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Angiogenesis and its targeting in rheumatoid arthritis[☆]

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Abstract

Angiogenesis, the development of new capillaries, is involved in leukocyte ingress into the synovium during the development and progression of rheumatoid arthritis. Several soluble and cell surface-bound mediators including growth factors, cytokines, chemokines, proteolytic matrix-degrading enzymes, cell adhesion molecules and others may promote synovial neovascularization. On the other hand, endogenous angiostatic factors, such as angiostatin, endostatin, interleukin-4 (IL-4), IL-13, interferons and some angiostatic chemokines are also produced within the rheumatoid synovium, however, their effects are insufficient to control synovial angiogenesis and inflammation. Several specific and non-specific strategies have been developed to block the action of angiogenic mediators. The first line of angiostatic agents include vascular endothelial growth factor (VEGF), angiopoietin, $\alpha_v\beta_3$ integrin antagonist, as well as non-specific angiogenesis inhibitors including traditional disease-modifying agents (DMARDs), anti-tumor necrosis factor biologics, angiostatin, endostatin, fumagillin analogues or thalidomide. Potentially any angiostatic compound could be introduced to studies using animal models of arthritis or even to human rheumatoid arthritis trials.

Keywords

Angiogenesis; Rheumatoid arthritis; Targeting; Angiogenic mediators; Angiostatic agents

1. Introduction

Angiogenesis, the formation of new capillaries from pre-existing vessels, is involved in the pathogenesis of inflammatory diseases, as well as in tumor progression (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Szekanecz et al., 2005; Walsh, 1999; Veale and Fearon, 2006; Lainer-Carr and Brahn, 2007). Rheumatoid arthritis (RA) has been associated with the perpetuation of new vessel formation that enables leukocyte transendothelial migration into the synovial tissue (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Szekanecz et al., 2005; Walsh, 1999; Veale and Fearon, 2006; Lainer-Carr and Brahn, 2007; Murakami et al., 2006). There are numerous angiogenic

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mediators and inhibitors in the RA synovium. There is an imbalance in RA yielding to increased turnover of capillary formation in arthritis (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Szekanecz et al., 2005; Walsh, 1999; Veale and Fearon, 2006; Lainer-Carr and Brahn, 2007; Murakami et al., 2006). One can block angiogenesis by either stimulating the synovial production of endogenous angiostatic mediators, or by externally administering synthetic inhibitors of neovascularization. There is evidence that the blockade of angiogenesis may lead to the suppression of synovial inflammation and pannus formation (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Szekanecz et al., 2005; Walsh, 1999; Yin et al., 2002).

In this review, we will briefly summarize the process and animal models of angiogenesis followed by a more extensive discussion of angiogenic mediators and inhibitors relevant for arthritis (Table 1). Then we will describe current and future anti-angiogenic strategies developed to suppress synovial angiogenesis (Table 2).

2. The process and models of angiogenesis

During the neovascularization process, cell surface-bound, as well as soluble angiogenic mediators (see later) activate endothelial cells lining the pre-existing vessels. In response, endothelial cells release matrix-degrading enzymes, which digest the underlying basal membrane and the interstitial matrix. This enables the emigration of endothelial cells, which then gather to form primary and, after continuous proliferation and migration of endothelial cells, further generation capillary sprouts. Lumen formation within the sprouts leads to capillary loops. Finally, new basal membrane is synthesized leading to the formation of new capillary generations (Szekanecz and Koch, 2001; Walsh, 1999; Szekanecz et al., 1998a,b,c; Szekanecz and Koch, 2004; Folkman and Klagsbrun, 1987).

Angiogenesis may be studied using *in vitro* or *in vivo* models. Various soluble mediators and cells carrying surface antigens with potential angiogenic effects, as well as angiostatic compounds can be tested in these models (Szekanecz et al., 1998a,b,c; Fearon and Veale, 2007). *In vitro* systems include endothelial cultures grown on substrata or endothelial cell chemotaxis assays (Koch, 1998; Szekanecz and Koch, 2001; Walsh, 1999; Szekanecz et al., 1998a,b,c; Szekanecz and Koch, 2004; Fearon and Veale, 2007). *In vivo* capillary formation has been investigated using rat, murine, rabbit, or guinea pig models. For example, in the Matrigel plug assay, mice are implanted subcutaneously with Matrigel-containing plugs containing the tested agent. In the rat corneal system, the angiogenic mediator is injected in the subcorneal micropocket and blood vessels grow toward the implant as a positive angiogenic response (Fig. 1). Other *in vivo* assays include chick embryo chorioallantoic membrane, hamster cheek pouch, mesenteric and aortic ring models (Koch, 1998; Szekanecz and Koch, 2001; Walsh, 1999; Szekanecz et al., 1998a,b,c; Fearon and Veale, 2007).

