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New insights in synovial angiogenesis

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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, cell adhesion molecules, proteases and others. Among the several potential angiogenesis inhibitors, targeting of VEGF, HIF-1, angiopoietin and the $\alpha_V\beta_3$ integrin, as well as some endogenous or synthetic compounds including angiostatin, endostatin, paclitaxel, fumagillin analogues, 2-methoxyestradiol and thalidomide seems to be promising for the management of synovial inflammation and angiogenesis. A complete review of antiangiogenic drugs used in animal models of arthritis or human RA is available in a table.

Keywords

Angiogenesis; Rheumatoid arthritis; Vascular endothelial growth factor; Angiostasis

1. Introduction

Angiogenesis is the formation of new capillaries from pre-existing blood vessels. Angiogenesis has been associated with inflammation and inflammatory diseases. A perpetuation of angiogenesis leading to enhanced endothelial surface and perpetuated leukocyte ingress into inflamed tissues has been described in rheumatoid arthritis (RA), as well as other types of arthritis and connective tissue diseases [1–6]. Thus, increased angiogenesis and defective vasculogenesis have important clinical relevance for rheumatic diseases including RA. The control of inflammatory neovascularization attenuates synovitis in RA [1–6].

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Conflicts of interest

The authors have no conflicts of interest to declare.

2. Angiogenesis: mechanisms and clinical relevances

2.1. Basic mechanisms

In RA, as well as in other types of arthritis, leukocytes migrate from the bloodstream through endothelia of vessels resembling high-endothelial venules generally found in lymphoid organs into the synovial tissue. Leukocyte-endothelial interactions are mediated by numerous cell adhesion receptors, primarily by integrins, selectins and their respective ligands [1,7]. As angiogenesis increases the number of blood vessels within a given compartment of the synovium, as well as the total endothelial surface, mechanistically, this process may result in the perpetuation of inflammatory leukocyte migration [7,8]. Synovial tissue macrophages and angiogenic mediators produced by these and other cells are primarily involved in RA-associated neovascularization [8].

Angiogenesis is a programmed sequence of events. Angiogenic mediators produced by various cell types in the synovium activate endothelial cells, which, in turn, release proteolytic enzymes. These enzymes degrade the endothelial basement membrane and the perivascular extracellular matrix. ECs then proliferate and emigrate into the interstitial tissue and thus “primary sprouts” are formed. The lumen formation within these sprouts leads to the formation of “capillary loops” followed by synthesis of new basement membrane and finally, capillary formation [2,4] (Fig. 1).

In vitro models used to investigate angiogenesis include EC cultures grown on extracellular matrix, such as Matrigel, as well as tissue culture systems or EC chemotaxis assays [2,4]. *In vivo* models include the animal corneal micropocket, chick embryo chorioallantoic membrane assays, aortic ring and sponge models, as well as other systems [2,4]. These models are used to investigate the role of angiogenesis in the pathogenesis of inflammatory diseases, as well as to design strategies for anti-angiogenic therapies.

2.2. Mediators of angiogenesis

Both soluble and cell surface-bound mediators may trigger neovascularization (Fig. 1). Angiogenic factors include growth factors, pro-inflammatory cytokines, chemokines, extracellular matrix molecules, matrix-degrading proteolytic enzymes, cellular adhesion molecules and others [1–5]. These mediators are primarily released by macrophages and ECs into the RA synovium [1–5,8]. Some of the most relevant molecules will be discussed in more detail (Table 1).

Growth factors are abundantly produced in synovial inflammation. These mediators are bound to heparin and heparan sulfate proteoglycans within the synovial extracellular matrix. Proteolytic enzymes, such as heparanase and plasmin release these growth factors from their binding during the process of angiogenesis [1,2]. With regards to angiogenesis and vasculogenesis, *vascular endothelial growth factor (VEGF)* is of outstanding importance. VEGF is probably the key regulator of neovascularization in inflammation. VEGF is produced within the synovium in response to pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) released by perivascular cells and synovial fibroblasts [1,2,9,10]. VEGF induces EC proliferation and migration, and it also stimulates angiogenesis, at least in part via cyclooxygenase-2 (COX-2) induction [1,10].

Hypoxia is a major feature of the inflamed joint. Intraarticular hypoxia induces branching of acts in part by the stimulation of hypoxia-inducible factors (HIF-1 and HIF-2) and HIFs then act predominantly via upregulation of VEGF [11,12]. Hypoxia has been detected within the RA joint [11]. Recently, a novel, HIF-independent regulatory pathway involving peroxisome-proliferator-activated receptor- γ (PPAR γ) and PPAR γ coactivator 1 α (PGC-1 α) has been described. PGC-1 α induces VEGF production and blood vessel reconstitution after

an ischemic insult in a HIF-independent manner [13]. Apart from hypoxia and HIFs, other angiogenic mediators including HGF, prostaglandins and nitric oxide (NO) also act via VEGF during neovascularization [1]. Thus, the hypoxia-HIF-VEGF pathway is crucial in the regulation of inflammation-associated angiogenesis.

Interaction between VEGF and angiopoietin-1 (Ang1)/Tie-2 is critical for the stabilization of newly formed blood vessels. In contrast, Ang2 antagonizes Ang1 and thus inhibits vessel maturation [1,2]. One more player of this network is survivin, an apoptosis inhibitor, which is also involved in VEGF-induced angiogenesis [2]. VEGF, HIF-1, HIF-2, Ang1, Tie2 and survivin are all expressed in the RA synovium [2,12]. Moreover, overexpression of VEGF and Ang1 in the arthritic joints is associated with distinct vascular morphology [2].

