Summary. There is much evidence that rheumatoid arthritis is closely linked to angiogenesis. Important angiogenic mediators have been demonstrated in synovium and tenosynovium of rheumatoid joints. VEGF (Vascular Endothelial Growth Factor), expressed in response to soluble mediators such as cytokines and growth factors and its receptors are the best characterized system in the angiogenesis regulation of rheumatoid joints. Moreover, other angiogenic mediators such as platelet-derived growth factor (PDGF), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), insulin-like growth factor (IGF), hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β), tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, IL-13, IL-15, IL-18, angiogenin, platelet activating factor (PAF), angiopoietin, soluble adhesion molecules, endothelial mediator (endoglin) play an important role in angiogenesis in rheumatoid arthritis. On the other hand, endostatin, thrombospondin-1 and -2 are angiogenic inhibitors in rheumatoid arthritis. The persistence of inflammation in rheumatoid joints is a consequence of an imbalance between these inducers and inhibitors of angiogenesis.

Key words: Angiogenesis, Anti-angiogenesis, Rheumatoid arthritis

Angiogenesis

Angiogenesis is a well-programmed cascade of events. Angiogenic factors activate EC and they produce proteolytic enzymes including matrix metalloproteinases and plasminogen activators. This results in the degradation of the basement membrane and the perivascular extracellular matrix. EC proliferate and migrate into the perivascular area to form primary sprouts whose lumenation leads to the formation of capillary loops and then the synthesis of new basement membrane and capillary formation. EC released from the primary sprouts and loops give rise to secondary and further generations. The new blood vessels supply nutrients and oxygen to the augmented inflammatory cell mass and promote the entry of leukocytes. This serves to perpetuate synovitis.
Vascular endothelial growth factor (VEGF)

Many endothelial growth factors have been demonstrated in the RA synovium and tenosynovium. An important mediator of angiogenesis is endothelial-selective VEGF (Koch, 2000), which also induces vascular permeability. VEGF is important in the development of RA joint destruction as shown by the significant correlation between serum VEGF at presentation and the magnitude of radiological deterioration within the first year as calculated from the corresponding hand and foot radiographs (Ballara et al., 2001; Taylor, 2002). These results suggest that early high serum VEGF levels are associated with the later increase in joint damage. VEGF expression is upregulated in macrophages and fibroblasts in the synovium of RA patients, and VEGF has also been described in their serum, synovial tissue and fluids (Fava et al., 1994; Koch et al., 1994; Jackson et al., 1997; Harada et al., 1998; Kikuchi et al., 1998; Bottomley et al., 1999). Neutrophils secrete VEGF and levels of neutrophil-associated VEGF in rheumatoid synovial fluids correlate well with free VEGF in joint effusions and with disease activity (Taichman et al., 1997; Kasama et al., 2000). In vitro, human peripheral blood mononuclear cells release VEGF in response to cytokines occurring in rheumatoid joints, including tumor necrosis factor alpha (TNF-α) (Bottomley et al., 1999). Release of VEGF from platelets has also been reported (Nagashima et al., 2000). VEGF levels are markedly higher in the serum and synovial fluids of RA patients than in osteoarthritis patients and normal controls (Koch et al., 1994; Nagashima et al., 1995; Harada et al., 1998; Paleolog et al., 1998; Lee et al., 2001). Serum VEGF concentrations in rheumatoid patients correlate with levels of C-reactive protein (Paleolog et al., 1998). Expression of VEGF mRNA by lining cells in RA has been reported, and immunohistochemical investigation of biopsies has revealed expression of VEGF by the lining itself and by EC lining small blood vessels in the pannus (Fava et al., 1994; Koch et al., 1994; Pufe et al., 2001). Moreover, microvascular EC in the vicinity of VEGF-positive cells express mRNA for VEGF receptors (Fava et al., 1994; Ikeda et al., 2000).