3. Angiogenic mediators and inhibitors in arthritis

3.1. Angiogenic factors

The most well-described angiogenic pathway is possibly the hypoxia-vascular endothelial growth factor (VEGF)-angiopoietin system. Numerous chemokines and chemokine receptors involved in neovascularization have also been characterized.

3.1.1. The hypoxia–VEGF–angiopoietin network—VEGF is a heparin-binding growth factor, which plays a central role in the regulation of neovascularization (Veale and Fearon, 2006). Hypoxia, as well as pro-inflammatory cytokines including tumor necrosis factor- α (TNF- α) and IL-1 stimulate VEGF production in arthritis (Koch, 1998; Auerbach

and Auerbach, 1994; Veale and Fearon, 2006). Hypoxia acts through the hypoxia-inducible factor heterodimer, HIF-1 α /HIF-1 β (Lainer-Carr and Brahn, 2007; Liu et al., 2002). Other mediators, such as hepatocyte (HGF) and epidermal growth factors (EGF), prostanoids or nitric oxide (NO) may also stimulate VEGF release (Koch, 1998; Veale and Fearon, 2006; Milkiewicz et al., 2006).

Angiopoietin 1 (Ang1) and Ang2 regulate endothelial functions upon stimulation by VEGF. Both Ang1 and Ang2 interact with Tie2, an endothelial tyrosine kinase receptor (Veale and Fearon, 2006; Suri et al., 1996). The interaction of Ang1 and Tie2 results in the stabilization of newly formed capillaries (Davis et al., 1996). In contrast, Ang2 antagonizes the effects of Ang1 and rather induces endothelial cell invasion and blocks vessel maturation (Suri et al., 1996; Holash et al., 1999). Interactions between VEGF, Ang1 and TNF- α may increase endothelial survival. Survivin is an inhibitor of apoptosis, which is also involved in VEGF-induced angiogenesis and endothelial cell survival (Veale and Fearon, 2006; Tran et al., 2002).

As far as the rheumatoid synovium is concerned, there are high amounts of VEGF in the inflamed synovial tissue (Koch et al., 1994). HIF-1, Ang1, Tie2 and surviving are also expressed in the arthritic synovium (Veale and Fearon, 2006; Koch et al., 1994; Giatromanolaki et al., 2003; Gravallesse et al., 2003; Shahrara et al., 2002).

3.1.2. Angiogenic chemokines and chemokine receptors—Chemokines are chemotactic inflammatory mediators, which have been classified as CXC, CC, C and CX₃C chemokines according to the position of cysteine residues in their structure (Szekanecz and Koch, 2001; Szekanecz et al., 1998a,b,c, 2002). Apart from their classical names, these chemokines are considered as chemokine ligands (CXCL, CCL, CL and CX₃CL), which bind to their respective receptors, CXCR, CCR, CR and CX₃CR (Szekanecz et al., 1998a,b,c, 2002).

Most CXC chemokines that contain the ELR amino acid sequence promote synovial angiogenesis. These angiogenic ELR⁺ CXC chemokines include interleukin-8 (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)/CXCL7. On the other hand, CXC chemokines lacking the ELR motif suppress neovascularization. ELR⁻ angiostatic CXC chemokines include platelet factor 4 (PF4)/CXCL4, IFN- γ -inducible protein 10 (IP-10)/CXCL10 and monokine induced by IFN- γ (Mig)/CXCL9 (Walz et al., 1996; Strieter et al., 1995). Interestingly, stromal cell-derived factor 1 (SDF-1)/CXCL12 lacks the ELR sequence, yet this chemokine stimulates capillary formation (Szekanecz and Koch, 2001; Strieter et al., 1995). IL-8/CXCL8, ENA-78/CXCL5, CTAP-III/CXCL7 and gro α /CXCL1 bind to their endothelial receptor, CXCR2. Thus, CXCR2 is a crucial chemokine receptor in angiogenesis (Szekanecz and Koch, 2001; Szekanecz et al., 2002; Walz et al., 1996; Strieter et al., 1995; Salcedo et al., 2000). All these chemokines have been detected in the arthritic synovium (Szekanecz and Koch, 2001; Szekanecz et al., 1998a,b,c, 2002).

