predict the formation of fully functional vessels. Indices of later stages of angiogenesis, e.g. vascular densities, are affected by a wide variety of processes, including vascular regression and the proliferation, infiltration and death of non-vascular cells. Vascular density may change little despite other evidence of rapid angiogenesis [59].

Studies of angiogenesis inhibitors in inflammatory synovitis have focused on changes in vascular density, a quantity used extensively in the study of angiogenesis inhibition in tumours [116, 118, 119]. Tumours differ from synovium, however, in being initially avascular. As discussed above, changes in vascular density may not be the most important outcome of angiogenesis in the pathogenesis of synovitis. Reduced expression of vasoregulatory systems, mismatching of perfusion and metabolic demand, and synovial hypoxia each may influence persistent synovitis and may be a target for angiogenesis inhibition.

Clinical outcomes that may be improved by angiogenesis inhibition include persistent synovial inflammation, pannus formation, bone erosion, and new bone formation. The relevance of any such effects to outcomes important to the patient, such as pain, functional ability and psychological well-being, requires further study. On the other hand, outcomes such as changes in joint scores and acute-phase response may not reflect direct effects of angiogenesis inhibition, and may be relatively insensitive indices for measuring response.

Angiogenesis is essential for a variety of beneficial processes. Potentially adverse clinical outcomes of angiogenesis inhibition may include delayed skin wound, fracture and gastrointestinal ulcer healing, infertility, teratogenicity and impaired muscle function. Vascular growth is important in healing gastrointestinal damage induced by non-steroidal anti-inflammatory agents [90, 120]. Angiogenesis inhibitors can prevent female reproduction and have been proposed as potential contraceptive agents [121]. The teratogenic and abortifacient potentials of anti-angiogenic agents are of concern in women of child-bearing age. Mice lacking genes for VEGF or its receptors die in utero with major cardiovascular abnormalities [122–124]. Thalidomide, a well-known teratogenic agent, has anti-angiogenic activity [125].

Specificity of angiogenesis inhibitors
Angiogenesis inhibitors developed to date often lack specificity, such that it is difficult to attribute improved outcomes to inhibition of angiogenesis. Inhibitors of angiogenesis may modify other biological processes, independently of their effect on angiogenesis, and they may have different effects on the angiogenesis associated with different physiological and pathological conditions or in different tissues.

Many slow-acting anti-rheumatic agents currently in use for the treatment of RA can inhibit angiogenesis. Gold, penicillamine, sulphasalazine, methotrexate and antimalarials each can reduce endothelial cell proliferation in vitro, and inhibit angiogenesis in vivo [126–131]. Other compounds currently under development for the treatment of arthritis may also inhibit angiogenesis. Examples include metalloproteinase inhibitors and cytokine antagonists [132, 133]. Non-steroidal anti-inflammatory agents also have anti-angiogenic activity in some in vivo models [84]. Each of these agents additionally affects processes not directly related to vascular growth and it is not yet possible to attribute disease-modifying activity of slow-acting anti-rheumatic agents to inhibition of vascular growth.

Several of those compounds that have been developed as angiogenesis inhibitors can inhibit synovitis in animal models. TNP-470 selectively inhibits the proliferation of endothelial cells, and ameliorates arthritic and collagen arthritides induced experimentally in rats [116, 117]. Integrin αβ3 antagonists also inhibit the arthritis which follows bilateral knee injection with ovalbumin and basic fibroblast growth factor (bFGF) in pre-immunized rabbits [118]. However, these agents also may have non-vascular effects. TNP-470 modulates lymphocyte and osteoclast functions, and integrin αβ3 is strongly expressed by lining cells in the chronically inflamed synovium [59, 134, 135]. The precise mechanisms through which these agents can inhibit synovitis therefore remain unknown. Thrombospondin, an anti-angiogenic agent in many systems, was not found to inhibit synovitis in rats [119]. However, thrombospondin is a large, multifunctional protein that can even promote angiogenesis under some circumstances [39].