Several isofoms of VEGF are generated by alternative splicing of VEGF mRNA. The gene for human VEGF is organized into eight exons, and the resultant mRNA undergoes alternative splicing events to generate five transcripts encoding VEGF proteins containing 121, 145, 165, 189, and 206 amino acids. These isoforms display different heparin-binding properties that determine whether the different glycoproteins are secreted or remain cell-associated. VEGF165 and VEGF121 are secreted efficiently from producing cells, whereas VEGF189 and VEGF206 remain bound to the cell surface and extracellular matrix, presumably due to the presence of a highly cationic 24-amino acid sequence encoded by exon 6. Covalent dimerization of VEGF is essential for its biological activity. Closely related members of the VEGF family include VEGF-B, VEGF-C, and placental growth factor (PlGF). VEGF-induced effects are mediated through receptor tyrosine kinases with seven extracellular immunoglobulin-like domains expressed predominantly, though not exclusively, on EC. VEGF (in particular VEGF165), PlGF, and VEGF-B are the primary ligands for Flt-1 (VEGFR-1), whose exact function is unclear. VEGFR-1 undergoes weak tyrosine autophosphorylation, but is thought unlikely to transmit a signal, whereas it may act as a decoy receptor and prevent VEGF binding to other receptors. The mitogenic effects of VEGF are mediated through an alternative VEGF receptor, the Flk-1/KDR (VEGFR-2). This also binds VEGF-C, though with a reduced affinity compared to VEGF (Ferrara, 2001). Activation through VEGFR-2 leads to the tyrosine phosphorylation of multiple components in EC, including p125 focal adhesion kinase, which could be important in the cytoskeletal changes preceding EC migration, and the Src homology domain, which contains molecules such as phospholipase C. Additional receptors act as coreceptors. For example, neuropilin-1 acts as a coreceptor for VEGFR-2 and enhances VEGF165 binding and biological activity. VEGFR-1 protein is expressed on microvessels in close proximity to VEGF protein and there is a close correlation between VEGF165 expression and that of VEGFR-2 and neuropilin (Ikeda et al., 2000). VEGF-C and VEGF-D have also been described. In contrast with VEGF, VEGF-C is inducible by interleukin-1 (IL-1)/TNF-α, but not by hypoxia. VEGF-C is chemotactic for monocytes and mediates angiogenesis and lymphangiogenesis. It is the ligand for VEGFR-3 and is strongly expressed in rheumatoid synovial lining, pericytes and vascular smooth muscle (Paavonen et al., 2002). VEGF-D binds VEGFR-2 and VEGFR-3 on lymphatic EC and is angiogenic. VEGF-D expression is not abundant in rheumatoid synovial tissue (Paavonen et al., 2002). VEGFR-2 and VEGFR-3 are expressed in rheumatoid synovium on blood vessels. VEGFR-3 is regarded as a relatively specific marker for lymphatic vessels in adult tissue. The significance of its expression by rheumatoid synovial EC, however, is unclear.

RT-PCR analysis shows that VEGF121 is expressed constitutively in rheumatoid synovial tissues, whereas VEGF165 is expressed in less than half the tissues examined (Ikeda et al., 2000; Pufe et al., 2001). Microvascular density is significantly higher in rheumatoid synovial tissues expressing VEGF165 (Ikeda et al., 2000). The proportion of CD31-expressing microvessels that are activated, as assessed by a monoclonal antibody recognising the VEGF/VEGFR complex, is significantly greater in RA than in osteoarthritis or normal synovial tissue (Giatromanolaki et al., 2001).

The role of hypoxia

Hypoxia in the rheumatoid joint suggests that...
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angiogenesis in the pannus may be driven by hypoxia-induced expression of VEGF. Expression of hypoxia-inducible factor-1 alpha (HIF-1α) by macrophages in rheumatoid synovium, predominantly close to the inimal layer but also in the subintimal area, has been described (Hollander et al., 2001).

To investigate the relation between tissue oxygen levels and synovial VEGF production in inflammatory arthritis in humans, patients were examined during knee arthroscopy. Synovial PO₂ levels were significantly lower in patients with active RA than in patients without RA, and release of VEGF from synovial cells prepared from tissue biopsies was likewise greater for RA patients. It would appear, therefore, that reduced intra-articular PO₂ is a stimulus for local VEGF production (Taylor et al., 2000).

Several molecular mechanisms are thought to be involved in hypoxia-induced up-regulation of VEGF expression, including transcriptional control, through transcriptional factors such as HIF-1, and post-transcriptional stabilization of VEGF mRNA (Levy et al., 1998). Furthermore, hypoxic culture conditions greatly augment the rate of VEGF secretion from cultured synovial fibroblasts stimulated by IL-1 and transforming growth factor beta (TGF-β) (Berse et al., 1999).