IP-10/CXCL10 blocks VEGF-induced angiogenesis, on the other hand, VEGF increases endothelial expression of IP-10/CXCL10. Thus, IP-10/CXCL10 and VEGF may form an autocrine regulatory loop during synovial neovascularization (Szekanecz et al., 2002; Walz et al., 1996). IP-10/CXCL10, Mig/CXCL9 and PF4/CXCL4 are released into the inflamed synovial tissue (Szekanecz and Koch, 2001; Szekanecz et al., 1998a,b,c, 2002).

SDF-1/CXCL12 is a unique CXC chemokine. This chemokine, unlike other CXC chemokines described above, exerts mainly a homeostatic function, as it plays a role in

lymphoid tissue organization. Yet, it has been implicated in inflammation, such as in RA (Pablos et al., 2003). SDF-1/CXCL12 and its receptor, CXCR4, are key regulators of angiogenesis (Pablos et al., 2003; Salcedo et al., 1999; Petit et al., 2007). This chemokine also acts in concert with the hypoxia-VEGF system discussed above. Hypoxia stimulates synovial fibroblasts to produce SDF-1/CXCL12 (Pablos et al., 2003). Furthermore, SDF-1/CXCL12-CXCR4 interaction results in the stimulation of VEGF production via the phosphatidyl inositol 3 kinase (PI3K)/Akt pathway (Liang et al., 2007; Zheng et al., 2007).

Among CC chemokines, MCP-1/CCL2 stimulates angiogenesis via its endothelial receptor, CCR2 (Szekanecz and Koch, 2001; Szekanecz et al., 2002; Salcedo et al., 2000; Stamatovic et al., 2006). MCP-1/CCL2-induced neovascularization and cell adhesion involves integrins, the Ets-1 transcription factor and ERK-1/2 activation (Stamatovic et al., 2006). MCP-1/CCL2 promotes neovascularization induced by growth factors, such as TGF- β and fibroblast growth factor 2 (FGF-2) (Ma et al., 2007). Myeloid progenitor inhibitory factor 1 (MPIF-1)/CCL23, another CC chemokine, has also been implicated in endothelial cell migration (Son et al., 2006).

The sole CX₃C chemokine, fractalkine/CX₃CL1, also promotes synovial capillary formation (Ruth et al., 2001; Volin et al., 2001). Fractalkine/CX₃CL1 is abundantly produced in the RA synovium (Ruth et al., 2001; Volin et al., 2001).

3.1.3. Cytokines and other growth factors—Growth factors not discussed above but implicated in angiogenesis include FGF-1, FGF-2, HGF, platelet-derived growth factor (PDGF), EGF, insulin-like growth factor-I (IGF-I), and TGF- β (Koch, 1998; Szekanecz and Koch, 2001; Szekanecz et al., 2005; Walsh, 1999). Interestingly, recent reports suggest that despite the angiogenic capacity of HGF, this growth factor may have anti-inflammatory properties in arthritis (Okunishi et al., 2007). Several pro-inflammatory cytokines, such as TNF- α , IL-1, IL-6, IL-15, IL-17, IL-18, granulocyte (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), oncostatin M and macrophage migration inhibitory factor (MIF) also promote synovial vessel formation (Koch, 1998; Szekanecz and Koch, 2001; Szekanecz et al., 2005; Szekanecz et al., 1998a,b,c; Brennan and Beech, 2007; Park et al., 2001; Angiolillo et al., 1997; Numasaki et al., 2005; Amin et al., 2007; Fearon et al., 2006; Amin et al., 2003; Morand et al., 2006). TNF- α may also regulate angiogenesis via the Ang1-Tie2 system (Markham et al., 2006), while IL-18 acts via the stimulation of angiogenic SDF-1/CXCL12, MCP-1/CCL2 and VEGF production by synovial fibroblasts (Amin et al., 2007). MIF upregulates the production of VEGF and IL-8/CXCL8 (Amin et al., 2003) (Fig. 2).

