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Angiogenesis in rheumatoid arthritis

ZOLTÁN SZEKANECZ¹, TIMEA BESENYEI¹, GYÖRGY PARAGH², and ALISA E. KOCH^{3,4}

¹Department of Rheumatology, Institute of Medicine, University of Debrecen Medical and Health Sciences Center, Debrecen H-4032, Hungary

²Department of Metabolic Diseases, Institute of Medicine, University of Debrecen Medical and Health Sciences Center, Debrecen H-4032, Hungary

³Veterans' Administration, Ann Arbor Healthcare System, Ann Arbor, MI, USA

⁴Department of Internal Medicine, Division of Rheumatology, University of Michigan Health System, Ann Arbor, MI, USA

Abstract

Angiogenesis is the formation of new capillaries from pre-existing vessels. A number of soluble and cell-bound factors may stimulate neovascularization. The perpetuation of angiogenesis involving numerous soluble and cell surface-bound mediators has been associated with rheumatoid arthritis (RA). These angiogenic mediators, among others, include growth factors, primarily vascular endothelial growth factor (VEGF) and hypoxia-inducible factors (HIFs), as well as pro-inflammatory cytokines, various chemokines, matrix components, cell adhesion molecules, proteases and others. Among the several potential angiogenesis inhibitors, targeting of VEGF, HIF-1, angiogenic chemokines, tumor necrosis factor- α and the $\alpha_v\beta_3$ integrin may attenuate the action of angiogenic mediators and thus synovial angiogenesis. In addition, some naturally produced or synthetic compounds including angiostatin, endostatin, paclitaxel, fumagillin analogues, 2-methoxyestradiol and thalidomide may be included in the management of RA.

Keywords

angiogenesis; rheumatoid arthritis; vascular endothelial growth factor; angiostasis therapy

Introduction

Increased angiogenesis has been associated with various inflammatory disease states including rheumatoid arthritis (RA), as well as malignancies and ocular angiogenic diseases [1-10]. Angiogenesis is new capillary outgrowth from existing vessels that is a well-orchestrated sequence of events. Angiogenic mediators released by or expressed on various types of cells within the synovium activate endothelial cells (ECs). ECs produce proteolytic enzymes that degrade the underlying endothelial basement membrane, as well as the synovial extracellular matrix. ECs then proliferate and emigrate into the interstitial tissue and form primary and then further generation capillary sprouts. After lumen is formed within the sprout resulting in capillary loops, ECs synthesize new basement membrane and

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Correspondence: Z. Szekanecz, Department of Rheumatology, Institute of Medicine, University of Debrecen Medical Center, 22 Móricz Zs Street, Debrecen H-4032, Hungary. Tel: + 36 52 255 091; Fax: + 36 52 255 091. szekanecz.zoltan@med.unideb.hu.

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thus new capillaries are generated [1,2,5,7,11]. Some mediators also produced during the inflammatory process, as well as externally administered organic or synthetic compounds may disrupt neovascularization. These agents may be used to suppress angiogenesis in inflammatory and malignant diseases [6,12].

Here we review the most important mechanisms, mediators and inhibitors underlying inflammatory angiogenesis. When describing the basic mechanisms, we concurrently present therapeutic options aiming at the suppression of angiogenesis by inhibiting the action of angiogenic mediators or by the administration of angiostatic agents. Some of these treatment modalities have already been administered to patients with arthritis, as well as cancer, or at least to arthritic animals, while others may be potentially used in the future.

Basic mechanisms

In inflammatory arthritis, leukocytes migrate from the bloodstream through the vessel wall into the synovium. Leukocyte transendothelial migration involves a number of cell adhesion molecules, such as integrins, selectins and their respective ligands [1,3,13]. The perpetuation of angiogenesis associated with inflammatory states may lead to increased number of blood vessels, as well as that of total endothelial surface. Mechanistically, this process may result in enhanced leukocyte ingress into the synovium [13,14]. Some inflammatory mediators including pro-inflammatory cytokines and chemokines may promote both EC adhesion receptor expression and angiogenesis. Furthermore, as discussed later, some adhesion molecules themselves induce neovascularization. Thus, there is a collaboration between various soluble and cell surface-bound mediators during the angiogenic process [1,3,4,8,13,14] (Figure 1).

Angiogenic mediators and possibilities of therapeutic inhibition

As mentioned here, both soluble and cell-bound mediators including numerous growth factors, proinflammatory cytokines and chemokines, components of the synovial matrix, matrix-degrading proteases, cellular adhesion molecules and others [1-3,5,8]. Most of these mediators are released by macrophages and vascular EC into the RA synovium [1-3,5,8,14] (Table I).

The hypoxia-vascular endothelial growth factor (VEGF)-angiopoietin-Tie2 system

VEGF may be of outstanding importance in angiogenesis associated with both malignancies and inflammation. As a consequence, VEGF inhibitors have been introduced to arthritis, as well as cancer clinical trials [1,10,15-18]. Pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) stimulate synovial fibroblasts and other cells to release VEGF [1,2,14-17]. As described later, numerous other mediators including IL-6, IL-17, IL-18, nitric oxide (NO), hepatocyte growth factor (HGF), macrophage migration inhibitory factor (MIF), endothelin-1 (ET-1) and prostaglandins act indirectly on angiogenesis by promoting VEGF production [1,3,17,19-23]. VEGF induces EC proliferation and migration in *in vitro* culture systems and it also stimulates capillary formation in *in vivo* models of angiogenesis [15,17]. VEGF-induced angiogenesis also involve cyclooxygenase-2 (COX-2) induction [1,16,18].

VEGF targeting is in the focus of cancer and inflammation research [9,10,16]. One can inhibit VEGF-mediated neovascularization by using monoclonal antibodies to VEGF or VEGF receptors (VEGFR), soluble VEGFR constructs, small molecule VEGF and VEGFR inhibitors or inhibitors of VEGF and VEGFR signaling [6,12,16,17,24-28]. Some of these compounds have been first administered to cancer patients, primarily in colorectal, lung, renal and liver malignancies [6,9,12,16,17]. VEGF or VEGFR inhibition has been introduced to the treatment of neovascular eye diseases [10] and recently also to arthritis

trials [6,25-28]. Bevacizumab, a human monoclonal antibody to VEGF has been approved for the treatment of various types of cancer, as well as angiogenic ocular diseases [6,10,16]. Anti-VEGFR antibodies are also under development [28]. The VEGF-Trap construct is a composite decoy receptor based on the fusion of VEGFR1 and VEGFR2 with IgG1-Fc (24).

Several small molecule VEGFR tyrosine kinase inhibitors including vatalanib, sunitinib malate, sorafenib, vandetanib (ZD6474), cediranib (AZD2171), axatinib (AG013736), KRN-951 and CEP-7055 have been developed [6,16,17]. In cancer studies, these orally administered compounds exerted favorable safety profiles [6,16,17]. Semaphorin-3A and soluble Fas ligand (sFasL, CD178) are functional inhibitors of the 165 amino-acid form of VEGF (VEGF165) [27,29]. Both agents suppressed EC survival and angiogenesis [27,29] (Table II).

Regarding experience in arthritis, a soluble VEGFR1 chimeric protein dose-dependently inhibited synovial EC proliferation [25]. An anti-VEGFR1 antibody suppressed arthritis including clinical scores, leukocyte infiltration and the number of CD31⁺ ECs in murine CIA [28]. Among VEGFR protein kinase inhibitors mentioned above, vatalanib also inhibited knee arthritis in rabbits [6,26]. sFasL suppressed VEGF165 production by RA synovial fibroblasts [27].