The cloning and molecular characterization of HIF-1 constitutes a significant advance in understanding cell adaptation to hypoxia (Wang et al., 1995). HIFs are heterodimeric transcription factors that regulate several adaptive responses to low oxygen tension. HIF-1 comprises two subunits, HIF-1α (120 kD) and HIF-1ß (91-94 kD), which contain a basic helix-loop-helix domain that permits recognition and binding to the HIF-1 DNA binding site within regulatory sequences of hypoxia-inducible genes. Intracellular HIF-1α concentrations are oxygen-independent, whereas HIF-1α is undetectable in aerobic conditions because of rapid ubiquitination followed by proteosomal degradation mediated by the von Hippel-Lindau tumor suppressor factor (Maxwell et al., 1999). This interaction requires the oxygen-dependent hydroxylation of three amino acid residues. Under normoxic conditions, the two subunits have a very short half-life, due to hydroxylation of conserved proline residues by prollyl 4-hydroxylase enzymes, which use oxygen as a cosubstrate. This allows binding of the von Hippel-Lindau E3 ubiquitin ligase complex, which targets HIF-1α for proteosomal destruction. Additional oxygen-dependent hydroxylation of asparaginyl residues within HIF-1α regulates recruitment of transcriptional coactivators. The critical dependence of prollyl and asparaginyl hydroxylation on oxygen means that under hypoxic conditions HIF-1α accumulates in the nucleus, where it binds to constitutively expressed HIF-1α, recruits coactivators and recognizes hypoxia-responsive elements (HRE) in the promoters of target genes. This critical hydroxylation event becomes rate-limiting in hypoxic conditions, so that HIF-1α is no longer degraded (Distler et al., 2004).

At oxygen concentrations below 6%, cellular HIF-1α levels rise exponentially to a maximum at approximately 0.5% corresponding to PO₂ values of 10 to 15 mmHg. HIF-1α is then free to bind its constitutively expressed partner HIF-1ß and thus completes the HIF-1 complex that translocates to the nucleus, where it binds HRE in the promoters of certain genes and up-regulates a gene program associated with angiogenesis, glycolysis and adaptation to pH (Kallio et al., 1998; Wiesemer et al., 1998).

VEGF mRNA levels are dramatically increased within a few hours when cell cultures are exposed to hypoxia and return to background when a normal oxygen supply is resumed. A HIF-1 binding site is crucial for the hypoxic induction of VEGF gene expression. However, sequences upstream from the HRE are required for full sensitivity to hypoxia induction, an effect apparently due to binding of other transcription factors. Additionally, within the 3'-untranslated region of VEGF mRNA, denylate-uridylate-rich RNA elements form a hypoxia-inducible RNA-protein complex that stabilizes VEGF mRNA. Tissue hypoxia in the rheumatoid joint results in increased VEGF mRNA stability and enhances VEGF gene transcription through the binding of HIF-1 or HIF-2 (Richard et al., 1999). Both factors are degraded within minutes of exposure to an oxygen tension >3-5%, but are stabilised under hypoxic conditions (<3% oxygen), then translocated to the nucleus, where they bind to HRE on hypoxia-inducible genes to up-regulate their expression (Mason et al., 2001).

The hypoxic environment in the rheumatoid joint, compounded by the high metabolic demands of synovial inflammation, may promote transcriptional changes permissive to perpetuation of synovitis. HIF-1 and HIF-2 are over-expressed in the synovial lining and stromal cells of patients with RA arthritis compared to synovial tissues from individuals without arthritis (Paleolog, 2002; Giatromonolaki et al., 2003). Furthermore, exposure of synovial tissue explants to hypoxia markedly enhances nuclear expression (Hitchon and Elbatalawly, 2004).

In this way, the hypoxic environment in the rheumatoid joint promotes transcriptional changes permissive for perpetuation of synovitis. In vitro experiments suggest that stabilization of HIF-1α is also mediated by several growth factors and cytokines important in RA pathogenesis, including IL-1ß. Furthermore, addition of IL-1ß or TNF-α to synovial fibroblast cultures up-regulates HIF-1α mRNA (Thornton et al., 2000). In vitro, hypoxia greatly augments VEGF secretion from synovial fibroblasts after stimulation by IL-1 and TGF-ß (Berse et al., 1999).

Direct measurements confirm that the rheumatoid joint is a hypoxic environment (Taylor et al., 2000). Synovial fluid samples from RA patients are hypoxic and acidic, with low glucose and high lactate concentrations indicative of local anaerobic metabolism (Falchuk et al., 1970; Lund-Olesen, 1970). Synovial fluid PO₂ in rheumatoid knee joints was reported to be
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as low as 27 mmHg compared with 43 mmHg in osteoarthritis and 63 mmHg in traumatic effusions in otherwise healthy control. Subsequent studies supported these findings and even recorded PO_2 values below 13 mmHg when silver microelectrodes were used in mice with established collagen-induced arthritis (an experimental RA model), and similar levels were detected in patients with inflammatory arthritis (Taylor et al., 2000; Etherington et al., 2002). Contributory factors include the high metabolic demands of inflamed synovial tissue and the rapid rate of synovial proliferation, such that cells become more distant from the closest blood vessels and compound hypoxia (Stevens et al., 1991).