3.1.4. Matrix macromolecules, adhesion receptors and proteolytic enzymes—Extracellular matrix components, cell adhesion molecules and matrix-degrading proteases are also involved in endothelial cell adhesion, basal membrane degradation, emigration, sprouting, lumen formation and thus angiogenesis. Among constituents of the synovial matrix, type I collagen, fibronectin, laminin, vitronectin, tenascin and proteoglycans promote capillary formation (Koch, 1998; Szekanecz et al., 1998a,b,c; Madri and Williams, 1983). Matrix metalloproteinases and plasminogen activators are involved in the degradation of the synovial connective tissue during the perpetuation of angiogenesis (Koch, 1998; Walsh, 1999; Szekanecz and Koch, 2007; Skotnicki et al., 1999). Several adhesion molecules including β_1 integrins, $\alpha_v\beta_3$, E-selectin, selectin-related glycoconjugates including Lewis^x/H and melanoma cell adhesion molecule (MUC18), vascular cell adhesion molecule 1 (VCAM-1), platelet-endothelial adhesion molecule 1 (PECAM-1; CD31) and endoglin have been implicated in the process of neovascularization (Koch, 1998; Szekanecz et al., 1998a, b; Madri and Williams, 1983).

Among adhesion receptors, the $\alpha_V\beta_3$ integrin is of outstanding importance. This integrin mediates synovial angiogenesis and osteoclast-mediated bone resorption in RA (Johnson et al., 1993). 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 susceptibility to RA in the European Caucasian population (Jacq et al., 2007). A recent genome-wide study confirmed the association of ITGAV with RA (Ahnert and Kirsten, 2007). The focal adhesion kinases (FAK) are predominant mediators of $\alpha_V\beta_3$ integrin signaling. Recent studies confirmed the expression of FAK family kinases in the RA synovium suggesting the role of FAK kinases in synovial inflammation and angiogenesis (Shahrara et al., 2007). Mast cells are also involved in integrin-dependent angiogenesis and joint destruction (Kneilling et al., 2007; Nigrovic and Lee, 2007). Mast cell silencing with salbutamol or cromolyn prevented $\alpha_V\beta_3$ integrin activation and angiogenesis in mice. On the other hand, mast cell reconstitution restored susceptibility of mice to integrin activation, vessel formation and joint destruction (Kneilling et al., 2007).

3.1.5. Other relevant angiogenic factors—Endothelin 1 is produced by endothelial cells. This mediator induces VEGF production, endothelial proliferation and angiogenesis (Koch and Distler, 2007; Haq et al., 1999). Increased levels of ET-1 have been detected in the synovial fluids and sera of RA patients (Koch and Distler, 2007; Haq et al., 1999).

Serum amyloid A (SAA) is a major acute-phase reactant, which has been implicated in the pathogenesis of inflammatory diseases, such as RA. The effects of SAA are mediated by the formyl peptide receptor-like 1 (FPRL1). The binding of SAA to FPRL1 stimulates synovial hyperplasia, endothelial cell proliferation, migration and neovascularization (Lee et al., 2006).

Other angiogenic factors not mentioned above include prostaglandin E_2 , angiogenin, angiotropin, pleiotrophin, platelet-activating factor (PAF), histamine, substance P, erythropoietin, adenosine, prolactin, thrombin and many others (Koch, 1998; Szekanecz et al., 2005; Walsh, 1999) (Table 1).