Hypoxia has been detected in the arthritic joint [19,30]. Intraarticular hypoxia induces branching of capillaries at least in part by the stimulation of hypoxia-inducible factor (HIF-1 and HIF-2) production. In response, HIFs induce the release of VEGF [30,31]. Hypoxia may also act via HIF-independent regulatory pathways. For example, after an ischemic insult peroxisome-proliferator-activated receptor- γ (PPAR γ) and PPAR γ coactivator 1 α (PGC-1 α) induce VEGF production and the reconstitution of capillaries [32]. The PPAR γ ligands rosiglitazone and pioglitazone inhibit VEGF-induced angiogenesis [33]. Furthermore, pioglitazone also improved joint and skin symptoms in psoriatic arthritis [34]. Apart from PPAR γ , endothelial PPAR β/δ has also been implicated in EC proliferation and angiogenesis [35].

Hypoxia-HIF-mediated neovascularization may also be targeted. For example, *YC-1*, a superoxide-sensitive stimulator of soluble guanylyl cyclase, also inhibits HIF-1. This compound has been developed for the treatment of hypertension and thrombosis but may potentially be used to suppress inflammatory angiogenesis [6,36]. There have been attempts to target HIF-1 signaling in inflammatory bowel disease [37] (Table II).

The angiopoietin-1 (Ang1)/Tie-2 complex interacts with VEGF during the stabilization of newly formed blood vessels [38,39]. In contrast, Ang2, an antagonist of Ang1, inhibits vessel maturation [17,38]. *Survivin*, an apoptosis inhibitor, is also involved in VEGF-induced angiogenesis [40]. VEGF, hypoxia, HIF-1, HIF-2, Ang1, Tie2 and survivin have been all detected within the RA synovium [3,5,30,31,39,41]. This system may also be targeted. A soluble Tie2 receptor transcript delivered via an adenoviral vector to mice attenuated the incidence and severity of collagen-induced arthritis (CIA) [42].

Other growth factors

Some growth factors including basic (bFGF), acidic fibroblast growth factors (aFGF) and HGF are bound to heparin and heparin sulfate within the synovial interstitial matrix. During inflammatory neovascularization, these growth factors are released by matrix-degrading heparanase and plasmin [1-3,5]. Other, non-heparin-bound angiogenic growth factors include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I), keratinocyte growth factor (KGF) and transforming growth factor- β (TGF- β) [1-3]. Recently, placenta growth factor (PlGF) has been implicated in arthritis-

associated inflammation and angiogenesis. PIGF, as well as VEGF, binds to the flt-1 receptor. There is abundant expression of PIGF in the synovial tissue of mice with CIA [43].

Imatinib mesylate is a specific inhibitor of PDGF receptor activation. This compound inhibits the excessive growth of RA synovial fibroblasts and thus pannus formation [44]. Imatinib mesylate both preventatively and therapeutically inhibited the development of arthritis in the murine CIA model [45]. The PPAR γ agonists rosiglitazone and pioglitazone suppressed bFGF-mediated neovascularization [33]. An anti-flt-1 hexapeptide, GNQWFI abrogated PIGF-induced angiogenesis, cytokine production and the development of CIA in mice [43] (Table II).

Angiogenic chemokines and chemokine receptors

Chemokines and chemokine receptors have also been implicated in synovial inflammation, as well as angiogenesis [3,5,8,46]. Chemokines have been classified into CXC, CC, CX3C and C families based on the location of cysteine (C) residues [8,46]. The angiogenic nature of most CXC chemokines has been associated with the glutamyl-leucyl-arginyl (ELR) amino acid motif within their structure [46]. These chemokines include IL-8/CXCL8, epithelial neutrophil activating protein-78 (ENA-78)/CXCL5, growth-related oncogene α (gro α)/CXCL1 and connective tissue activating protein-III (CTAP-III)/CXCL6 [8]. Stromal cell-derived factor-1 (SDF-1)/CXCL12, a key regulator of lymphoid neogenesis and vasculogenesis within the RA synovium, does not contain the ELR sequence, however, yet this chemokine promotes synovial angiogenesis [47,48]. Hypoxia stimulates RA synovial fibroblasts to produce SDF-1/CXCL12 [47,48]. SDF-1/CXCL12 expression in tumors may have prognostic value [49]. Among CC chemokines, MCP-1/CCL2 is involved in angiogenesis as this chemokine supports the angiogenic effects of growth factors [8,50,51]. Fractalkine/CX3CL1 also promotes synovial neovascularization [52]. These chemokines are also involved in leukocyte recruitment into the inflamed synovium [8,46,47,50,52].

Regarding chemokine receptors, CXCR2 is the most important receptor for ELR⁺ CXC chemokines on ECs [1,8]. CXCR4, the receptor for SDF-1/CXCL12 and CCR2, the receptor for MCP-1/CCL2 are also involved in chemokine-dependent angiogenesis [8,47,53].

Chemokine and chemokine receptor targeting may suppress synovitis, as well as synovial angiogenesis. For example, CXCR2 blockade resulted in the attenuation of tumor-induced angiogenesis [8,54]. Mig/CXCL9 gene therapy in addition to cytotoxic agents exerted strong angiostatic and tumor-suppressive effects in cancer trials [55]. Bicyclam (AMD3100), a highly selective antagonist of SDF-1/CXCL4, inhibits EC proliferation and migration [56] (Table II).

Extracellular matrix constituents, cell adhesion molecules and proteases

The ingress of inflammatory leukocytes into the synovium, as well as synovial angiogenesis involves basal membrane and interstitial matrix macromolecules, cellular adhesion receptors, as well as matrix-degrading proteases [11,57]. Among extracellular matrix components including matrix molecules within the EC basement membrane, such as type I collagen, laminin, fibronectin, vitronectin, tenascin and various proteoglycans have been implicated in EC migration during angiogenesis [1,2,11,57]. Some studies suggest that partial loss of basement membrane constituents results in the widening and enlargement of vessels that enables the process of new capillary formation [11].

Among adhesion receptors, most β_1 and β_3 integrins, E-selectin, the L-selectin ligand CD34, selectin-related glycoconjugates including Lewis^x/H and MUC18, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-2 (ICAM-2), platelet-EC adhesion

molecule-1 (PECAM-1), endoglin and junctional cell adhesion molecules A (JAM-A) and C (JAM-C) are expressed on the endothelial surface and promote angiogenesis [1-3,13,58-64]. The $\alpha_V\beta_3$ integrin seems to be of significant importance. This adhesion receptor is involved in osteoclast activation leading to the development of erosions, as well as synovial angiogenesis in RA [13,58,61]. The α_V subunit of this integrin is encoded by the ITGAV gene. A significant genetic association has been found between the ITGAV rs3738919-C allele and RA in the European Caucasian population [65]. Focal adhesion kinases (FAK) are involved in $\alpha_V\beta_3$ integrin signaling. FAKs have been detected in the RA synovium suggesting their role in synovial inflammation and angiogenesis [66]. Mast cells are also involved in integrin-dependent angiogenesis and joint destruction as mast cell silencing with salbutamol or cromolyn prevented $\alpha_V\beta_3$ integrin activation and angiogenesis in mice [67].

Adhesion receptor blockade may effectively suppress synovial angiogenesis, as well as leukocyte migration in RA. For example, Vitaxin, a humanized antibody to the $\alpha_V\beta_3$ integrin inhibited synovial neovascularization in animal models of arthritis [6,13,58], yet, very little efficacy was observed in a phase II human RA trial [6]. Statins, currently used for the treatment of dyslipidemia, also modify endothelial function and adhesion receptor expression [68] (Table II).

Angiogenic proteases include matrix metalloproteinases (MMPs) and plasminogen activators [5,8]. Numerous protease inhibitors including tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs) that antagonize the effects of proteases have been tried in angiogenesis models [5,6,69].