Other factors boosting intra-articular pressure to as high as 300 mmHg include movement and accumulation of synovial fluid in the joints involved. This further impairs the vasculature and exacerbates hypoxia in an already ischemic environment. On completion of movement, intra-articular pressure normalizes and small vessels refill. A high proportion of vessels in rheumatoid synovia express neovascular markers, however, and lack the accessory cells associated with mature vasculature that in health permit autoregulation of blood flow in response to tissue demand. In the joints of mice with collagen-induced arthritis, tissue oxygenation is dysregulated in response to movement in comparison with healthy nonarthritis mice (Etherington et al., 2002). Similarly, dysfunctional vasculature in the rheumatoid joint fails to maintain adequate tissue oxygen homeostasis, and the joint may be susceptible to hypoxic-reperfusion injury, thus favoring a redox environment in which cell systems generate reactive oxygen species by nicotinamide-adenine dinucleotide phosphate-mechanisms (Rowley et al., 1984; Blake et al., 1997; Simonini et al., 2001). If present in high concentrations, reactive oxygen species can lead to tissue damage.

Many growth factors and cytokines regulate VEGF expression. Fibroblast expression of VEGF is up-regulated by IL-1 and TNF-α. In rheumatoid patients treated with anti-TNF-α, vascular deactivation occurs and serum VEGF levels fall in step with clinical improvement. Bone morphogenetic proteins (BMPs), which induce formation of cartilage and bone, and seem to down-regulate IL-1, induced VEGF production. Recent studies have indicated that VEGF partly stimulates angiogenesis via cyclooxygenase-2 (COX-2) induction (Hernández et al., 2001). VEGF up-regulated decay accelerating factor (DAF) expression on EC, which is cytotoxic against activated complement and may regulate endothelial proliferation and angiogenesis (Mason et al., 2001). Other investigators characterized a subpopulation of circulating human CD34+ cells expressing the VEGFR-2. These cells seem to be functional endothelial precursors involved in angiogenesis (Peichay et al., 2000). Significant serum VEGF reduction in response to treatment of RA suggests that an imbalance between inducers and inhibitors of angiogenesis contributes to persistence of joint inflammation. Concentrations of endostatin, an angiogenesis inhibitor, in fact, are not elevated in serum or synovial fluid samples from patients with high serum VEGF concentrations, and serum endostatin levels rise after a single infusion of the anti-TNF-α agent infliximab (Walsh, 1999; Nagashima et al., 2000; Kucharz et al., 2003).

Diminished vascular permeability accompanying rapid suppression of VEGF levels may well contribute to the early reduction of joint swelling after anti-TNF-α therapy (Paleolog et al., 1998). Furthermore, TNF-α blockade is followed by reduced synovial angiogenesis and vascular regression in RA after infliximab (Taylor et al., 1998; Canete et al., 2004; Goedkoop et al., 2004).

Other drugs used to treat RA may also modulate VEGF production. Cyclosporin treatment of rheumatoid synovial fibroblasts exposed to TGF-β results in decreased production of VEGF (Cho et al., 2002). Experimentally, modulation of a VEGF receptor through a VEGFR-1 Fc molecule suppressed rheumatoid synovial endothelial proliferation (Sekimoto et al., 2002). Another naturally occurring inhibitor of angiogenesis is soluble sFlt-1, an alternatively spliced form of Flt-1 (Kendall et al., 1996). Serum sFlt-1 is increased in patients with both early and chronic RA compared with control individuals and its levels positively correlate with VEGF concentrations in RA patients (Ballara et al., 2001). Elevated sFlt-1 in RA sera presumably reflects an attempt to achieve homeostasis that fails to inhibit VEGF activity. Anti-Flt-1 or sFlt-1 reduced synovial angiogenesis and arthritis (Miotla et al., 2000; Lutten et al., 2002).

