Antagonising angiogenesis in rheumatoid arthritis

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Angiogenesis in the inflamed joint

Rheumatoid arthritis (RA) is a chronic systemic disease characterised by an inflammatory erosive synovitis. Early changes in the synovium are marked by neovascularisation, inflammatory cell infiltration, and associated synoviocyte hyperplasia, which produce a pannus of inflammatory vascular tissue. This pannus covers and erodes articular cartilage, eventually leading to joint destruction.

Angiogenesis is a complex, highly regulated physiological process, which in normal adults is restricted to the female reproductive cycle and wound healing. A coordinated sequence of endothelial cell division, selective degradation of vascular basement membranes and surrounding extracellular matrix (ECM) with migration of endothelial cells[1] results in new capillary growth from existing vessels. Pathological angiogenesis is now recognised as a fundamental component of pannus development in RA,[2] with evidence of both focal endothelial proliferation and apoptosis in the synovium.[3]

The importance of angiogenesis in providing the vascular network to sustain synovial and immune cell proliferation is confirmed by pharmacological inhibition of angiogenesis, resulting in suppression of arthritis in experimental models.[4–7] More recently, specific inhibition of vascular endothelial growth factor (VEGF) by soluble receptor, sflt-1,[8] and by anti-VEGF antibody[9] has been shown to attenuate collagen induced arthritis in mice, supporting the major role of VEGF in promoting angiogenesis.

Angiogenic growth factors in RA

The initiation of angiogenesis is associated with expression of a number of angiogenic growth factors of which basic fibroblast growth factor-2 (FGF-2) and VEGF are the most potent.[10]

In RA, high levels of VEGF,[11] FGF-1,[12] FGF-2,[13] transforming growth factor β_1 ($TGF\beta_1$),[14] and hepatocyte growth factor[15] are present in synovial fluid (SF) or synovial membrane, secreted by synoviocytes or infiltrating leucocytes. The presence of these angiogenic factors confirms earlier studies showing that SF induces capillary-like structures in endothelial cell cultures.[16]

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VEGF as a primary modulator of RA angiogenesis

We have investigated the control of VEGF production in patients with RA by a hypoxia related stimulus and by other cytokines in a study of genetic markers of VEGF. Hypoxia, a potent stimulus for VEGF production,[17] independent of immune control, is commonplace in the joints of severely affected patients and is often accentuated where blood flow is compromised by joint swelling.[18 19]

Hypoxic induction of VEGF is mediated in part through a heterodimeric transcription factor, hypoxia-inducible factor-1 (HIF-1), which is activated in cells exposed to hypoxia or cobalt ions, thus allowing binding to a consensus sequence (5'-TACGTGCT-3') first described in the Epo 3' enhancer.[20] For VEGF, a cis-acting DNA sequence of 35 bp has been identified that allows hypoxia induced transcription of reporter genes and therefore constitutes a hypoxia response element . The hypoxia response element has an HIF-1 site with essential flanking sequences which includes the Epo sequence 5'-CACAG-3' present both upstream and downstream of this HIF-1 site.[21] Together with these promoter region sites that induce VEGF expression in response to hypoxia, five sites in the 3'-UTR have been identified that enhance mRNA VEGF stability,[22] with a consequent increase in translation.

Peripheral blood mononuclear cells (PBMCs) from patients with RA show a spontaneous increase in VEGF secretion, which can be further up regulated by cobalt ions mimicking hypoxia.[23] In a recent study we compared the cobalt stimulated VEGF production from PBMCs from 43 patients with RA and 21 osteoarthritic age matched controls (unpublished data). Patients with RA with more than five years' history of disease were categorised as either showing mild disease (Larsen score <50) or severe disease (Larsen score >90). Patients with severe disease had a significant increase in cobalt stimulated VEGF production (median 259 pg/ml, interquartile range (IR) 125–359) compared with patients with minimal disease (median 107 pg/ml, IR 10–218), p=0.0096 Mann-Whitney U test. The osteoarthritic control group were no different from patients with minimal disease, VEGF (median 82 pg/ml, IR 34–175). There was no significant difference in endotoxin stimulated VEGF production between the groups, and cobalt stimulated VEGF production was not associated with disease activity as assessed by C reactive protein level.

VEGF is also intimately linked into the processes of immune regulation as a number of cytokines and growth factors up regulate its expression in different cell types, including interleukin 1β (IL1β),[24] TGFβ,[25] FGF-2.[26] In studies investigating the effects of IL1β, $TGF\beta_1$, and hypoxia on VEGF induction and secretion from synoviocytes, synergistic interaction with growth factor and hypoxia is described.[27 28]

We have recently shown that tumour necrosis factor α (TNFα) and $TGF\beta_1$ up regulate VEGF production, whereas IL4 and IL10 down regulate it.[23] Interestingly, TNFα in

synovial fluid appears to be the main inducer of VEGF from blood monocytes.

We have described polymorphisms[29] in the VEGF promoter and 5'-UTR and have investigated two common polymorphisms at –460 and +405 for association with RA and for influence on VEGF production by cobalt. In a group of 160 patients with RA and 157 healthy controls we found no significant difference from controls in genotype for either polymorphism in the RA group and no association with genotype and cobalt stimulated VEGF production (unpublished data).