Pro-inflammatory and angiogenic cytokines

Pro-inflammatory cytokines may exert direct angiogenic activity or may act indirectly via VEGF-dependent pathways. TNF- α , IL-1, IL-6, IL-15, IL-17, IL-18, oncostatin M, MIF, granulocyte (G-CSF) and granulocyte-macrophage colony-stimulating factors (GM-CSF) have been implicated in synovial inflammation and angiogenesis [1-3,70-76]. TNF- α itself promotes neovascularization [74] and it may also regulate capillary formation via the Ang1-Tie2-VEGF network [75]. IL-6 may also act via the induction of VEGF production [76]. IL-17 synergizes with TNF- α in stimulating VEGF, EGF, HGF and KGF production by synovial fibroblasts [1,70]. IL-17 also acts through CXCR2-dependent pathways [77]. IL-18 induces the synthesis of VEGF, as well as the SDF-1/CXCL12 and MCP-1/CCL2 angiogenic chemokines by synovial fibroblasts [20]. Oncostatin M induces ICAM-1 expression on RA synovial ECs and fibroblasts and it also stimulates EC migration and capillary formation [78]. MIF is primarily produced by synovial macrophages and is involved in macrophage-derived synovial angiogenesis [14,21,79,80]. MIF acts via the induction of VEGF and IL-8/CXCL8 release by RA synovial fibroblasts [21].

Anti-cytokine therapy influences several inflammatory mechanisms including angiogenesis in arthritis. For example, TNF- α blockade by infliximab administered in combination with methotrexate reduced VEGF expression and vascularity within the RA synovium [6,81]. Anti-TNF therapy reduced synovial Ang1-Tie2 and survivin but upregulated Ang2 expression [6,75]. Suppression of circulating IL-6 levels by various DMARDs has been associated with decreased EC activation in RA [82]. The anti-IL-6 receptor antibody tocilizumab, which has recently been approved for the treatment of RA, decreased serum levels of VEGF [76] (Table II).

Other angiogenic mediators

Other mediators not mentioned above include ET-1, serum amyloid A (SAA), members of the COX-2-prostaglandin E₂ network, angiogenin, angiotropin, pleiotrophin, platelet-

activating factor (PAF), substance P, erythropoietin, adenosine, histamine, prolactin and thrombin [1-3,5,7,83-86]. For example, *ET-1* stimulates VEGF production, EC proliferation and angiogenesis [83,84]. ET-1 is abundantly produced in RA and scleroderma [83,84]. SAA is an important acute-phase reactant also implicated in arthritis. SAA acts via the formyl peptide receptor-like 1 (FPRL1). Interaction of SAA with FPRL1 stimulates EC proliferation, migration and neovascularization, as well as synovitis [85,86].

ET-1 antagonists currently used in the treatment of primary and scleroderma-associated pulmonary hypertension may also exert anti-angiogenic effects [83] (Table II).

Angiogenesis inhibitors and their use in arthritis therapy

There is a great number of naturally produced or synthetic angiostatic molecules. Most angiostatic molecules block the action of angiogenic mediators, such as VEGF, HIFs or the $\alpha_v\beta_3$ integrin, and thus they indirectly suppress neovascularization [5,6,12,17]. Some of these compounds have already been tried to target tumor- or inflammation-associated neovascularization [1,5,6,12] (Tables I and II).

Angiostatic cytokines include interferon- α (IFN- α), IFN- γ , IL-4, IL-12 and leukemia inhibitory factor (LIF). These cytokines inhibit the release of VEGF and other angiogenic mediators [5,6,12,70,87]. For example, IL-4 blocks VEGF release by RA synovial fibroblasts [87]. IL-4 and IL-13 gene transfer inhibited synovial inflammation and angiogenesis in rats [88,89] (Table II).

Angiostatic CXC chemokines that lack the ELR motif include platelet factor-4 (PF4)/CXCL4, monokine induced by interferon- γ (MIG)/CXCL9 and interferon- γ -inducible protein 10 (IP-10)/CXCL10 [2,8,90,91]. These chemokines may regulate VEGF-dependent angiogenesis. For example, VEGF stimulates the release of IP-10/CXCL10 and, in turn, IP-10/CXCL10 suppresses VEGF-induced angiogenesis [16,90]. Secondary lymphoid tissue chemokine (SLC)/CCL21 is an angiostatic CC chemokine that inhibits tumor progression [92]. CXCR3 that binds most ELR⁻ CXC chemokines is possibly the most important chemokine receptor that confers angiostasis [8]. Angiostatic chemokines may also be used in cancer or arthritis therapy. For example, PF4/CXCL4 has been tried in rodent models of arthritis [8,91] (Table II).

Among currently used antirheumatic drugs, rofecoxib, dexamethasone, chloroquine, sulfasalazine, methotrexate, azathioprine, cyclophosphamide, leflunomide, thalidomide, minocycline and some anti-TNF agents, apart from several other anti-inflammatory effects, also inhibit EC migration and synovial angiogenesis [1,2,12,93]. Results are rather conflicting regarding methotrexate. This DMARD inhibited tumor angiogenesis but exerted no such effects in psoriatic arthritis [12] (Table II).

Thalidomide is a potent TNF- α antagonist and an angiogenesis inhibitor [6,94]. The net effect of thalidomide on synovial angiogenesis is not fully clear as in one rodent study it suppressed, while in another report it did not influence VEGF production [6,95]. Yet, thalidomide suppressed both synovitis and angiogenesis *in vivo* [6,95] suggesting that its angiostatic effects may be, in part, independent of VEGF. CC1069, a thalidomide analogue, is an even more potent angiogenesis inhibitor than thalidomide itself [6,96]. Regarding human thalidomide trials, it showed little efficacy in RA and moderate clinical response was observed in systemic lupus erythematosus (SLE) [6] (Tables I and II).

Thrombospondin-1 (TSP1) and TSP2 that are angiostatic extracellular matrix components naturally produced within the RA synovium [6,97,98]. A TSP1 peptide suppressed synovial inflammation and angiogenesis in a rat arthritis model [97]. TSP2 inhibited synovial

neovascularization in the severe combined immunodeficiency (SCID) mouse model of arthritis [98] (Tables I and II).

Antibiotic derivatives including minocyclin, fumagillin analogues, deoxyspergualin and clarithromycin also inhibit the release of VEGF and other angiogenic mediators and thus neovascularization [6,12,99-101]. Fumagillin is a naturally occurring product of *Aspergillus fumigatus*. Its synthetic derivatives, TNP-470 and PPI-2458 inhibit VEGF, as well as methionine aminopeptidase-2, an enzyme involved in angiogenesis [6,99]. In a study of 60 early RA patients, minocycline was clinically more effective than hydro-xychloroquine [102]. Minocycline treatment was associated with clinically significant improvement in RA, yet, its effects are rather moderate in comparison to other currently used DMARDs (103). In a 6-month study on 81 early RA patients, clarithromycin was more effective than placebo [104]. In rodent models, deoxyspergualin, as well as the fumagillin analogs TNP-470 and PPI-2458 suppressed the development of arthritis [100,101,105,106] (Table II).

Angiostatin, a fragment of plasminogen, endostatin, a fragment of type XIII collagen and their derivatives block $\alpha_v\beta_3$ integrin-dependent angiogenesis [6,12,107-109]. Endostatin also interferes with VEGFR 2 signaling [6,109] and induces apoptosis of synovial fibroblasts [110]. Some of these agents, particularly angiostatin and endostatin, gave promising results in cancer therapy trials, as well as pre-clinical arthritis studies [1,6,12,107,108]. For example, angiostatin gene transfer attenuated murine CIA [6,108]. Endostatin both prophylactically and therapeutically suppressed arthritis scores, pannus formation and the development of joint erosions in rodent arthritis models [6,107,109,111,112]. The angiostatin-like protease-activated kringle 1-5 (K1-5) may be a more potent angiogenesis inhibitor than angiostatin itself [113]. Serum levels and synovial tissue expression of kallistatin was increased in RA [114]. Intraarticular injection of kallistatin attenuated rat arthritis using gene therapy [114]. Type IV collagen derivatives including arrestin, canstatin and tumstatin also inhibit neovascularization [115]. Regarding human studies, both angiostatin and endostatin have been introduced to clinical trials in cancer [6,12] (Tables I and II).