Other growth factors involved in angiogenesis include the heparin-bound basic (bFGF), acidic fibroblast growth factors (aFGF) and hepatocyte growth factor (HGF), as well as the non-heparin bound 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) [1,2,14].

Some *chemokines and chemokine receptors* have also been implicated in synovial inflammation and angiogenesis [1,2,5]. Most CXC chemokines containing the glutamyl-leucyl-arginyl (ELR) amino acid sequence stimulate, while chemokines lacking this motif rather suppress neovascularization [15]. ELR⁺ angiogenic CXC 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 [5,15]. Stromal cell-derived factor-1 (SDF-1)/CXCL12, a key regulator of lymphoid neogenesis within the RA synovium, is an ELR⁻, but still angiogenic CXC chemokine [1,16]. Hypoxia stimulates the release of SDF-1/CXCL12 by RA synovial fibroblasts [16]. Among CC chemokines, MCP-1/CCL2 is involved in angiogenesis as this chemokine supports the angiogenic effects of growth factors [5,17]. Fractalkine/CX3CL1, a CX₃C chemokine also promotes vessel formation [1]. All these chemokines are also involved in leukocyte recruitment into the inflamed synovium [5]. As CXCR2 is the most important endothelial receptor for ELR⁺ angiogenic CXC chemokines, this chemokine receptor plays a major role in synovial angiogenesis [1,2,5]. CXCR4, the receptor for SDF-1/CXCL12 and CCR2, the receptor for MCP-1/CCL2 are also involved in chemokine-induced angiogenesis [5,18].

Synovial inflammation is associated with chemokine-driven recruitment of inflammatory leukocytes into the synovium. This process involves cellular adhesion molecules (CAMs), synovial matrix macromolecules, as well as matrix-degrading proteolytic enzymes. Among *extracellular matrix constituents*, type I collagen, fibronectin, laminin, vitronectin, tenascin and proteoglycans are also involved in EC migration during angiogenesis [1,7,19]. Regarding *CAMs*, numerous 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-endothelial cell adhesion molecule-1 (PECAM-1; CD31), endoglin (CD105) and junctional cell adhesion molecules A (JAM-A) and C (JAM-C) are expressed by ECs and promote angiogenesis [5,12,20–23].

Among all these CAMs, the $\alpha_V\beta_3$ *integrin* seems to be of outstanding importance. This integrin mediates osteoclast-mediated bone resorption and thus the development of erosions, as well as synovial angiogenesis in RA [20,22]. Furthermore, the α_V subunit of this integrin is encoded by the ITGAV gene. A significant association has been found between the ITGAV rs3738919-C allele and RA in the European Caucasian population [24]. Focal

adhesion kinases (FAK) are predominant mediators of $\alpha_v\beta_3$ integrin signaling. FAKs have recently been detected in the RA synovium suggesting their role in synovial inflammation and angiogenesis [25]. 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 [26]. *Angiogenic proteases* include some matrix metalloproteinases (MMPs), as well as plasminogen activators [2,5].

Pro-inflammatory cytokines may either directly induce neovascularization or may, as described above, act via VEGF-dependent pathways. Among these cytokines, TNF- α , IL-1, IL-6, IL-8, IL-15, IL-17, IL-18, oncostatin M, macrophage migration inhibitory factor (MIF), granulocyte (G-CSF) and granulocyte-macrophage colony-stimulating factors (GM-CSF) are involved in angiogenesis, as well as rheumatoid synovitis [1,2,14,27]. Among these cytokines, TNF- α may also regulate capillary formation via the Ang1-Tie2-VEGF system described above [28]. IL-6 may also act via the induction of VEGF production as blockade of the IL-6 receptor resulted in diminished serum VEGF levels in RA patients [29]. IL-17 synergizes with TNF- α in stimulating VEGF, EGF, HGF and KGF production by synovial fibroblasts [14]. IL-18 induces SDF-1/CXCL12, MCP-1/CCL2 and VEGF production by synovial fibroblasts [30]. Oncostatin M enhances ICAM-1 expression on RA synovial fibroblasts and endothelium and it also stimulates EC migration and tubule formation [31]. MIF has recently drawn a lot of attention as it is primarily produced by synovial macrophages and is involved in macrophage-dependent synovial angiogenesis [8,32,33]. MIF acts via the induction of VEGF and IL-8/CXCL8 chemokine release by RA synovial fibroblasts [8,33].

There are numerous *other angiogenic mediators* not classified above. The most relevant ones include endothelin 1 (ET-1), serum amyloid A (SAA), the cyclooxygenase-2 (COX-2)-prostaglandin E₂ system, angiogenin, angiotropin, pleiotrophin, platelet-activating factor (PAF), substance P, histamine, erythropoietin, adenosine, prolactin and thrombin [1,2]. For example, *ET-1*, also released by ECs, induces VEGF production, EC proliferation and angiogenesis [34]. ET-1 is abundantly produced in RA [34]. SAA is an important acute-phase reactant, which has been implicated in the pathogenesis of arthritis. SAA acts through the formyl peptide receptor-like 1 (FPRL1). The binding of SAA to FPRL1 stimulates EC proliferation, migration, enhanced EC sprouting activity and neovascularization, as well as synovitis [35].