Importance of CD40-CD40L interactions

CD40-CD40L interactions in rheumatoid synovitis have been regarded as important because CD40 is expressed on synovial fibroblasts. This interaction increases the expression of adhesion molecules such as ICAM-1, VCAM-1, and E-selectin, which can recruit circulating leukocytes to the antigenic challenge site. Furthermore, activation of T cells via antigenic receptors enhances CD40L expression as well as interferon alpha (IFN-α) production, which further augments the inflammation. T cells expressing CD40L stimulate synovial fibroblasts and monocytes to generate a series of proinflammatory cytokines and increase the expression of matrix metalloproteinase (MMP) (Chul-Soo et al., 2000). CD40L on activated T cells is responsible for the induction of VEGF from fibroblast-like synovial cells.

At the synovitis site, activated T cells are recruited adjacent to the resident synoviocytes by the stimulatory effect of a set of cytokines or chemokines. Formation of the CD40L-CD40 bridge may thus allow infiltrating T cells to induce the proliferation of synovial fibroblasts and up-regulate VEGF, which could further augment the recruitment of inflammatory cells into the synovium by promoting neovascularization. In this context, CD40L could be responsible for establishing a critical
amplification loop, which leads to the persistence of synovitis. Anti-CD40L monoclonal antibody (mAb) treatment is reported to suppress the development of collagen-induced arthritis (Durie et al., 1993) by blocking joint inflammation, infiltration of inflammatory cells into synovial tissue and erosion of cartilage and bone. Prevention of collagen-induced arthritis by this mAb may also be mediated by the suppression of circulating Abs to collagen and decreased production of inflammatory mediators such as nitric oxide and MMP by macrophages or synovial cells (Stout et al., 1996).

Since IL-1 and TNF-α are also involved in the modulation of VEGF in vivo and in vitro (Chul-Soo et al., 2000), an increase of VEGF by CD40 ligation might be expected to be indirectly imposed by cytokines (IL-1, TNF-α) released following CD40 ligation. It also seems likely that prostaglandin E2 (PGE2) and TGF-β potent inducers of VEGF, are not involved in the production of VEGF by CD40 ligation because indomethacin or anti-TGF-β antibodies did not block the ability of CD40L to induce VEGF production. Together, these observations suggest that CD40L induces VEGF, independently of fibroblast-derived endogenous inducers of VEGF. Notably, the combined effect of CD40L+ L cells (mouse fibroblastic L cells transfected with the human CD40L) with these cytokines was additive and not synergistic. It may be that two kinds of stimuli, CD40L and cytokines, promote VEGF production via distinct pathways. This possibility is also supported by the observation that VEGF production by CD40 ligation was not mediated by IL-1, TNF-α, and TGF-β (Chul-Soo et al., 2000).

CD40L trimer induces clustering of the receptors to initiate signal transduction. Exactly how signal transduction via CD40 occurs is unknown, but multiple pathways may be involved. CD40 ligation results in the activation of transcription factors including NF-κB, NF-AT, and AP-1. However, the relative importance of these factors as CD40 effectors is still unclear. VEGF mRNA expression was increased by CD40 ligation, indicating that up-regulated VEGF production by CD40 ligation is attributable to transcriptional activation of the VEGF gene. In addition, dexamethasone completely abrogated up-regulation of VEGF mediated by CD40L. The exact way in which glucocorticoids act is not clear. They may somehow block the function of NF-κB, perhaps by direct physical association of the glucocorticoid receptor with the transcription factor. Furthermore, dexamethasone stimulates the transcription of IkBa, an inhibitor of NF-κB. CD40 ligation may stimulate NF-κB expression in fibroblast-like synovial cells, and NF-κB inhibition by dexamethasone may abrogate VEGF production. However, since glucocorticoid receptors may also interfere with AP-1 and NF-AT, a decrease in the level of VEGF caused by dexamethasone cannot be solely attributed to NF-κB inhibition (Chul-Soo et al., 2000).

Other growth factors

Other growth factors, such as platelet-derived growth factor (PDGF), FGF-2, epidermal growth factor (EGF), insulin-like growth factor (IGFI), hepatocyte growth factor (HGF) and TGF-β may also promote capillary formation.

The synovium of RA patients and joints from rats with adjuvant-induced arthritis contain increased amounts of FGF-2 (Yamashita et al., 2002). Moreover, a Sendai virus containing the FGF-2 gene aggravated adjuvant-induced arthritis, but had no effect on normal joints. Hence, it seems that FGF-2 worsens the progression of experimental arthritis, rather than being important when it starts (Koch, 2003).