2-Methoxyestradiol (2-ME) is a natural metabolite of estrogen with low affinity for estrogen receptors. 2-ME inhibits angiogenesis by disrupting microtubules and by suppressing HIF-1 α activity [116]. In recent preclinical studies, 2-ME suppressed arthritis in the rat CIA model [117]. On the other hand, 2-ME suppressed arthritis, but synovial vascularity in another study [118] suggesting that 2-ME may also have angiogenesis-independent effects on synovial inflammation.

Paclitaxel (taxol) is a microtubule disrupting agent that diminishes HIF-1 α expression and activity and thus indirectly inhibits angiogenesis [6,116]. This anti-cancer agent has been found effective and safe in a phase I RA clinical trial [6]. Further compounds not discussed here include osteonectin, opioids, troponin I, and chondromodulin-1 [6,12,17].

Regulation of synovial angiogenesis

Macrophages and mediators produced by these cells definitely play a central role in the regulation of synovial angiogenesis [14]. Synovial macrophages express CXCR2 and CXCR4, chemokine receptors implicated in CXC chemokine-mediated neovascularization [5,8,14]. In addition, macrophages also release important soluble angiogenic mediators, such as IL-8/CXCL8, ENA-78/CXCL5, gro α /CXCL1, CTAP-III/CXCL7, MCP-1/CCL2, TNF- α , IL-15, IL-18, VEGF, bFGF, aFGF, HGF, PDGF and MMPs [2,5,8,14,53]. On the other hand, macrophages are also involved in angiostasis, as they produce IP-10/CXCL10, MIG/CXCL9, IFN- γ and TIMPs [8,14].

Several regulatory interactions and loops exist in sites of angiogenesis and inflammation. Regarding specific antagonistic pairs, there is an abundance of ELR⁺ over ELR⁻ CXC chemokines, MMPs over TIMPs, pro-inflammatory, angiogenic over anti-inflammatory, angiostatic cytokines [2,5,6]. Also, different angiogenic mediators may have additive effects. For example, TNF- α stimulates the production of various angiogenic chemokines, growth factors, other cytokines or adhesion receptors [2,5,6,13]. Furthermore, as described here, numerous angiogenic mediators indirectly promote, while various angiostatic agents inhibit VEGF-dependent neovascularization [1,5,16]. Apart from natural regulatory networks described here, the administration of compounds with angiostatic activity may also interfere with neovascularization [6,12].

Conclusions

In this review, we discussed the putative role of angiogenesis in RA. Synovial angiogenesis may increase the total vascular endothelial surface and thus may enhance the ingress of leukocytes into the synovial tissue. Several soluble and cell surface-bound mediators of angiogenesis including VEGF and other growth factors, cytokines, chemokines, extracellular matrix constituents, cell adhesion molecules, proteases and others have been described in relation to synovitis.

When describing the action of the most relevant angiogenic mediators we also presented therapeutic strategies that may suppress the production of these mediators and thus attenuate synovial angiogenesis, as well as inflammation. On the other hand, some endogenous angiogenesis inhibitors are naturally produced to counterbalance excessive neovascularization associated with RA. Yet, the amount and activity of these endogenous agents are insufficient to control inflammatory angiogenesis in arthritis. Inhibitors of VEGF and VEGFR, angiogenic chemokines and chemokine receptors, integrins, currently used DMARDs and biologics, thalidomide, taxol, 2-ME, fumagillin analogues, angiostatin and endostatin may be included in future anti-angiogenic, as well as anti-inflammatory treatment.

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References

1. Koch AE. Angiogenesis: Implications for rheumatoid arthritis. *Arthritis Rheum* 1998;41:951–962. [PubMed: 9627005]
2. Szekanecz Z, Koch AE. Endothelial cells in inflammation and angiogenesis. *Curr Drug Targ* 2005;4:319–323.
3. Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: 'vascular rheumatology'. *Arthritis Res Ther* 2008;10:224. [PubMed: 18947376]
4. Foster W, Carruthers D, Lip GY, Blann AD. Relationship between endothelial inflammatory and angiogenesis markers in rheumatoid arthritis: Implications for cardiovascular pathophysiology. *Thromb Res* 2009;4:659–664. [PubMed: 18692223]
5. Szekanecz Z, Koch AE. Mechanism of disease: Angiogenesis in inflammatory diseases. *Nat Clin Pract Rheumatol* 2007;3:635–643. [PubMed: 17968334]
6. Lainer-Carr D, Brahn E. Angiogenesis inhibition as a therapeutic approach for inflammatory synovitis. *Nat Clin Pract Rheumatol* 2007;3:434–442. [PubMed: 17664950]
7. Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987;235:442–447. [PubMed: 2432664]
8. Szekanecz Z, Koch AE. Chemokines and angiogenesis. *Curr Opin Rheumatol* 2001;13:202–208. [PubMed: 11333349]

9. Naumov GN, Folkman J, Straume O, Akslen LA. Tumor-vascular interactions and tumor dormancy. *APMIS* 2008;116:569–585. [PubMed: 18834403]
10. Shams N, Ianchulev T. Role of vascular endothelial growth factor in ocular angiogenesis. *Ophthalmol Clin North Am* 2006;19:335–344. [PubMed: 16935208]
11. Jakobsson L, Claesson-Welsh L. Vascular basement membrane components in angiogenesis—an act of balance. *Sci World J* 2008;8:1246–1249.
12. Veale DJ, Fearon U. Inhibition of angiogenic pathways in rheumatoid arthritis: Potential for therapeutic targeting. *Best Pract Res Clin Rheumatol* 2006;20:941–947. [PubMed: 16980216]
13. Agarwal SK, Brenner MB. Role of adhesion molecules in synovial inflammation. *Curr Opin Rheumatol* 2006;18:268–276. [PubMed: 16582691]
14. Szekanecz Z, Koch AE. Macrophages and their products in rheumatoid arthritis. *Curr Opin Rheumatol* 2007;19:289–295. [PubMed: 17414958]
15. Koch AE, Harlow LA, Haines GK, Amento EP, Unemori EN, Wong WL, Pope RM, Ferrara N. Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. *J Immunol* 1994;152:4149–4156. [PubMed: 7511670]
16. Kiselyov A, Balakin KV, Tkachenko SE. VEGF/VEGFR signaling as a target for inhibiting angiogenesis. *Expert Opin Investig Drugs* 2007;16:83–107.
17. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. *BMB Rep* 2008;41:278–286. [PubMed: 18452647]
18. Hernandez GL, Volpert OV, Iñiguez MA, Lorenzo E, Martínez-Martínez S, Grau R, Fresno M, Redondo JM. Selective inhibition of vascular endothelial growth factor-mediated angiogenesis by cyclosporin A: Roles of the nuclear factor of activated T cells and cyclooxygenase 2. *J Exp Med* 2001;193:607–612. [PubMed: 11238591]
19. Taylor PC, Sivakumar B. Hypoxia and angiogenesis in rheumatoid arthritis. *Curr Opin Rheumatol* 2005;17:293–298. [PubMed: 15838239]
20. Amin MA, Mansfield PJ, Pakozdi A, Campbell PL, Ahmed S, Martinez RJ, Koch AE. Interleukin-18 induces angiogenic factors in rheumatoid arthritis synovial tissue fibroblasts via distinct signaling pathways. *Arthritis Rheum* 2007;56:1787–1797. [PubMed: 17530707]
21. Kim HR, Park MK, Cho ML, Yoon CH, Lee SH, Park SH, Leng L, Bucala R, Kang I, Choe J, Kim HY. Macrophage migration inhibitory factor upregulates angiogenic factors and correlates with clinical measures in rheumatoid arthritis. *J Rheumatol* 2007;34:927–936. [PubMed: 17407222]
22. Koch AE, Distler O. Vasculopathy and disordered angiogenesis in selected rheumatic diseases: Rheumatoid arthritis and systemic sclerosis. *Arthritis Res Ther* 2007;9(Suppl. 2):S3. [PubMed: 17767741]
23. Haq A, El-Ramahi K, Al-Dalaan A. Serum and synovial fluid concentrations of endothelin-1 in patients with inflammatory arthritides. *J Med* 1999;30:51–60. [PubMed: 10515240]
24. Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E, Huang T, Radziejewski C, Bailey K, Fandl JP, Daly T, Wiegand SJ, Yancopoulos GD, Rudge JS. VEGF-Trap: A VEGF blocker with potent antitumor effects. *Proc Natl Acad Sci USA* 2002;99:11393–11398. [PubMed: 12177445]
25. Manley PW, Martiny-Baron G, Schlaeppli JM, Wood JM. Therapies directed at vascular endothelial growth factor. *Expert Opin Investig Drugs* 2002;11:1715–1736.
26. Grosios K, Wood J, Esser R, Raychaudhuri A, Dawson J. Angiogenesis inhibition by the novel VEGF tyrosine kinase inhibitor, PTK787/ZK222584, causes significant anti-arthritis effects in models of rheumatoid arthritis. *Inflamm Res* 2004;53:133–142. [PubMed: 15060719]
27. Kim WU, Kwok SK, Hong KH, Yoo SA, Kong JS, Choe J, Cho CS. Soluble Fas ligand inhibits angiogenesis in rheumatoid arthritis. *Arthritis Res Ther* 2007;26:R42. [PubMed: 17459170]
28. Choi ST, Kim JH, Seok JY, Park YB, Lee SK. Therapeutic effect of anti-vascular endothelial growth factor receptor I antibody in the established collagen-induced arthritis mouse model. *Clin Rheumatol* 2009;3:333–337.
29. Guttmann-Raviv N, Shraga-Heled N, Varshavsky A, Guimaraes-Sternberg C, Kessler O, Neufeld G. Semaphorin-3A and semaphorin-3F work together to repel endothelial cells and to inhibit their survival by induction of apoptosis. *J Biol Chem* 2007;282:26294–26305. [PubMed: 17569671]