2.3. Angiogenesis inhibitors

There is a great number of naturally produced or synthetic angiostatic molecules. Some of them have already been tried to target tumor- or inflammation-associated neovascularization. Some examples for current or future anti-angiogenesis therapy will be presented later [1–3,6] (Table 1). Angiostatic *cytokines* include interferon- α (IFN- α), IFN- γ , IL-4, IL-12 and leukemia inhibitory factor (LIF). These cytokines inhibit the release of angiogenic mediators and thus they indirectly block neovascularization [2,3,6]. For example, IL-4 suppresses VEGF production by synovial fibroblasts [36].

As described above, CXC *chemokines* lacking the ELR sequence, such as platelet factor-4 (PF4)/CXCL4, monokine induced by interferon- γ (MIG)/CXCL9 and interferon- γ -inducible protein 10 (IP-10)/CXCL10 are angiogenesis inhibitors [2,5]. VEGF and these angiostatic chemokines may form a regulatory loop: VEGF stimulates the release of IP-10/CXCL10, on the other hand, IP-10/CXCL10 suppresses VEGF-induced angiogenesis [10,37]. Among CC chemokines, secondary lymphoid tissue chemokine (SLC)/CCL21 is angiostatic and it inhibits tumor progression [38]. Regarding chemokine receptors, as CXCR3 binds most angiostatic ELR⁻ CXC chemokines, this receptor is primarily involved in angiogenesis inhibition [2,5].

Protease inhibitors including tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs) antagonize the effects of proteolytic enzymes during angiogenesis [2,3]. Thrombospondin-1 and the PF4/CXCL4 chemokine inhibit growth factor binding to heparin and thus synovial capillary formation [3].

Among currently used *antirheumatic drugs*, dexamethasone, chloroquine, sulfasalazine, methotrexate (MTX), azathioprine, cyclophosphamide, leflunomide, thalidomide, minocycline and some anti-TNF agents, apart from several other anti-inflammatory effects, also inhibit EC migration and synovial angiogenesis [3,6]. There are some conflicting results regarding MTX, as it suppressed cancer-related angiogenesis but failed to do so in psoriatic arthritis [6].

Some *antibiotic derivatives* including minocyclin, fumagillin analogues, deoxyspergualin and clarithromycin also inhibit the release of VEGF and other angiogenic mediators and thus neovascularization [2,3,6].

Further compounds not mentioned above include angiostatin (a fragment of plasminogen), endostatin (a fragment of type XIII collagen), paclitaxel, osteonectin, opioids, troponin I, and chondromodulin-1 [2,3,6]. Most angiostatic molecules block the action of angiogenic mediators, such as VEGF, HIFs or the $\alpha_v\beta_3$ integrin [2,3,6]. Some of these agents, particularly angiostatin and endostatin, gave promising results in cancer therapy trials, as well as preclinical arthritis studies [2,3,6,39].

2.4. Regulation of synovial angiogenesis

The central role of *macrophages* in inflammatory angiogenesis is indisputable [8]. These cells express CXCR2 and CXCR4, which recognize many angiogenic CXC chemokines described above [2,5,8,18]. Macrophages also release important soluble angiogenic mediators, such as IL-8/CXCL8, ENA-78/CXCL5, gro α /CXCL1, CTAP-III/CXCL7, MCP-1/CCCL2, TNF- α , IL-15, IL-18, VEGF, bFGF, aFGF, HGF, PDGF and MMPs [2,5,8,18]. On the other hand, macrophages are also involved in the down-regulation of neovascularization as they produce angiostatic IP-10/CXCL10, MIG/CXCL9, IFN- γ and TIMPs [5,8].

Several *regulatory interactions* exist in sites of angiogenesis and inflammation. For example, there is an imbalance between specific antagonistic pairs, such as ELR⁺ versus ELR⁻ CXC chemokines, MMPs and TIMPs, pro-inflammatory, angiogenic versus anti-inflammatory, angiostatic cytokines [2,3]. Different angiogenic mediators may have additive effects. For example, the pro-inflammatory and angiogenic TNF- α stimulates the production of several angiogenic chemokines and growth factors, as well as the expression of angiogenic CAMs [2,3,7]. Furthermore, as described above, numerous angiogenic mediators indirectly promote neovascularization via VEGF- and Ang1/Tie-2-dependent mechanisms [2,10]. Regarding negative feedback, the autocrine loop between VEGF and IP-10/CXCL10 was described above [10,37]. Apart from intrinsic regulatory networks described here, external administration of angiostatic compounds may also interfere with neovascularization. As discussed later, these agents may be used clinically as anti-angiogenic therapy [3,6].

2.5. Therapeutic targeting of angiogenesis in arthritis

Inflammation and angiogenesis may be controlled simultaneously. Neovascularization may be targeted by the use of endogenous inhibitors or by specific targeting of angiogenic mediators [1–3,6]. *Endogenous angiogenesis inhibitors* including cytokines, chemokine, protease inhibitors and others produced within the synovial tissue were described above. These inhibitors try to counterbalance the effects of angiogenesis mediators, however, there

is an excess of angiogenic factors within the inflamed synovium. Therefore, these endogenous angiostatic molecules need to be administered in excess in order to attenuate neovascularization [2,3,6] (Tables 2 and 3).