FGF-2, VEGF and HGF are bound to heparin or heparan sulphate in the interstitial matrix. During angiogenesis, these mediators are released from the matrix by endothelial-derived plasmin and heparanase (Grant et al., 1993; Paleolog and Fava, 1998; Szekanecz et al., 1998; Walsh, 1999; Brenchley, 2000). Heparanase promotes angiogenesis through cleavage of extracellular matrix-heparan sulfate and mobilisation of growth factors resident in the extracellular matrix. It has been identified in human peripheral blood mononuclear cells and in T lymphocytes, B cells, neutrophils, and mast cells. Fibroblasts and EC can be induced to express heparanase (Brenchley, 2001).

The extracellular matrix has an important physiological role as a storage depot for a range of heparin binding growth factors. They are protected from proteolysis and prevented from diffusing between tissue compartments, but can be rapidly mobilised to supply relevant cells. In vitro addition of FGF-2 to EC shows that 70% or more binds to the extracellular matrix, with only 7% binding to the cell surface receptor (Brenchley, 2001). Interestingly, the extracellular matrix-bound FGF-2 subsequently released is significantly more active as a mitogen than equivalent native growth factor, and the kinetics of growth factor release modulates target cell function. Heparan sulphate proteoglycans (HSPGs) on EC, such as syndecan and ryuedeacan, bind and present growth factor to receptors on the same cell. In the absence of heparanase, FGF-2 does not bind its receptor and is not active (Brenchley, 2001).

Human synovium and cultured synovial cells express perlecan, a specialised HSPG originally identified in basement membranes. Approximately 25% of the proteoglycan produced by synovial cells contain heparan sulphate chains. Similarly, rheumatoid synovial endothelium and isolated EC express significant levels of HSPG.

The heparan sulphate chain is endowed with a number of structural features that allow it to bind extracellular matrix proteins, growth factors, and serine proteases. The ability to bind these ligands is conferred by particular structural features of the chain, including the organization 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 sulphation patterns.

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 (Clark et al., 1992). VEGF, however, binds to sequences of at least 18-saccharide residues, with maximum affinity for VEGF reported with sequences of 22- or more residues. The range of potent mediators that bind to heparan sulphate include FGF-2, VEGF, PIGF, HGF, PDGF, TGF-β, the proinflammatory cytokines TNF-α and IL-1α, IL-2, IL-4, IL-8 and IL-12. The high synovial levels of a wide range of these heparan sulphate binding cytokines/growth factors in RA patients may thus reflect growth factor overproduction leading to saturation of heparanase sites in the extracellular matrix, or underproduction of heparanase with poor extracellular matrix growth factor retention. Alternatively, excessive mobilisation of heparanase-growth factor complexes may be the result of aberrant heparanase expression (Brenchley, 2001).

**Other angiogenic molecules**

Of the pro-inflammatory cytokines active in RA, IL-18, TNF-α, IL-1, IL-6, IL-8, IL-13, IL-15 are involved in angiogenesis. IL-18 is important in the induction of rheumatoid synovial angiogenesis (Park et al., 2001). It is a potent inducer of endothelial chemotaxis in vitro and angiogenesis in vivo in the Matrigel plug assay and sponge angiogenesis assay in rodents. Immunodepletion of IL-18 from rheumatoid synovial fluids resulted in reduced endothelial migration in vitro, suggesting that IL-18 is important in RA-mediated angiogenesis. IL-18 appears to act on endothelium through an αvβ3 mediated mechanism (Koch, 2003).

Other angiogenic molecules produced by synovial cells, including angiogenin, platelet activating factor (PAF) and substance P may play a role in RA-associated angiogenesis (Bodalay et al., 2002). Prolactin and prolactin-like polypeptides have been detected in rheumatoid synovial tissues and prolactin has an important role in T cell activation, cell communication and synovial angiogenesis (Takahara et al., 2004).

**The mechanism of action of some angiogenic factors**

EC invasion, migration, and proliferation are partly regulated by the integrin family of cell adhesion molecules. αvβ3 is minimally expressed on normal blood vessels, but highly expressed on rheumatoid synovial blood vessels. FGF-2, TNF-α, and IL-18 may act through αvβ3 integrins (Brooks et al., 1994; Koch, 2003), whereas VEGF or TGF-β appear to act through an αvβ5 integrin mechanism using protein kinase C. This may prove to be not the main mode of action of VEGF in RA, since αvβ5 integrin is expressed in normal and osteoarthritic synovial tissue, but not in rheumatoid synovial tissue (Koch, 2003). Nonetheless, the mechanisms through which some of these cytokines promote angiogenesis are rapidly becoming identified.