30. Fearon U, Veale DJ. Angiogenesis in arthritis: Methodological and analytical details. *Methods Mol Med* 2007;135:343–357. [PubMed: 17951670]
31. Giatromanolaki A, Sivridis E, Maltezos E, Athanassou N, Papazoglou D, Gatter KC, Harris AL, Koukourakis MI. Upregulated hypoxia inducible factor-1 α and -2 α pathway in rheumatoid arthritis and osteoarthritis. *Arthritis Res Ther* 2003;5:R193–R198. [PubMed: 12823854]
32. Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, Cooper M, Laznik D, Chinsomboon J, Rangwala SM, Baek KH, Rosenzweig A, Spiegelman BM. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1 α . *Nature* 2008;451:1008–1012. [PubMed: 18288196]
33. Aljada A, O'Connor L, Fu YY, Mousa SA. PPAR γ ligands, rosiglitazone and pioglitazone inhibit bFGF- and VEGF-mediated angiogenesis. *Angiogenesis* 2008;11:361–367. [PubMed: 18810647]
34. Bongartz T, Coras B, Vogt T, Schölmerich J, Müller-Ladner U. Treatment of active psoriatic arthritis with the PPAR γ ligand pioglitazone: An open-label pilot study. *Rheumatology* 2005;44:126–129. [PubMed: 15479756]
35. Piqueras L, Reynolds AR, Hodivala-Dilke KM, Alfranca A, Redondo JM, Hatae T, Tanabe T, Warner TD, Bishop-Bailey D. Activation of PPAR β/δ induces endothelial cell proliferation and angiogenesis. *Arterioscler Thromb Vasc Biol* 2007;27:63–69. [PubMed: 17068288]
36. Yeo EJ, Chun YS, Cho YS, Kim J, Lee JC, Kim MS, Park JW. YC-1: A potential anticancer drug targeting hypoxia-inducible factor 1. *J Natl Cancer Inst* 2003;95:516–525. [PubMed: 12671019]
37. Hirota SA, Beck PL, MacDonald JA. Targeting hypoxia-inducible factor-1 (HIF-1) signaling in therapeutics: Implications for the treatment of inflammatory bowel disease. *Recent Pat Inflamm Allergy Drug Discov* 2009;3:1–16. [PubMed: 19149741]
38. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, Wiegand SJ. Vessel cooption, regression and growth in tumors mediated by angiopoietins and VEGF. *Science* 1999;284:1994–1998. [PubMed: 10373119]
39. Gravalles EM, Pettit AR, Lee R, Madore R, Manning C, Tsay A, Gaspar J, Goldring MB, Goldring SR, Oettgen P. Angiopoietin-1 is expressed in the synovium of patients with rheumatoid arthritis and is induced by tumour necrosis factor α . *Ann Rheum Dis* 2003;62:100–107. [PubMed: 12525377]
40. Tran J, Master Z, Yu JL, Rak J, Dumont DJ, Kerbel RS. A role for surviving in chemoresistance of endothelial cells mediated by VEGF. *Proc Natl Acad Sci USA* 2002;99:4349–4354. [PubMed: 11917134]
41. Shahrara S, Volin MV, Connors MA, Haines GK, Koch AE. Differential expression of the angiogenic Tie receptor family in arthritic and normal synovial tissue. *Arthritis Res* 2002;4:201–208. [PubMed: 12010571]
42. Chen Y, Donnelly E, Kobayashi H, Debusk LM, Lin PC. Gene therapy targeting the Tie2 function ameliorates collagen-induced arthritis and protects against bone destruction. *Arthritis Rheum* 2005;52:1585–1594. [PubMed: 15880817]
43. Yoo SA, Yoon HJ, Kim HS, Chae CB, De Falco S, Cho CS, Kim WU. Role of placenta growth factor and its receptor flt-1 in rheumatoid inflammation: A link between angiogenesis and inflammation. *Arthritis Rheum* 2009;60:345–354. [PubMed: 19180491]
44. Kameda H, Ishigami H, Suzuki M, Abe T, Takeuchi T. Imatinib mesylate inhibits proliferation of rheumatoid synovial fibroblast-like cells and phosphorylation of Gab adapter proteins activated by platelet-derived growth factors. *Clin Exp Immunol* 2006;144:335–341. [PubMed: 16634808]
45. Koyama K, Hatsushika K, Ando T, Sakuma M, Wako M, Kato R, Haro H, Sugiyama H, Hamada Y, Ogawa H, Nakao A. Imatinib mesylate both prevents and treats the arthritis induced by type II collagen antibody in mice. *Mod Rheumatol* 2007;17:306–310. [PubMed: 17694264]
46. Strieter RM, Polverini PJ, Kunkel SL, Arenberg DA, Burdick MD, Kasper J, Dzuiba J, Van Damme J, Walz A, Marriott D, Chan S-Y, Rocznik S, Shanafelt AB. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995;270:27348–27357. [PubMed: 7592998]
47. Petit I, Jin D, Rafii S. The SDF-1-CXCR4 signaling pathway: A molecular hub modulating neo-angiogenesis. *Trends Immunol* 2007;28:299–307. [PubMed: 17560169]