3.2. Inhibitors of angiogenesis

Endogenous inhibitors of neovascularization include cytokines, such as interferon- α (IFN- α), IFN- γ , IL-4, IL-12, IL-13 and leukemia inhibitory factor (LIF). These angiostatic cytokines suppress the production and action of angiogenic mediators described above (Koch, 1998; Auerbach and Auerbach, 1994; Haas et al., 2007) (Table 1). For example, IL-4 inhibits VEGF production by synovial fibroblasts (Hong et al., 2007). Tissue inhibitors of metalloproteinases (TIMP) and plasminogen activator inhibitors (PAI) antagonize the effects of angiogenic proteases described above (Koch, 1998; Szekanecz and Koch, 2001; Szekanecz et al., 2005; Veale and Fearon, 2006; Szekanecz and Koch, 2007; Mabjeesh et al., 2003). Thrombospondin-1 (TSP1) and the chemokine PF4/CXCL4 block the activity of heparin-binding growth factors (Koch, 1998; Szekanecz and Koch, 2001; Szekanecz et al., 2005; Veale and Fearon, 2006). ELR⁻, angiostatic CXC chemokines, such as PF4/CXCL4, Mig/CXCL9 and IP-10/CXCL10 were already mentioned above (Szekanecz and Koch, 2001; Szekanecz et al., 2005; Strieter et al., 1995). 2-methoxyestradiol, a metabolite of estrogen, blocks angiogenesis by disrupting microtubules and by suppressing HIF-1 α activity (Mabjeesh et al., 2003).

Other angiostatic compounds include angiostatin (a fragment of plasminogen), endostatin (a fragment of type XIII collagen), paclitaxel, osteonectin, opioids, troponin I, and chondromodulin-1 (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Szekanecz et al., 2005) (Table 1). Angiostatin and endostatin will be discussed in more detail later.

4. Targeting of synovial angiogenesis in RA

There may be two major strategies to control the perpetuation of neovascularization in “angiogenic diseases”, such as RA, as well as malignancies (Koch, 1998; Szekanecz et al., 2005; Walsh, 1999; Lainer-Carr and Brahn, 2007). First, there are endogenous inhibitors described above that include cytokines, chemokines, protease inhibitors and others. These angiostatic molecules are naturally produced in the RA synovium, however, their amounts are not enough to counterbalance the excessive neovascularization observed in arthritis. On the other hand, numerous synthetic compounds currently used to control inflammation and to treat arthritis may, among other effects, block neovascularization as well. These externally administered angiostatic compounds include some corticosteroids, traditional disease-modifying antirheumatic drugs (DMARDs), anti-TNF biologics, antibiotic derivatives and others (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Szekanecz et al., 2005) (Table 2).

4.1. Use of endogenous angiogenesis inhibitors and their derivatives

Angiostatin and endostatin act via $\alpha_v\beta_3$ integrin-dependent mechanisms (Veale and Fearon, 2006; Milkiewicz et al., 2006). Both molecules inhibited the development of arthritis in various animal models (Koch, 1998; Szekanecz and Koch, 2001; Auerbach and Auerbach, 1994; Lainer-Carr and Brahn, 2007; Yin et al., 2002; Takahashi et al., 2005). Angiostatin gene transfer inhibited synovial inflammation, vascularization and pannus formation in the murine type II collagen-induced arthritis (CIA) model (Lainer-Carr and Brahn, 2007; Takahashi et al., 2005). An angiostatin-related inhibitor, protease-activated kringle 1–5 (K1–5), suppressed CIA more potently than angiostatin itself (Sumariwalla et al., 2003). Endostatin interferes with VEGF receptor 2 signaling (Veale and Fearon, 2006; Yin et al., 2002; O'Reilly et al., 1997). In murine and rat models of arthritis, endostatin suppressed synovial proliferation and joint destruction (Lainer-Carr and Brahn, 2007; Yin et al., 2002; Yue et al., 2007). Angiostatin and endostatin have been tried in human cancer (Szekanecz et al., 2005; Veale and Fearon, 2006; Guttman-Raviv et al., 2007). Kallistatin, another endogenous inhibitor of angiogenesis, has been detected in the joints of RA patients. Local injection of the kallistatin gene into rat ankles attenuated the progression of arthritis (Wang et al., 2007). Type IV collagen-derived inhibitors including arresten, canstatin and tumstatin also inhibit neovascularization (Mundel and Kalluri, 2007).

TSP1 and TSP2 are angiostatic extracellular matrix components produced by RA synovial macrophages and fibroblasts. TSPs link connective tissue molecules to adhesion receptors, such as integrins. A TSP1-derived peptide ameliorated inflammation and angiogenesis in peptidoglycan-induced rat arthritis (Rico et al., 2007). TSP2 inhibited synovial neovascularization in the SCID mouse model of arthritis (Park et al., 2004).