A proapoptotic αvβ3 antagonist, composed of an arginine-glycine-aspartic acid (RGD) peptide linked to a heptapeptide dimer, is therapeutic in mice with collagen-induced arthritis and is selective with their articular synovium compared with normal synovium and control organ endothelium (Koch, 2003).

**Chemokines as angiogenic mediators**

Chemokines are other important mediators of angiogenesis. Angiogenic chemokines facilitate inflammatory cell recruitment into the synovium by stimulating leukocyte chemotaxis and increasing the number of newly formed vessels.

The distinction between the CXC, CC, and CX3C chemokine families is based on the presence or absence of an amino acid, X, between a pair of cysteine residues near the amino terminus. CXC chemokines containing the ELR motif (glutamyl-leucyl-arginyl sequence) are angiogenic, along with the CC chemokines MCP-1, vMIP-I and vMIP-II (Volin et al., 2001), whereas those lacking the motif are angiostatic. The CXC chemokine stem cell derived factor-1 (SDF-1), however, induces endothelial chemotaxis in vitro and dermal angiogenesis in mice (Bodalay et al., 2002) despite its lack of the motif (Koch, 2000) and may be the first ELR-less angiogenic CXC chemokine.

Several CXC chemokines are involved in RA angiogenesis (Bodalay et al., 2002). They bind to EC via specific endothelial chemokine receptors, such as CXCR2. Those lacking the ELR motif, such as PF4, IP-10 and MIG, are potent angiostatic factors. In the rheumatoid synovium, chemokine-expressing cells are found near EC. The role of CC chemokines in RA angiogenesis has not been fully determined.

Fractalkine is the only characterized CX3C chemokine. It is expressed on cytokine-activated endothelia and enhances angiogenesis both in vitro and in vivo. When fractalkine is immunodepleted from rheumatoid synovial fluids, its ability to chemotacttract EC is decreased (Bodalay et al., 2002).

The expression of several adhesion molecules is upregulated in rheumatoid synovial tissue. The interactions between cytokines, chemokines and adhesion molecules may have additive stimulatory effects on angiogenesis (Bodalay et al., 2002).

**The angiopoietin-Tie system**

The angiopoietin ligand and Tie receptor families are also important regulators of blood vessel growth, maturation, and function (Takahara et al., 2004). Tie-1 and Tie-2 were originally identified as EC-specific tyrosine kinase receptors. Their dysfunction results in defective capillary network formation. Tie-1 is still an orphan receptor. Tie-2, on the other hand, is a specific receptor for angiopoietins (Ang) 1, 2, 3 and 4. In keeping with this function, genetic analysis has underscored the essential roles of Ang-1 and Ang-2 in the developing vessel: Ang-1 is agonistic, whereas Ang-2 acts as both a Tie-2 antagonist and an agonist (Tanaka
In rheumatoid synovium, Tie-1 and Tie-2 are expressed in the synovial lining and stromal cells in addition to blood vessels (Takahara et al., 2004). Tie-2 expression is almost restricted to a fibroblastic phenotype compared with the ubiquitous expression of Tie-1. Ang-1 and -2 are coexpressed with Tie-2 in many cellular components of rheumatoid synovia. They are also detected in vimentin-positive fibroblastic synovial lining and sublining cells. Their expression in synovial tissue and cultured synovial fibroblasts from RA patients has been examined by quantitative RT-PCR and immunohistochemistry (Takahara et al., 2004). Both ligands are exclusively coexpressed with Tie-2, indicating that Tie-2 mediates Ang-1 and -2 signaling by an autocrine/paracrine mechanism in the rheumatoid synovium. Furthermore, although actively proliferating lesions express abundant Ang-1, -2, and Tie-2, the level of expression does not necessarily reflect the extent of disease activity.

Although the biological significance of Ang-1 and -2 coexpression in rheumatoid synovium remains unclear, Ang/Tie-2 signaling is important for the up-regulated angiogenesis and subsequent synovial proliferation. High expression of Ang/Tie-2 may well be induced by local tissue hypoxia and/or high-level expression of TNF-α, both of which induce its expression in cultured EC and synoviocytes. However, it is also possible that up-regulated Ang/Tie-2 itself may play a central role in the tumor-like expansion of rheumatoid synovitis, as demonstrated in tumor-derived EC (Takahara et al., 2004).

Since angiopoietin-positive synovial lining cells display a fibroblastic phenotype and are also highly positive for proliferating cell nuclear antigen (PCNA), the Ang/Tie-2 system may be active in cultured fibroblastic synoviocytes.