48. Pablos JL, Santiago B, Galindo M, Torres C, Brehmer M, Blanco FJ, Garcia-Lazaro FJ. Synovial cell-derived CXCL12 is displayed on endothelium and induces angiogenesis in rheumatoid arthritis. *J Immunol* 2003;170:2147–2152. [PubMed: 12574387]
49. Calatozzolo C, Maderna E, Pollo B, Gelati M, Marras C, Silvani A, Croci D, Boiardi A, Salmaggi A. Prognostic value of CXCL12 expression in 40 low-grade oligodendrogliomas and oligoastrocytomas. *Cancer Biol Ther* 2006;5:827–832. [PubMed: 16760646]
50. Salcedo R, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK, Oppenheim JJ, Murphy WJ. Human endothelial cells express CCR2 and respond to MCP-1: Direct role of MCP-1 in angiogenesis and tumor progression. *Blood* 2000;96:34–40. [PubMed: 10891427]
51. Stamatovic SM, Keep RF, Mostarica-Stojkovic M, Andjelkovic AV. CCL2 regulates angiogenesis via activation of Ets-1 transcription factor. *J Immunol* 2006;177:2651–2661. [PubMed: 16888027]
52. Volin MV, Woods JM, Amin MA, Connors MA, Harlow LA, Koch AE. Fractalkine: A novel angiogenic chemokine in rheumatoid arthritis. *Am J Pathol* 2001;159:1521–1526. [PubMed: 11583978]
53. Nanki T, Hayashida K, El-Gabalawy HS, Suson S, Shi K, Girschick HJ, Yavuz S, Lipsky PE. Stromal cell-derived factor-1-CXC chemokine receptor 4 interactions play a central role in CD4 + T-cell accumulation in rheumatoid arthritis synovium. *J Immunol* 2000;165:6590–6598. [PubMed: 11086103]
54. Wente MN, Keane MP, Burdick MD, Friess H, Büchler MW, Ceyhan GO, Reber HA, Strieter RM, Hines OJ. Blockade of the chemokine receptor CXCR2 inhibits pancreatic cancer cell-induced angiogenesis. *Cancer Lett* 2006;241:221–227. [PubMed: 16458421]
55. Zhang R, Tian L, Chen LJ, Hou JM, Li G, Li J, Zhang L, Chen XC, Luo F, Jiang Y, Wei YQ. Combination of MIG (CXCL9) chemokine gene therapy with low-dose cisplatin improves therapeutic efficacy against murine carcinoma. *Gene Ther* 2006;13:1263–1271. [PubMed: 16672984]
56. Yin Y, Huang L, Zhao X, Fang Y, Yu S, Zhao J, Cui B. AMD3100 mobilizes endothelial progenitor cells in mice, but inhibits its biological functions by blocking an autocrine/paracrine regulatory loop of stromal cell derived factor-1 *in vitro*. *J Cardiovasc Pharmacol* 2007;50:61–67. [PubMed: 17666917]
57. Madri JA, Williams KS. Capillary endothelial cell cultures: Phenotypic modulation by matrix components. *J Cell Biol* 1983;97:153–165. [PubMed: 6190818]
58. Szekanecz Z, Szegedi G, Koch AE. Cellular adhesion molecules in rheumatoid arthritis. Regulation by cytokines and possible clinical importance. *J Investig Med* 1996;44:124–135.
59. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. *Nat Rev Immunol* 2004;4:432–444. [PubMed: 15173832]
60. Koch AE, Halloran MM, Haskell CJ, Shah MR, Polverini PJ. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature* 1995;376:517–519. [PubMed: 7543654]
61. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin $\alpha v \beta 3$ for angiogenesis. *Science* 1994;264:569–571. [PubMed: 7512751]
62. Huang MT, Mason JC, Birdsey GM, Amsellem V, Gerwin N, Haskard DO, Ridley AJ, Randi AM. Endothelial intercellular adhesion molecule (ICAM)-2 regulates angiogenesis. *Blood* 2005;106:1636–1643. [PubMed: 15920013]
63. Naik TU, Naik MU, Naik UP. Junctional adhesion molecules in angiogenesis. *Front Biosci* 2008;13:258–262. [PubMed: 17981544]
64. Rabquer BJ, Pakozdi A, Michel JE, Gujar BS, Haines GK III, Imhof BA, Koch AE. Junctional adhesion molecule C mediates leukocyte adhesion to rheumatoid arthritis synovium. *Arthritis Rheum* 2008;58:3020–3039. [PubMed: 18821692]
65. Jacq L, Garnier S, Dieudé P, Michou L, Pierlot C, Migliorini P, Balsa A, Westhovens R, Barrera P, Alves H, Vaz C, Fernandes M, Pascual-Salcedo D, Bombardieri S, Dequeker J, Radstake TR, Van Riel P, van de Putte L, Lopes-Vaz A, Glikmans E, Barbet S, Lasbleiz S, Lemaire I, Quillet P, Hilliquin P, Teixeira VH, Petit-Teixeira E, Mbarek H, Prum B, Bardin T, Cornélis F. European Consortium on Rheumatoid Arthritis Families. The ITGAV rs3738919-C allele is associated with

- rheumatoid arthritis in the European Caucasian population: A family-based study. *Arthritis Res Ther* 2007;9:R63. [PubMed: 17615072]
66. Shahrara S, Castro-Rueda HP, Haines GK, Koch AE. Differential expression of the FAK family kinases in rheumatoid arthritis and osteoarthritis synovial tissues. *Arthritis Res Ther* 2007;9:R112. [PubMed: 17963503]
 67. Kneilling M, Hültner L, Pichler BJ, Mailhammer R, Morawietz L, Solomon S, Eichner M, Sabatino J, Biedermann T, Krenn V, Weber WA, Illges H, Haubner R, Röcken M. Targeted mast cell silencing protects against joint destruction and angiogenesis in experimental arthritis in mice. *Arthritis Rheum* 2007;56:1806–1816. [PubMed: 17530709]
 68. McCarey DW, Sattar N, McInnes IB. Do the pleiotropic effects of statins in the vasculature predict a role in inflammatory diseases? *Arthritis Res Ther* 2005;7:55–61. [PubMed: 15743490]
 69. Skotnicki JS, Zask A, Nelson FC, Albright JD, Levin JJ. Design and synthetic considerations of matrix metalloproteinase inhibitors. *Ann NY Acad Sci* 1999;878:61–72. [PubMed: 10415720]
 70. Szekanecz Z, Strieter RM, Koch AE. Cytokines in rheumatoid arthritis: Potential targets for pharmacological intervention. *Drugs Aging* 1998;12:377–390. [PubMed: 9606615]
 71. Brennan F, Beech J. Update on cytokines in rheumatoid arthritis. *Curr Opin Rheumatol* 2007;19:296–301. [PubMed: 17414959]
 72. Park CC, Morel JC, Amin MA, Connors MA, Harlow LA, Koch AE. Evidence of IL-18 as a novel angiogenic mediator. *J Immunol* 2001;167:1644–1653. [PubMed: 11466388]
 73. Angiolillo AL, Kanegane H, Sgadari C, Reaman GH, Tosato G. Interleukin15 promotes angiogenesis *in vivo*. *Biochem Biophys Res Commun* 1997;233:231–237. [PubMed: 9144429]
 74. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N. Macrophage-induced angiogenesis is mediated by tumour necrosis factor- α . *Nature* 1987;329:630–632. [PubMed: 2443857]
 75. Markham T, Mullan R, Golden-Mason L, Rogers S, Bresnihan B, Fitzgerald O, Fearon U, Veale DJ. Resolution of endothelial activation and down-regulation of Tie2 receptor in psoriatic skin after infliximab therapy. *J Am Acad Dermatol* 2006;54:1003–1012. [PubMed: 16713454]
 76. Nakahara H, Song J, Sugimoto M, Hagihara K, Kishimoto T, Yoskizaki K, Nishimoto N. Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. *Arthritis Rheum* 2003;48:1521–1529. [PubMed: 12794819]
 77. Numasaki M, Watanabe M, Suzuki T, Takahashi H, Nakamura A, McAllister F, Hishinuma T, Goto J, Lotze MT, Kolls JK, Sasaki H. IL-17 enhances the net angiogenic activity and *in vivo* growth of human non-small cell lung cancer in SCID mice through promoting CXCR2-dependent angiogenesis. *J Immunol* 2005;175:6177–6189. [PubMed: 16237115]
 78. Fearon U, Mullan R, Markham T, Connolly M, Sullivan S, Poole AR, Fitzgerald O, Bresnihan B, Veale DJ. Oncostatin M induces angiogenesis and cartilage degradation in rheumatoid arthritis synovial tissue and human cartilage cocultures. *Arthritis Rheum* 2006;54:3152–3162. [PubMed: 17009243]
 79. Amin MA, Volpert OV, Woods JM, Kumar P, Harlow LA, Koch AE. Migration inhibitory factor mediates angiogenesis via mitogen-activated protein kinase and phosphatidylinositol kinase. *Circ Res* 2003;93:321–329. [PubMed: 12881477]
 80. Morand EF, Leech M, Bernhagen J. MIF: A new cytokine link between rheumatoid arthritis and atherosclerosis. *Nat Rev Drug Discov* 2006;5:399–410. [PubMed: 16628200]
 81. Goedkoop AY, Kraan MC, Picavet DI, de Rie MA, Teunissen MB, Bos JD, Tak PP. Deactivation of endothelium and reduction in angiogenesis in psoriatic skin and synovium by low dose infliximab therapy in combination with stable methotrexate therapy. *Arthritis Res Ther* 2004;6:R326–R334. [PubMed: 15225368]
 82. Dessein PH, Joffe BI. Suppression of circulating interleukin-6 concentrations is associated with decreased endothelial activation in rheumatoid arthritis. *Clin Exp Rheumatol* 2006;24:161–167. [PubMed: 16762151]
 83. Koch AE, Distler O. Vasculopathy and disordered angiogenesis in selected rheumatic diseases: Rheumatoid arthritis and systemic sclerosis. *Arthritis Res Ther* 2007;9(Suppl. 2):S3. [PubMed: 17767741]