Among these compounds, *angiostatin* and *endostatin* block $\alpha_V\beta_3$ integrin-dependent angiogenesis [2,3]. Endostatin interferes with VEGF receptor 2 signaling [3]. The administration of either of these compounds abrogated arthritis in various rodent models [2,3,5,39]. For example, angiostatin gene transfer attenuated murine collagen-induced arthritis (CIA) [3], while endostatin also suppressed pannus formation and joint destruction in rodent arthritis models [3,39]. The angiostatin-like protease-activated kringles 1–5 (K1–5) suppressed CIA more effectively than angiostatin itself [40]. Intraarticular injection of kallistatin, an endogenous angiogenesis inhibitor also expressed within the RA joint, attenuated rat ankle arthritis using gene therapy [41]. Type IV collagen derivatives including arresten, canstatin and tumstatin also inhibit neovascularization [42]. Regarding human studies, both angiostatin and endostatin have been introduced to clinical trials in cancer [3,6].

Other endogenous angiogenesis inhibitors include *thrombospondin-1 (TSP1)* and *TSP2* that are angiostatic extracellular matrix components naturally produced by RA synovial macrophages and fibroblasts [2,3]. A peptide derived from TSP1 suppressed synovial inflammation and angiogenesis in peptidoglycan-polysaccharide-induced rat arthritis, while TSP2 inhibited synovial neovascularization in the severe combined immunodeficiency (SCID) mouse model of arthritis [2,3,43]. *IL-4* and *IL-13* gene transfer inhibits synovial inflammation and angiogenesis in rats [2,44,45]. The *PF4/CXCL4* angiostatic chemokine already described above has also been tried in rodent models of arthritis [2].

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 [46]. In recent preclinical therapeutic studies, 2-ME suppressed VEGF and bFGF gene expression, as well as arthritis in the rat CIA model [2,3]. On the other hand, while 2-ME also suppressed rat adjuvant-induced arthritis, it did not affect synovial vascularity in this model [47]. Thus, 2-ME may also have angiogenesis-independent effects on synovitis.

Exogenously administered agents are not naturally found within the synovium. These synthetic compounds include antirheumatic drugs, biological monoclonal antibodies and receptor antagonists, as well as small molecule inhibitors that block the activities of VEGF, chemokines, integrins and other angiogenic mediators [2,3,6] (Table 3).

Certainly today targeting of *VEGF* and VEGF-induced angiogenesis, as well as the hypoxia-HIF-VEGF-Ang1-Tie2 system is in the focus of cancer and inflammation research. There have been several attempts to target VEGF by using synthetic VEGF and VEGFR inhibitors, anti-VEGF antibodies and inhibitors of VEGF and VEGFR signaling, primarily in colorectal, lung, renal and liver cancers [3,6,10]. The VEGF-Trap construct is a composite decoy receptor based on the fusion of VEGFR1 and VEGFR2 with IgG1-Fc [3,48]. Bevacizumab, a human monoclonal antibody to VEGF, has been approved for the treatment of various types of cancer [3]. A soluble VEGFR1 chimeric protein dose-dependently inhibited the proliferation of synovial ECs [48]. Vatalanib, sunitinib malate, sorafenib, vandetanib (ZD6474), cediranib (AZD2171), axatinib (AG013736), KRN-951 and CEP-7055 are small molecule inhibitors of VEGFR tyrosine kinases [3,10,48]. In cancer studies, these small molecules have been administered orally and exerted favorable safety profiles [3,48]. Among these VEGFR protein kinase inhibitors, vatalanib also inhibited knee arthritis in rabbits [49]. Semaphorin-3A blocks the function of the 165 amino-acid form of

VEGF (VEGF165). This compound also suppressed EC survival and neovascularization [50]. Soluble Fas ligand (CD178) also inhibited VEGF165 production by RA synovial fibroblasts, as well as neovascularization [3].

Hypoxia-HIF-mediated neovascularization may also be targeted. YC-1, a superoxide-sensitive stimulator of soluble guanylyl cyclase originally developed to treat hypertension and thrombosis, is also a HIF-1 inhibitor [3,51]. Microtubule destabilizers, such as 2-ME mentioned above, as well as *paclitaxel*, an anti-cancer agent, also diminish HIF-1 α expression and activity [3,46]. In a phase I human trial, paclitaxel was effective and safe in RA patients [3].

Regarding the *Ang-Tie* system, a soluble Tie2 receptor transcript was delivered via an adenoviral vector to mice with CIA. Tie2 inhibition attenuated the incidence and severity of arthritis [52].

The suppression of *chemokine- and chemokine receptor-mediated neovascularization* may also be feasible. For example, blockade of CXCR2 inhibited tumor-induced angiogenesis [53]. Mig/CXCL9 gene therapy and cytotoxic agents had additive effects in cancer trials [3]. Bicyclam (AMD3100), a highly selective antagonist of SDF-1/CXCL4, inhibits endothelial cell proliferation and migration [3,54].

As discussed above, the $\alpha_v\beta_3$ *integrin* may be a key adhesion molecule that stimulates angiogenesis. Vitaxin, a humanized antibody to this CAM, inhibited synovial angiogenesis in animal models of arthritis [1,3], however, it showed very little efficacy in a phase II human RA trial [3]. Numerous *MMP inhibitors* have been tried in angiogenesis models [55]. *ET-1 antagonists* currently used in the treatment of primary and scleroderma-associated pulmonary hypertension may also exert anti-angiogenic effects [34].

The angiostatic effects of currently used *antirheumatic agents* including classical disease-modifying drugs (DMARDs) and biologics are described above. In addition, the COX-2 inhibitor rofecoxib inhibited EC tube formation induced by RA synovial fibroblast culture supernatants [3]. Infliximab treatment in combination with MTX reduced synovial VEGF expression and vascularity [3,6,56]. Anti-TNF therapy in arthritic patients reduced Ang1-Tie2 and surviving but stimulated Ang2 expression [3,28]. Recently, the anti-IL-6 receptor antibody tocilizumab decreased serum levels of VEGF [29].