The chemotactic properties of Ang-1 and -2 are considered important for close spatial and polarized interaction between EC and supporting cells. Fibrous synoviocytes display chemotactic migration in response to various inflammatory cytokines, including IL-1 and TNF-α. Ang-1 and -2 induce the chemotaxis of cultured synoviocytes. This seems specific for rheumatoid synovium, because skin fibroblasts do not respond, probably due to a lack of Tie-2 expression (Witzenbichler et al., 1998). The in vivo significance of the chemotactic reaction seen in cultured synoviocytes has not been established. The Ang/Tie-2 expression pattern observed, however, together with the chemotaxis property of cultured rheumatoid synoviocytes, suggests that Ang/Tie-2 signaling may serve two purposes in the rheumatoid synovium. First, angiopoietin expression in synovial lining and sublining cells may be important in recruiting EC to the avascular lining layer for the initiation and acceleration of capillary sprouting. In this case, cooperative activation with other angiogenic factors, would be required for effective vascular development. Moreover, the system may participate in the local inflammatory reaction by regulating leukocyte infiltration into synovial tissue (Takahara et al., 2004).

**Soluble adhesion molecules as angiogenic mediators**

EC shed and secrete soluble adhesion molecules in response to cytokine stimulation. The function of these molecules is uncertain. CD146 (Mucl8), a new endothelial antigen potentially involved in endothelial-leukocyte adhesion, is expressed in RA (Koch, 2003). These molecules may exert an anti-inflammatory function role by binding leukocytes so as to prevent them from adhering to endothelium and entering inflamed tissues, but may also be proinflammatory in some cases. The cellular adhesion molecules sE-selectin and soluble vascular cell adhesion molecule-1 (sVCAM-1) are angiogenic. Activated cells in the synovial milieu carry both molecules and shed them into the synovial fluid, where they interact with vascular EC via sialyl Lex, in the case of sE-selectin, and VLA-4, in the case of sVCAM-1, to mediate angiogenesis.

**Endoglin as an angiogenic mediator**

Endoglin, an endothelial glycoprotein, which contains an RGD motif and acts as an adhesion molecule, is a receptor for TGF-β. Mice lacking the endoglin gene die from defective vascular development. Endoglin is upregulated in rheumatoid synovial EC (Koch, 2000).

**Angiogenesis inhibitors**

Endostatin, a fragment of collagen type XVIII, decreases mouse joint mRNA levels of a number of angiogenic factors, including VEGF and FGF-2 (Koch, 2000). Thrombospondin-1 and -2 (TSP-1, TSP-2) are endogenous angiogenesis inhibitors. TSP-1 may act as an adhesive glycoprotein that mediates cellular interactions, or may counteract the effects of the angiogenic factors or proteinases produced by cells within diseased synovial tissues. TSP-1-expressing fibroblast-like synoviocytes have a direct role in the activation and clonal expansion of inflammatory T cells.

Overexpression of TSP-2 protein in a human RA model suppressed angiogenesis, but also disrupted immune stimulation and inflammation. These data suggest that TSP-2 is produced by resident synovial cells as a physiological regulator of local vascularization and has a protective role by preventing tissue inflammation (Park et al., 2004). Physiological production of TSP-2 by synovial fibroblasts may be the mechanism through which the synovial microenvironment protects itself from excessive blood vessel formation, and may also be a defense mechanism against tissue inflammation.

Fumagillin is a fungal contaminant that was shown to be angiostatic when it inhibited endothelial growth on a culture dish. Its derivatives AGM-1470 or TNP-470 inhibit both angiogenesis and arthritis in rodents.
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(Nagashima et al., 2002; Koch, 2003).

Three angiogenesis-modulating agents (thalidomide, taxol and 2-methoxyestradiol) have recently been investigated in rodent arthritis models. Thalidomide suppressed rat collagen-induced arthritis, though inhibition of TNF-α or VEGF. Taxol, a chemotherapeutic agent, inhibited both collagen-induced arthritis and synovial angiogenesis in rats. 2-methoxyestradiol decreased collagen-induced arthritis in mice (Koch, 2003).

COX-2 inhibitors have recently been released for the treatment of RA. Since they inhibit angiogenesis in tumors, they may be able to reduce it in RA (Koch, 2003).

Sulfasalazine, commonly used to treat a variety of diseases including RA, reduces endothelial proliferation as well as chemotaxis (Koch, 2003).

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