84. Haq A, El-Ramahi K, Al-Dalaan A. Serum and synovial fluid concentrations of endothelin-1 in patients with inflammatory arthritides. *J Med* 1999;30:51–60. [PubMed: 10515240]
85. Lee MS, Yoo SA, Cho CS, Suh PG, Kim WU, Ryu SH. Serum amyloid A binding to formyl peptide receptor-like 1 induces synovial hyperplasia and angiogenesis. *J Immunol* 2006;177:5585–5594. [PubMed: 17015746]
86. Mullan RH, Bresnihan B, Golden-Mason L, Markham T, O'Hara R, FitzGerald O, Veale DJ, Fearon U. Acute-phase serum amyloid A stimulation of angiogenesis, leukocyte recruitment and matrix degradation in rheumatoid arthritis through an NF- κ B-dependent signal transduction pathway. *Arthritis Rheum* 2006;54:105–114. [PubMed: 16385502]
87. Hong KH, Cho ML, Min SY, Shin YJ, Yoo SA, Choi JJ, Kim WU, Song SW, Cho CS. Effect of interleukin-4 on vascular endothelial growth factor production in rheumatoid synovial fibroblasts. *Clin Exp Immunol* 2007;147:573–579. [PubMed: 17302909]
88. Haas CS, Amin MA, Allen BB, Ruth JH, Haines GK III, Woods JM, Koch AE. Inhibition of angiogenesis by interleukin-4 gene therapy in rat adjuvant-induced arthritis. *Arthritis Rheum* 2006;54:2402–2414. [PubMed: 16869003]
89. Haas CS, Amin MA, Ruth JH, Allen BL, Ahmed S, Pakozdi A, Woods JM, Shahrara S, Koch AE. *In vivo* inhibition of angiogenesis by interleukin-13 gene therapy in a rat model of rheumatoid arthritis. *Arthritis Rheum* 2007;56:2535–2548. [PubMed: 17665443]
90. Boulday G, Haskova Z, Reinders ME, Pal S, Briscoe DM. Vascular endothelial growth factor-induced signaling pathways in endothelial cells that mediate overexpression of the chemokine IFN- γ -inducible protein of 10 kDa *in vitro* and *in vivo*. *J Immunol* 2006;176:3098–3107. [PubMed: 16493069]
91. Maurer AM, Zhou B, Han ZC. Roles of platelet factor 4 in hematopoiesis and angiogenesis. *Growth Factors* 2006;24:242–252. [PubMed: 17381065]
92. Vicari AP, Ait-Yahia S, Chemin K, Mueller A, Zlotnik A, Caux C. Antitumor effects of the mouse chemokine 6Ckine/SLC through angiostatic and immunological mechanisms. *J Immunol* 2000;165:1992–2000. [PubMed: 10925282]
93. Woods JM, Mogollon A, Amin MA, Martinez RJ, Koch AE. The role of COX-2 in angiogenesis and rheumatoid arthritis. *Exp Mol Pathol* 2003;74:282–290. [PubMed: 12782016]
94. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci USA* 1994;91:4082–4085. [PubMed: 7513432]
95. Komorowski J, Jerczyńska H, Siejka A, Barańska P, Ławnicka H, Pawłowska Z, Stepień H. Effect of thalidomide affecting VEGF secretion, cell migration, adhesion and capillary tube formation of human endothelial EA.hy926 cells. *Life Sci* 2006;78:2558–2663. [PubMed: 16310808]
96. Oliver SJ, Cheng TP, Banquerigo ML, Brahn E. The effect of thalidomide and two analogs on collagen induced arthritis. *J Rheumatol* 1998;25:964–969. [PubMed: 9598899]
97. Rico MC, Castaneda JL, Manns JM, Uknis AB, Sainz IM, Safadi FF, Popoff SN, Dela Cadena RA. Amelioration of inflammation, angiogenesis and CTGF expression in a arthritis model by a TSP-1-derived peptide treatment. *J Cell Physiol* 2007;211:504–512. [PubMed: 17219411]
98. Park YW, Kang YM, Butterfield J, Detmar M, Goronzy JJ, Weyand CM. Thrombospondin 2 functions as an endogenous regulator of angiogenesis and inflammation in rheumatoid arthritis. *Am J Pathol* 2004;165:2087–2098. [PubMed: 15579451]
99. Ingber D, Fujita T, Kishimoto S, Sudo K, Kanamaru T, Brem H, Folkman J. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature* 1990;348:555–557. [PubMed: 1701033]
100. Peacock DJ, Banquerigo ML, Brahn E. Angiogenesis inhibition suppresses collagen arthritis. *J Exp Med* 1992;175:1135–1138. [PubMed: 1372645]
101. Hannig G, Bernier SG, Hoyt JG, Doyle B, Clark E, Karp RM, Lorusso J, Westlin WF. Suppression of inflammation and structural damage in experimental arthritis through molecular targeted therapy with PPI-2458. *Arthritis Rheum* 2007;56:850–860. [PubMed: 17328059]
102. O'Dell JR, Blakely KW, Mallek JA, Eckhoff PJ, Leff RD, Wees SJ, Sems KM, Fernandez AM, Palmer WR, Klassen LW, Paulsen GA, Haire CE, Moore GF. Treatment of early seropositive rheumatoid arthritis: A two-year, double-blind comparison of minocycline and hydroxychloroquine. *Arthritis Rheum* 2001;44:2235–2241. [PubMed: 11665963]