Thalidomide, currently in use to treat multiple myeloma but also tried in lupus and RA, is a potent TNF- α antagonist and angiogenesis inhibitor [3,57]. The effects of thalidomide on neovascularization may be controversial, as in one study it suppressed, while in a rat CIA study it did not affect VEGF production [3,57]. Nevertheless, thalidomide suppressed both synovitis and angiogenesis [3] suggesting that its anti-angiogenic effects may be, in part, VEGF-independent. CC1069, a thalidomide analogue, even more potently inhibited rat AIA than thalidomide itself [3]. Regarding human arthritis, thalidomide has been tried in RA showing little efficacy, while in systemic lupus erythematosus (SLE), it had some effect on disease activity [3].

Fumagillin is a naturally occurring product of *Aspergillus fumigatus*. TNP-470 and PPI2458 are synthetic derivatives of fumagillin that inhibit methionine aminopeptidase-2, an enzyme involved in angiogenesis [58]. These fumagillin analogues also act by inhibiting VEGF production and VEGF-induced capillary formation [3,58]. In rodent models, TNP-470 prevented arthritis when administered before the onset of the disease [3]. PPI2458 also suppressed the development of arthritis and joint erosions [3].

Statins and glitazones are currently used for the treatment of metabolic diseases, such as hyperlipidemia and diabetes, respectively. *Statins* modify EC function, as well as endothelial CAM expression [59]. The PPAR γ agonist *pioglitazone* improved joint and skin symptoms in psoriatic arthritis [60].

Theoretically, most angiostatic agents may have therapeutic relevance for arthritis-associated angiogenesis and many of these compounds have already been introduced to either preclinical or clinical therapeutic trials (Table 3). However, it is unlikely that specific targeting of one single angiogenic mediator could exert dramatic effects on angiogenesis and inflammation. Non-specific approaches using DMARDs or anti-TNF- α biologics that suppress angiogenesis, as well as numerous other inflammatory mechanisms may be more effective in controlling synovitis and joint destruction [3,6].

3. Conclusions

In this review, we first discussed the putative role of angiogenesis in the pathogenesis of inflammatory conditions, such as RA. Neovascularization may increase the total vascular endothelial surface and thus may promote the ingress of leukocytes into inflammatory sites leading to the progression of the disease. Several soluble and cell surface-bound mediators of angiogenesis, including growth factors, cytokines, chemokines, CAMs, proteases and others have been described in relation to synovitis. On the other hand, naturally occurring angiogenesis inhibitors, as well as exogenously administered angiostatic compounds may counterbalance excessive neovascularization in inflammation, as well as in malignancies. Inhibitors of VEGF and VEGF receptor, angiogenic chemokines and chemokine receptors, integrins, currently used DMARDs and biologics, thalidomide, taxol, 2-ME, fumagillin analogues, angiostatin and endostatin are in the forefront in cancer-and arthritis-associated angiogenesis research.

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References

1. Szekanecz Z, Koch AE. Vascular involvement in rheumatic diseases: 'vascular rheumatology'. *Arthritis Res Ther* 2008;10:224. [PubMed: 18947376]
2. Szekanecz Z, Koch AE. Mechanism of disease: angiogenesis in inflammatory diseases. *Nat Clin Pract Rheumatol* 2007;3:635–643. [PubMed: 17968334]
3. Lainer-Carr D, Brahn E. Angiogenesis inhibition as a therapeutic approach for inflammatory synovitis. *Nat Clin Pract Rheumatol* 2007;3:434–442. [PubMed: 17664950]
4. Folkman J, Klagsbrun M. Angiogenic factors. *Science* 1987;235:442–447. [PubMed: 2432664]
5. Szekanecz Z, Koch AE. Chemokines and angiogenesis. *Curr Opin Rheumatol* 2001;13:202–208. [PubMed: 11333349]
6. 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]
7. Agarwal SK, Brenner MB. Role of adhesion molecules in synovial inflammation. *Curr Opin Rheumatol* 2006;18:268–276. [PubMed: 16582691]
8. Szekanecz Z, Koch AE. Macrophages and their products in rheumatoid arthritis. *Curr Opin Rheumatol* 2007;19:289–295. [PubMed: 17414958]
9. Koch AE, Harlow LA, Haines GK, et al. Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. *J Immunol* 1994;152:4149–4156. [PubMed: 7511670]