Among cytokines, chemokines and growth factors, IL-4 and IL-13 gene transfer resulted in the attenuation of synovial inflammation and angiogenesis in rat adjuvant-induced arthritis (AIA) (Haas et al., 2007, 2006). The PF4/CXCL4 chemokine has also been tried in rodent models of arthritis (Szekanecz and Koch, 2001; Szekanecz et al., 2005). Interestingly, the otherwise angiogenic HGF may exert immunosuppressive and anti-inflammatory effects as it attenuated arthritis in the murine CIA model (Okunishi et al., 2007).

TNP-470 and PPI2458 are two angiostatic derivatives of fumagillin, a naturally occurring product of *Aspergillus fumigatus*. These compounds inhibit methionine aminopeptidase-2, an enzyme involved in neovascularization (Ingber et al., 1990). Fumagillin analogues also suppress VEGF release and capillary formation (Koch, 1998; Lainer-Carr and Brahn, 2007). In rodents, TNP-470 prevented arthritis when administered before the onset of the disease

(Lainer-Carr and Brahn, 2007; Peacock et al., 1992). PPI2458 also suppressed arthritis and the development of joint erosions (Hannig et al., 2007).

4.2. External blockade of neovascularization

4.2.1. Non-specific strategies—Traditional DMARDs and biologics exert various anti-inflammatory effects. In addition, these compounds may also inhibit synovial vessel formation by non-specifically blocking the action of angiogenic mediators (Szekanecz and Koch, 2001; Szekanecz et al., 2005; Veale and Fearon, 2006). For example, infliximab treatment in combination with methotrexate inhibited systemic and synovial VEGF release resulting in attenuated synovial vascularization (Szekanecz et al., 2005; Veale and Fearon, 2006; Goedkoop et al., 2004). Anti-TNF therapy of RA patients also reduced Ang1-Tie2 and survivin, but stimulated Ang2 expression (Markham et al., 2006).

Thalidomide, currently used in the management of multiple myeloma and also introduced into the treatment of RA and lupus, is a potent TNF- α antagonist and angiogenesis inhibitor (Lainer-Carr and Brahn, 2007; D'Amato et al., 1994). However, the net effect of thalidomide on capillary formation is not fully clear, as in some studies it suppressed VEGF production, capillary formation and synovitis (Lainer-Carr and Brahn, 2007; Komorowski et al., 2006), while in rat CIA it did not influence the release of angiogenic VEGF or TNF- α (Oliver et al., 1998). CC1069, a thalidomide analogue, even more potently inhibited rat AIA (Lainer-Carr and Brahn, 2007). Thalidomide has been suggested as a treatment option in RA, however, studies demonstrated only limited efficacy (Lainer-Carr and Brahn, 2007).

The hypoxia-HIF pathway may also be targeted using non-specific inhibitor compounds. For example, YC-1, a superoxide-sensitive stimulator of soluble guanylyl cyclase is also an inhibitor of HIF-1 (Yeo et al., 2003). 2-methoxyestradiol mentioned above and paclitaxel (taxol), a drug already used in human cancer, on one hand destabilize the intracellular cytoskeleton, on the other hand also block HIF-1 α expression and activity (Lainer-Carr and Brahn, 2007). In a phase I study, paclitaxel was effective and safe in RA patients (Lainer-Carr and Brahn, 2007). Soluble Fas ligand (CD178) inhibited synovial VEGF165 production and angiogenesis (Kim et al., 2007). Pioglitazone, an anti-diabetic PPAR γ agonist, is also angiostatic. This agent effectively controlled psoriatic arthritis in ten patients (Bongartz et al., 2005).

4.2.2. Specific blockade of angiogenic mediators—As described above, VEGF plays a central role in synovial, as well as tumor angiogenesis. Thus, VEGF could be a primary therapeutic target. VEGF has been targeted by using synthetic VEGF and VEGF receptor inhibitors, anti-VEGF antibodies, as well as inhibitors of VEGF and VEGF receptor signaling (Koch, 1998; Kiselyov et al., 2007). Numerous small molecular VEGF receptor tyrosine kinase inhibitors are under development for the treatment of malignancies. These compounds include vatalanib, sunitinib malate, sorafenib, vandetanib and AG013736 (Lainer-Carr and Brahn, 2007; Kiselyov et al., 2007). Small molecular VEGF receptor inhibitors are administered orally and generally exert favorable safety profiles. To date vatalanib has been tried and attenuated knee arthritis in rabbits (Lainer-Carr and Brahn, 2007; Grosios et al., 2004). The VEGF-Trap construct is a composite decoy receptor developed by the fusion of VEGF receptors 1 and 2 with IgG1-Fc (Holash et al., 2002). An anti-VEGF monoclonal antibody, bevacizumab is currently used for the treatment of colon and lung cancer (Lainer-Carr and Brahn, 2007). There have been no completed and published studies with either bevacizumab, or VEGF-Trap in arthritis. Semaphorin-3A blocks the effects of the 165 aminoacid form of VEGF (VEGF165) and inhibits neovascularization (Guttmann-Raviv et al., 2007).