103. Stone M, Fortin PR, Pacheco-Tena C, Inman RD. Should tetracycline treatment be used more extensively for rheumatoid arthritis? *J Rheumatol* 2003;30:2112–2122. [PubMed: 14528503]
104. Ogrendik M. Effects of clarithromycin in patients with active rheumatoid arthritis. *Curr Med Res Opin* 2007;23:515–522. [PubMed: 17355733]
105. Nemoto K, Mae T, Abe F, Takeuchi T. Successful treatment with a novel immunosuppressive agent, deoxyspergualin, in type II collagen-induced arthritis in mice. *Ann NY Acad Sci* 1993;685:148–154. [PubMed: 8363219]
106. Bernier SG, Lazarus DD, Clark E, Doyle B, Labenski MT, Thompson CD, Westlin WF, Hannig G. A methionine aminopeptidase-2 inhibitor, PPI-2458, for the treatment of rheumatoid arthritis. *Proc Natl Acad Sci USA* 2004;101:10768–10773. [PubMed: 15249666]
107. Yin G, Liu W, An P, Li P, Ding I, Planelles V, Schwarz EM, Min W. Endostatin gene transfer inhibits joint angiogenesis and pannus formation in inflammatory arthritis. *Mol Ther* 2002;5:547–554. [PubMed: 11991745]
108. Takahashi H, Kato K, Miyake K, Hirai Y, Yoshino S, Shimada T. Adeno-associated virus vector-mediated anti-angiogenic gene therapy for collagen-induced arthritis in mice. *Clin Exp Rheumatol* 2005;23:455–461. [PubMed: 16095112]
109. Yue L, Shen YX, Feng LJ, Chen FH, Yao HW, Liu LH, Wu Q, Wang H. Blockage of the formation of new blood vessels by recombinant human endostatin contributes to the regression of rat adjuvant arthritis. *Eur J Pharmacol* 2007;567:166–170. [PubMed: 17490637]
110. Huang XY, Chen FH, Li J, Xia LJ, Liu YJ, Zhang XM, Yuan FL. Mechanism of fibroblast-like synoviocyte apoptosis induced by recombinant human endostatin in rats with adjuvant arthritis. *Anat Rec (Hoboken)* 2008;291:1029–1037. [PubMed: 18509875]
111. Kurosaka D, Yoshida K, Yasuda J, Yasuda C, Noda K, Furuya K, Ukichi T, Kingetsu I, Joh K, Yamaguchi N, Saito S, Yamada A. The effect of endostatin evaluated in an experimental animal model of collagen-induced arthritis. *Scand J Rheumatol* 2007;36:434–441. [PubMed: 18092264]
112. Kurosaka D, Yasuda J, Yoshida K, Yasuda C, Toyokawa Y, Yokoyama T, Kingetsu I, Yamada A. Kinetics of circulating endothelial progenitor cells in mice with type II collagen arthritis. *Blood Cells Mol Dis* 2005;35:236–240. [PubMed: 16023391]
113. Sumariwalla PF, Cao Y, Wu HL, Feldmann EM, Paleolog E. The angiogenesis inhibitor protease-activated kringles 1-5 reduces the severity of murine collagen-induced arthritis. *Arthritis Res Ther* 2003;5:R32–R39. [PubMed: 12716451]
114. Wang CR, Chen SY, Shiau AL, Wu CL, Jou IM, Chao L, Chao J. Upregulation of kallistatin expression in rheumatoid joints. *J Rheumatol* 2007;34:2171–2176. [PubMed: 17937475]
115. Mundel TM, Kalluri R. Type IV collagen-derived angiogenesis inhibitors. *Microvasc Res* 2007;74:85–89. [PubMed: 17602710]
116. Mabeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* 2003;3:363–375. [PubMed: 12726862]
117. Brahn E, Banquerigo ML, Lee JK, Park EJ, Fogler WE, Plum SM. An angiogenesis inhibitor, 2-methoxyestradiol involutes rat collagen-induced arthritis and suppresses gene expression of synovial vascular endothelial growth factor and basic fibroblast growth factor. *J Rheumatol* 2008;35:2119–2128. [PubMed: 18792999]
118. Issekutz AC, Sapru K. Modulation of adjuvant arthritis in the rat by 2-methoxyestradiol: An effect independent of an anti-angiogenic action. *Int Immunopharmacol* 2008;8:708–716. [PubMed: 18387513]

Figure 1.
Soluble and cell surface-bound angiogenesis mediators and inhibitors in synovial neovascularization.

Table IRelevant mediators and inhibitors of angiogenesis in RA.^a

	Mediators	Inhibitors
Growth factors	VEGF, aFGF, bFGF, HGF, HIF-1, HIF-2, PDGF, EGF, KGF, IGF-I, TGF- β , PIGF	–
Cytokines	TNF- α , IL-1, IL-6, IL-8, IL-15, IL-17, IL-18, G-CSF, GM-CSF, oncostatin M, MIF	IFN- α , IFN- γ , IL-4, IL-12, LIF
Chemokines/receptors	IL-8/CXCL8, ENA-78/CXCL5, gro α /CXCL1, CTAP-III/CXCL6, SDF-1/CXCL12, MCP-1/CCL2, fractalkine/CX3CL1 CXCR2, CXCR4, CCR2	PF4/CXCL4, M ϕ g/CXCL9, IP-10/CXCL10, SLC/CCL21, CXCR3
Matrix molecules	Type I collagen, fibronectin, laminin, vitronectin, tenascin, proteoglycan	Thrombospondin-1, -2
Cell adhesion molecules	β 1 and β 3 integrins, E-selectin, VCAM-1, ICAM-2, CD34, Lewis ^x /H, MUC18, PECAM-1, endoglin, JAM-A, JAM-C	–
Proteolytic enzymes	MMPs, plasminogen activators	TIMPs, PAIs
Antirheumatic drugs	–	Dexamethasone, rofecoxib, classical DMARDs, thalidomide, minocycline, anti-TNF biologics
Antibiotic derivatives	–	Minocycline, fumagillin analogs, deoxyspergualin, clarithromycin
Environmental factors	Hypoxia	–
Others	Angiopoietin 1/Tie-2, angiotropin, pleiotrophin, angiogenin, survivin, COX/prostaglandin E2, PAF, NO, ET-1, Serum amyloid A, histamine, substance P, adenosine, erythropoietin, prolactin, thrombin	Angiopoietin 2, angiostatin, endostatin, kallistatin, type IV collagen derivatives, paclitaxel, 2-methoxyestradiol, osteonectin, opioids, troponin I, chondromodulin

^aSee text for abbreviations.

Table II

Angiogenesis targeting strategies in animal models of arthritis and human RA.^a

Compound	Animal (A)/Human (H) study ^b	Reference(s)
<i>Endogenous inhibitors</i>		
Angiostatin	A	[6,108]
Endostatin	A	[6,107,109,111,112]
Protease-activated kringle 1-5 (K1-5) (angiostatin analogue)	A	[113]
Kallistatin	A	[114]
Thrombospondin-1-derived peptide	A	[97]
Thrombospondin-2	A	[98]
IL-4 gene	A	[88]
IL-13 gene	A	[89]
PF4/CXCL4 chemokine	A	[8,91]
2-methoxyestradiol (2-ME)	A	[117,118]
<i>Exogenous inhibitors</i>		
Anti-VEGFR1 antibody	A	[28]
Soluble VEGFR 1	H (culture)	[26]
Vatalanib (VEGF-R tyrosine kinase inhibitor)	A	[26]
Anti-flt-1 hexapeptide (PIGF and VEGF antagonist)	A	[43]
Imatinib mesylate (PDGF receptor signaling)	H (culture), A	[44,45]
sFasL	H (culture)	[27]
Bicyclam (AMD3100)	H (culture)	[56]
Soluble Tie2 vector	A	[42]
Vitaxin (anti- $\alpha_v\beta_3$ integrin)	A, H (RA)	[6,13,58]
Traditional DMARDs (methotrexate, sulfasalazine, etc.)	H (RA)	[1,2,12]
Rofecoxib	H (culture)	[93]
Infliximab	H (RA)	[6,75,81]
Tocilizumab	H (RA)	[76]
Thalidomide	A, H (RA)	[6,95]
CC1069 (thalidomide analogue)	A	[96]
TNP-470 (fumagillin analogue)	A	[6,99]
PPI2458 (fumagillin analogue)	A	[99,101,106]
Statins	H (culture)	[68]
ET-1 antagonists	H	[83]
Paclitaxel	H	[6,116]
Minocycline	H (RA)	[102,103]
Clarithromycin	H (RA)	[104]
Deoxyspergualin	H (RA)	[105]
PPAR γ agonists (pioglitazone, rosiglitazone)	H (arthritis)	[33,34]

^a See text for abbreviations^b Human studies include both *in vitro* studies with isolated cell cultures, as well as *in vivo* RA studies.