10. Kiselyov A, Balakin KV, Tkachenko SE. VEGF/VEGFR signaling as a target for inhibiting angiogenesis. *Expert Opin Investig Drugs* 2007;16:83–107.
11. Taylor PC, Sivakumar B. Hypoxia and angiogenesis in rheumatoid arthritis. *Curr Opin Rheumatol* 2005;17:293–298. [PubMed: 15838239]
12. Giatromanolaki A, Sivridis E, Maltezos E, et al. Upregulated hypoxia inducible factor-1 α and -2 α pathway in rheumatoid arthritis and osteoarthritis. *Arthritis Res Ther* 2003;5:R193–R198. [PubMed: 12823854]
13. Arany Z, Foo SY, Ma Y, et al. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1 α . *Nature* 2008;451:1008–1012. [PubMed: 18288196]
14. Honorati MC, Neri S, Cattini L, et al. Interleukin-17, a regulator of angiogenic factor release by synovial fibroblasts. *Osteoarthritis Cartilage* 2006;14:345–352. [PubMed: 16311048]
15. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995;270:27348–27357. [PubMed: 7592998]
16. Pablos JL, Santiago B, Galindo M, et al. Synovioyte-derived CXCL12 is displayed on endothelium and induces angiogenesis in rheumatoid arthritis. *J Immunol* 2003;170:2147–2152. [PubMed: 12574387]
17. Salcedo R, Ponce ML, Young HA, et al. 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]
18. Nanki T, Hayashida K, El-Gabalawy HS, et al. 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]
19. Madri JA, Williams KS. Capillary endothelial cell cultures: phenotypic modulation by matrix components. *J Cell Biol* 1983;97:153–165. [PubMed: 6190818]
20. Imhof BA, Aurrand-Lions M. Adhesion mechanisms regulating the migration of monocytes. *Nat Rev Immunol* 2004;4:432–444. [PubMed: 15173832]
21. Koch AE, Halloran MM, Haskell CJ, et al. Angiogenesis mediated by soluble forms of E-selectin and vascular cell adhesion molecule-1. *Nature* 1995;376:517–519. [PubMed: 7543654]
22. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin α v β 3 for angiogenesis. *Science* 1994;264:569–571. [PubMed: 7512751]
23. Naik TU, Naik MU, Naik UP. Junctional adhesion molecules in angiogenesis. *Front Biosci* 2008;13:258–262. [PubMed: 17981544]
24. Jacq L, Garnier S, Dieudé P, et al. 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]
25. Shahrara S, Castro-Rueda HP, Haines GK, et al. Differential expression of the FAK family kinases in rheumatoid arthritis and osteoarthritis synovial tissues. *Arthritis Res Ther* 2007;9:R112. [PubMed: 17963503]
26. Kneilling M, Hültner L, Pichler BJ, et al. Targeted mast cell silencing protects against joint destruction and angiogenesis in experimental arthritis in mice. *Arthritis Rheum* 2007;56:1806–1816. [PubMed: 17530709]
27. Brennan F, Beech J. Update on cytokines in rheumatoid arthritis. *Curr Opin Rheumatol* 2007;19:296–301. [PubMed: 17414959]
28. Markham T, Mullan R, Golden-Mason L, et al. 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]
29. Nakahara H, Song J, Sugimoto M, et al. Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. *Arthritis Rheum* 2003;48:1521–1529. [PubMed: 12794819]
30. Amin MA, Mansfield PJ, Pakozdi A, et al. Interleukin-18 induces angiogenic factors in rheumatoid arthritis synovial tissue fibroblasts via distinct signaling pathways. *Arthritis Rheum* 2007;56:1787–1797. [PubMed: 17530707]

31. Fearon U, Mullan R, Markham T, et al. Oncostatin M induces angiogenesis and cartilage degradation in rheumatoid arthritis synovial tissue and human cartilage cocultures. *Arthritis Rheum* 2006;54:3152–3162. [PubMed: 17009243]
32. Amin MA, Volpert OV, Woods JM, et al. Migration inhibitory factor mediates angiogenesis via mitogen-activated protein kinase and phosphatidylinositol kinase. *Circ Res* 2003;93:321–329. [PubMed: 12881477]
33. 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]
34. 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]
35. Mullan RH, Bresnihan B, Golden-Mason L, et al. 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]
36. Hong KH, Cho ML, Min SY, et al. Effect of interleukin-4 on vascular endothelial growth factor production in rheumatoid synovial fibroblasts. *Clin Exp Immunol* 2007;147:573–579. [PubMed: 17302909]
37. Boulday G, Haskova Z, Reinders ME, et al. Vascular endothelial growth factor-induced signaling pathways in endothelial cells that mediate overexpression of the chemokine IFN-gamma-inducible protein of 10 kDa in vitro and in vivo. *J Immunol* 2006;176:3098–3107. [PubMed: 16493069]
38. Vicari AP, Ait-Yahia S, Chemin K, et al. Antitumor effects of the mouse chemokine 6Ckine/SLC through angiostatic and immunological mechanisms. *J Immunol* 2000;165:1992–2000. [PubMed: 10925282]
39. Yin G, Liu W, An P, et al. Endostatin gene transfer inhibits joint angiogenesis and pannus formation in inflammatory arthritis. *Mol Ther* 2002;5:547–554. [PubMed: 11991745]
40. Sumariwalla PF, Cao Y, Wu HL, et al. The angiogenesis inhibitor protease-activated kringle 1–5 reduces the severity of murine collagen-induced arthritis. *Arthritis Res Ther* 2003;5:R32–R39. [PubMed: 12716451]
41. Wang CR, Chen SY, Shiau AL, et al. Upregulation of kallistatin expression in rheumatoid joints. *J Rheumatol* 2007;34:2171–2176. [PubMed: 17937475]
42. Mundel TM, Kalluri R. Type IV collagen-derived angiogenesis inhibitors. *Microvasc Res* 2007;74:85–89. [PubMed: 17602710]
43. Park YW, Kang YM, Butterfield J, et al. Thrombospondin 2 functions as an endogenous regulator of angiogenesis and inflammation in rheumatoid arthritis. *Am J Pathol* 2004;165:2087–2098. [PubMed: 15579451]
44. Haas CS, Amin MA, Allen BB, et al. Inhibition of angiogenesis by interleukin-4 gene therapy in rat adjuvant-induced arthritis. *Arthritis Rheum* 2006;54:2402–2414. [PubMed: 16869003]
45. Haas CS, Amin MA, Ruth JH, et al. 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]
46. Mabeesh NJ, Escuin D, LaVallee TM, et al. 2ME2 inhibits tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell* 2003;3:363–375. [PubMed: 12726862]
47. 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]
48. Manley PW, Martiny-Baron G, Schlaeppli JM, et al. Therapies directed at vascular endothelial growth factor. *Expert Opin Investig Drugs* 2002;11:1715–1736.
49. Grosios K, Wood J, Esser R, et al. 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]