Role of heparan sulphate glycosaminoglycan (HSGAG) in modulation of growth factor biology

ECM has an important physiological role as a storage depot for a wide range of heparin binding growth factors, which are protected from proteolysis[30] and prevented from diffusing between tissue compartments, yet these growth factors can be rapidly mobilised to supply relevant cells. In vitro addition of FGF-2 to endothelial cells shows that 70% or more binds to the ECM, with only 7% binding to the cell surface receptor.[31] Interestingly, ECM-bound FGF-2 that is subsequently released is significantly more active as a mitogen than equivalent native growth factor, and the kinetics of growth factor release modulate target cell function.[32] Heparan sulphate proteoglycans (HSPGs) on endothelial cells such as syndecan and ryudecan bind and present growth factor to receptors on the same cell. In the absence of HSGAG, FGF-2 does not bind its receptor and is not active.[33]

The ECM component responsible for binding growth factors such as FGF-2[30] and VEGF[34] is HSGAG. Human synovium and cultured synovial cells express perlecan,[35] a specialised HSPG, originally identified in basement membranes. Approximately 25% of the proteoglycan produced by synovial cells contain heparan sulphate chains. Similarly, RA synovial endothelium and isolated endothelial cells express significant levels of HSPG.[36]

The heparan sulphate chain is endowed with a number of structural features that allow the molecule to bind ECM proteins, growth factors, and serine proteases. The ability to bind these ligands is conferred by particular structural features of the chain, including the organisation of N- and O-sulphate residues into domains of high and low charge density, the flexibility of the chain provided by the iduronic residues, and tissue-specific patterns of sulphation.

There is evidence that protein ligands bind to specific oligosaccharide sequences. FGF-2 binds to a five disaccharide sequence of N-sulphated glucosamine-iduronic acid 2-O-sulphate, with an affinity comparable with intact heparan sulphate.[37] VEGF, however, binds to sequences of at least 18 saccharide residues,[38] with maximum affinity for VEGF reported with sequences of 22 or more saccharide residues. The range of potent mediators that bind to heparan sulphate include the growth factors FGF-1, FGF-2, VEGF, placenta growth factor, hepatocyte growth factor, platelet derived growth factor, $TGF\beta_1$, the proinflammatory cytokines TNFα and IL1β, Th1 cytokines IL2, IL12, and interferon γ, Th2 cytokine IL4, and the chemokines IL8, monocyte chemotactic protein 1, and midkine. Therefore the high SF levels of a wide range of these heparan sulphate binding cytokines/growth factors in patients with RA[39] might result from overproduction of growth factors, leading to saturation of HSGAG sites in ECM or underproduction of HSGAG with poor ECM retention of growth factor. Alternatively, excessive mobilisation of HSGAG-growth factor complexes may be the result of aberrant heparanase expression.

Heparanase and angiogenesis

Heparanase (a generic term for enzymes degrading HSGAG) promotes angiogenesis through cleavage of the ECM-heparan sulphate and mobilisation of ECM resident growth factors—for example, FGF-2.[40] As far as we know we were first to describe heparanase in human PBMCs,[41] and heparanase has been identified in all leucocytes, including T lymphocytes, B cells, neutrophils, and mast cells. Non-immune cells such as fibroblasts, endothelial cells, and tumour cells—for example, melanoma, can be induced to express heparanase. The gene for human heparanase has been cloned from a placental library and simultaneously reported by research groups in July 1999.[42–44] The gene is on chromosome 4 and consists of 12 exons with a 2.3 kb sequence upstream of the coding region containing the promoter. The enzyme is synthesised as a latent 65 kDa protein which is N-terminally processed by proteases at a glu_{157}-lys_{158} cleavage site to an active form of 45–50 kDa.

We have developed a new solid phase quantitative assay specific for heparanase which is unaffected by proteases, chondroitinases, and hyaluronidase. For the first time (Behzad and Brenchley, unpublished data) we have identified heparanase activity in SF and found significantly higher levels in SF from patients with RA (n=7, median 4.44 mIU, IR 4.28–4.50) than from non-inflammatory SF (n=10, median 3.28 mIU, IR 3.26–3.31), p=0.001 Mann-Whitney test. We are currently identifying the cellular source of heparanase in SF and the factors that promote its up regulation and activation.

Strategies for inhibiting angiogenesis in RA

Evidence for the involvement of VEGF in promoting angiogenesis in RA has been discussed. Clearly, there are several places in the pathway from hypoxic induction or cytokine up regulation of VEGF that could be targeted to block the effect of VEGF driven angiogenesis. Interference with hypoxic triggering of VEGF would be a new and as yet unreported treatment. The mechanism of action of anti-TNFα treatments currently in use has not been fully described, but by neutralising TNFα, the ability of TNFα to up regulate VEGF may be

contained. The conventional approaches to neutralise the effects of VEGF by use of monoclonal anti-VEGF or soluble recombinant receptor, sflt-1, or to inhibit VEGF receptor signal transduction by use of specific tyrosine kinase inhibitors are in progress. Efforts are currently targeted at inhibiting VEGF driven angiogenesis in cancer and, if successful clinically, this experience could be rapidly translated to angiogenesis in other diseases—for example, RA.

The problem with antagonising angiogenesis through inhibition of a single growth factor such as VEGF is the inherent degeneracy of cytokine/growth factor biology with the danger that by blocking one growth factor, such as VEGF, that over time another—for example, FGF-2, may predominate, thus rendering the specific antagonism ineffective. The attraction of blocking angiogenesis through inhibition of heparanase, which in theory might control all HSGAG binding angiogenic factors may overcome this problem. Competitive substrate inhibitors or active site-specific sFvs are under development to antagonise active heparanase. Alternatively, it would be possible to inhibit activation of the latent heparanase precursor by blocking the protease cleavage step.

There is significant support for the idea that pathological angiogenesis is an important contributor to the mechanism of inflammatory joint erosion. Anti-TNFα treatments may block TNFα driven VEGF angiogenesis, but would be ineffective where hypoxia is the main stimulus for VEGF up regulation. In those patients who respond poorly to TNFα antagonist treatment, an anti-angiogenesis agent may prove to be of benefit.

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