Several studies therefore indicate that inhibition of angiogenesis may be a useful therapeutic strategy in inflammatory arthritis. However, it is not yet possible specifically to eliminate angiogenic processes by interventions that do not independently modify other components of inflammation. Such multiplicity of action may have therapeutic advantages, but limits our ability to demonstrate the roles of angiogenesis in synovitis.

Targeting individual angiogenic pathways provides one approach to the selective inhibition of pathological, rather than physiological, angiogenesis. Different pathways may be important in angiogenesis under different circumstances. bFGF-enhanced angiogenesis is dependent on integrin αβ3, whereas VEGF-enhanced angiogenesis requires integrin αβ3 [136]. A variety of factors that stimulate plasma extravasation appear to enhance
angiogenesis through pathways which involve nitric oxide. Such factors include VEGF and substance P [14, 137]. Vascular growth can be inhibited by antagonizing the specific interactions between growth factors and their receptors [138]. Inhibition of individual factors or common pathways may be less prone to adverse clinical events than may be expected with global inhibitors of angiogenesis.

Further research is required to determine which angiogenic pathways are important in the increased angiogenesis observed in various tissues in the inflamed joint. The angiogenic factors that are generated early during the acute inflammatory response differ from those which are upregulated during chronic inflammation (Table 1). For example, substance P is generated and released during acute inflammation, but may be depleted in chronically inflamed tissues [9, 139]. On the other hand, VEGF is present at low levels in normal synovium, but is upregulated during chronic inflammation [19, 59]. Different factors may therefore be important in the initiation and the maintenance of angiogenesis. The pathways that mediate vessel growth in early arthritis may differ from those in persistent synovitis. The regulation of angiogenesis during ossification is likely to differ from that in synovial inflammation, since normal cartilage is known to generate a variety of anti-angiogenic agents including metalloproteinase inhibitors [77, 78]. Such diversity creates the potential to inhibit angiogenesis associated with specific pathological processes, while leaving intact those angiogenic pathways that may be beneficial to the organism.

Other approaches to the regulation of vessel growth include pharmacological manipulation of endothelial cell death. The mechanisms remain unclear by which endothelial cell proliferation and cell death are balanced, permitting increased cell turnover without large changes in vascular density. Several vascular survival factors have been identified, including integrin αβ3 and VEGF [140, 141]. Inhibiting vascular turnover by reducing endothelial cell death may allow vasoregulatory systems to develop. Stimulation of nerve growth by topical application of nerve growth factor accelerates the healing of corneal ulcers [142]. Would stimulating the growth of perivascular nerves facilitate the repair of mesenchymal tissues such as the synovium?

Under some circumstances, it may be desirable to stimulate vessel growth. bFGF can enhance the healing rate of gastrointestinal ulcers, and genetic transfer of VEGF can increase perfusion and oxygenation of ischemic limbs [143, 144]. It remains to be determined whether such approaches could ameliorate synovial hypoxia without exacerbating inflammation or pannus growth and invasion.

Conclusions

Angiogenesis is enhanced in rheumatoid synovitis, and is one component of the increased vascular turnover observed during chronic inflammation. Vessel growth may contribute to the pathogenesis of synovitis, pannus growth, bone and cartilage destruction, and ossification of articular cartilage and osteophytes. Angiogenesis also has many beneficial actions during the repair of injured tissues and the normal female reproductive cycle. Several lines of evidence indicate that inhibition of angiogenesis may be a productive therapeutic strategy in human arthritis. Future studies should address the specific pathways that mediate pathological angiogenesis. Measured outcomes should reveal the effects of interventions on angiogenesis, as well as clinical efficacy of consequence to the patient. Major advances in our understanding of angiogenesis have been catalysed by recent research in oncology, wound healing and vascular biology. However, the inflamed joint differs in many respects from tumours and healing tissues. Angiogenesis is a complex process rather than a single entity, and our increasing understanding of the various roles of vascular growth raises the exciting possibility of novel therapeutic strategies in human arthritis.

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