50. Guttmann-Raviv N, Shraga-Heled N, Varshavsky A, et al. 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]
51. Yeo EJ, Chun YS, Cho YS, et al. YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. *J Natl Cancer Inst* 2003;95:516–525. [PubMed: 12671019]
52. Chen Y, Donnelly E, Kobayashi H, et al. Gene therapy targeting the Tie2 function ameliorates collagen-induced arthritis and protects against bone destruction. *Arthritis Rheum* 2005;52:1585–1594. [PubMed: 15880817]
53. Wente MN, Keane MP, Burdick MD, et al. Blockade of the chemokine receptor CXCR2 inhibits pancreatic cancer cell-induced angiogenesis. *Cancer Lett* 2006;241:221–227. [PubMed: 16458421]
54. Yin Y, Huang L, Zhao X, et al. 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]
55. Skotnicki JS, Zask A, Nelson FC, et al. Design and synthetic considerations of matrix metalloproteinase inhibitors. *Ann NY Acad Sci* 1999;878:61–72. [PubMed: 10415720]
56. Goedkoop AY, Kraan MC, Picavet DI, et al. 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]
57. D'Amato RJ, Loughnan MS, Flynn E, et al. Thalidomide is an inhibitor of angiogenesis. *Proc Natl Acad Sci U S A* 1994;91:4082–4085. [PubMed: 7513432]
58. Ingber D, Fujita T, Kishimoto S, et al. Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature* 1990;348:555–557. [PubMed: 1701033]
59. 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]
60. Bongartz T, Coras B, Vogt T, et al. Treatment of active psoriatic arthritis with the PPAR γ ligand pioglitazone: an open-label pilot study. *Rheumatology* 2005;44:126–129. [PubMed: 15479756]

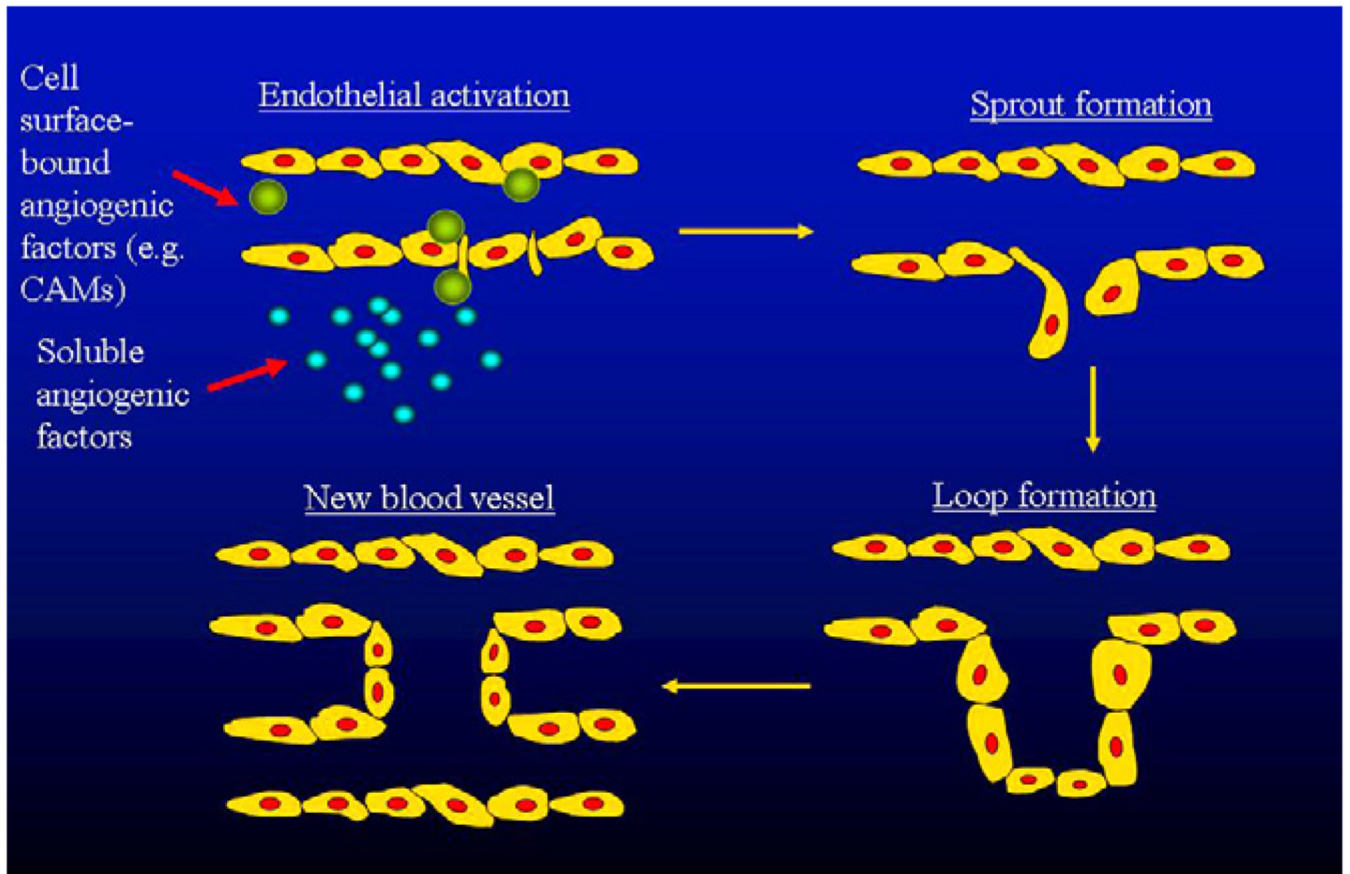


Fig 1. The process of angiogenesis. Both soluble and cell surface-bound angiogenic factors trigger endothelial activation, sprouting, lumen formation and then the formation of new vessels.

Table 1Some important mediators and inhibitors of angiogenesis in rheumatoid arthritis^a.

	Mediators	Inhibitors
Growth factors	VEGF, aFGF, bFGF, HGF, HIF-1 ^a , HIF-2, PDGF, EGF, KGF, IGF-I, TGF- β	-
Cytokines	TNF- α , IL-1, IL-6 ^a , 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 ^a , MCP-1/CCL2, fractalkine/CX3CL1 CXCR2, CXCR4 ^a , CCR2	PF4/CXCL4, M β g/CXCL9, IP-10/CXCL10, SLC/CCL21, CXCR3
Matrix molecules	Type I collagen, fibronectin, laminin, vitronectin, tenascin, proteoglycan	Thrombospondin-1
Cell adhesion molecules	β 1 and β 3 integrins ^a , E-selectin ^a , 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, classical DMARDs, anti-TNF biologics
Antibiotic derivatives	-	Minocycline, fumagillin, deoxyspergualin, clarithromycin
Environmental factors	Hypoxia	-
Others	Angiopoietin 1 /Tie-2, angiotropin, pleiotrophin, angiogenin, survivin, COX/prostaglandin E2, PAF, Nitric oxide (NO), endothelin-1, Serum amyloid A, histamine, substance P, adenosine, erythropoietin, prolactin, thrombin, etc.	Angiopoietin 2, angiostatin, endostatin, taxol, osteonectin, opioids, troponin I, chondromodulin, etc.

VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; HIF: hypoxia-inducible factors; PDGF: platelet-derived growth factor; EGF: epidermal growth factor; KGF: keratinocyte growth factor; IGF: insulin-like growth factor; TGF- β : transforming growth factor- β ; TNF: tumor necrosis factor; G-CSF: granulocyte colony-stimulating factors; GM-CSF: granulocyte-macrophage colony-stimulating factors; MIF: migration inhibitory factor; JAM: junctional cell adhesion molecules; PECAM-1: platelet-endothelial cell adhesion molecule-1; MMP: matrix metalloproteinases; TIMP: tissue inhibitors of metalloproteinases; PAI: plasminogen activator inhibitors.

^a Also key regulators of vasculogenesis.

Table 2**Inhibitors of angiogenesis.**

Endogenous inhibitors	Angiostatin, endostatin and related agents
	Thrombospondin 1 and 2
	IL-4, IL-13
	PF4/CXCL4
	2-methoxyestradiol
Exogenous inhibitors	VEGF inhibitors
	Ang1/Tie2 inhibitors
	Classical DMARDs
	Anti-TNF agents
	Thalidomide
	Fumagillin analogues
	Chemokine and chemokine receptor blockade (e.g. bicyclam)
	$\alpha\text{v}\beta_3$ integrin inhibitors (e.g. vitaxin)
	Protease inhibitors
	Endothelin-1 antagonists
	Microtubule destabilizers (e.g. paclitaxel)
	Others (soluble FasL, PPAR γ agonists, statins)

Table 3

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

Compound	Animal (A)/Human (H) study ^a	Reference(s)
<i>Endogenous inhibitors</i>		
Angiostatin	A	5,54
Endostatin	A	5,53,55
Protease-activated kringle 1–5 (K1–5) (angiostatin analogue)	A	56
Kallistatin	A	57
Thrombospondin-1-derived peptide	A	59
Thrombospondin-2	A	60
Interleukin-4 gene	A	61
Interleukin-13 gene	A	62
PF4/CXCL4 chemokine	A	4
2-methoxyestradiol (2-ME)	A	64,65
<i>Exogenous inhibitors</i>		
Soluble VEGF receptor 1	H (culture)	67
Vatalanib (VEGF-R tyrosine kinase inhibitor)	A	68
Soluble Fas ligand	H (culture)	70
Paclitaxel	H	5
Soluble Tie2 vector	A	72
Vitaxin (anti- $\alpha_v\beta_3$ integrin)	A, H (RA)	1,5
Traditional DMARDs (methotrexate, sulfasalazine, etc.)	H (RA)	1,2,5,8
Rofecoxib	H (culture)	77
Infliximab	H (RA)	5,39
Tocilizumab	H (RA)	40
Thalidomide	A, H (RA)	5,80,81
CC1069 (thalidomide analogue)	A	5
TNP-470 (fumagillin analogue)	A	5,83
PPI2458 (fumagillin analogue)	A	84
Statins	H (culture)	85
Pioglitazone	H (arthritis)	86

VEGF: vascular endothelial growth factor.

^aHuman studies include both in vitro studies with isolated cell cultures, as well as in vivo RA studies.