The Ang-Tie system may also be targeted. A soluble Tie2 receptor transcript was delivered via an adenoviral vector to mice with CIA. The inhibition of Tie2 delayed the onset and attenuated the severity of arthritis (Chen et al., 2005).

Among anti-chemokine blockade strategies, specific inhibition of CXCR2 suppressed tumor-induced angiogenesis (Wente et al., 2006). Mig/CXCL9 gene transfer promoted the effects of cytotoxic agents in cancer studies (Zhang et al., 2006).

As discussed above, the $\alpha_v\beta_3$ integrin is a key regulator of endothelial cell adhesion, migration and angiogenesis. Vitaxin, a humanized antibody to this integrin, inhibited synovial angiogenesis in animal models of arthritis (Koch, 1998; Szekanecz et al., 2005; Lainer-Carr and Brahn, 2007), however, in a phase II human RA trial Vitaxin showed limited efficacy (Lainer-Carr and Brahn, 2007). Numerous specific inhibitors of metalloproteinases have been tried in angiogenesis models (Skotnicki et al., 1999). Endothelin-1 antagonists currently used in the therapy of primary pulmonary hypertension, as well as scleroderma may also exert anti-angiogenic effects (Koch and Distler, 2007).

5. Conclusions

RA, as well as malignancies, may be considered as “angiogenic diseases”, as they are associated with active tissue neovascularization. The perpetuation of angiogenesis involves numerous soluble and cell surface-bound mediators, which are also abundantly produced in the arthritic synovium. On the other hand, numerous endogenous mediators with angiostatic activity are released in the RA joint, however, their effects are insufficient to control high turnover synovial angiogenesis. Theoretically, higher amounts of these endogenous inhibitors or their derivatives, as well as externally administered non-specific or specific angiogenesis blockers may be used to control synovial inflammation and capillary formation. Among the several potential angiogenesis inhibitors, the targeting of VEGF, HIF-1, angiopoietin and the $\alpha_v\beta_3$ integrin seem to be of primary interest. However, endogenous or synthetic compounds including angiostatin, endostatin, paclitaxel, fumagillin analogues and thalidomide may also be used to treat arthritis. Many of these compounds are already in pre-clinical or clinical trials. However, there may be limitations of angiogenesis inhibition for the treatment of RA. As there is a complex regulatory network of angiogenesis in the inflamed synovium consisting of hundreds of mediators, targeting strategies using agents with multiple actions may be more effective than blocking one single player in the angiogenic cascade. Yet, potentially any angiostatic compound could be introduced to studies using animal models of arthritis or even to human RA trials.

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Fig. 1.
The rat corneal micropocket assay. (A) A micropocket is prepared by cutting the cornea; (B) the tested angiogenic compound is inserted into the pocket; (C) after 7–10 days vessel proliferation is induced towards the angiogenic compound.

Fig. 2.
Monocyte migration inhibitory factor (MIF) expression on rheumatoid synovial tissue endothelium (arrow) (immunohistochemistry, 400 $\times$).

Table 1

Some important mediators and inhibitors of angiogenesis in rheumatoid arthritis.

Class	Mediators	Inhibitors
Growth factors	VEGF, HGF, EGF, HIF-1, PDGF, aFGF, bFGF, IGF-I, TGF- β	–
Pro-inflammatory cytokines	TNF- α , IL-1, IL-6, IL-15, IL-17, IL-18, G-CSF, GM-CSF, oncostatin M, MIF	Interferons, IL-4, IL-12, IL-13, LIF
Chemokines	ELR ⁺ CXC chemokines, SDF-1/CXCL12, MCP-1/CCL2, fractalkine/CXC3CL1	ELR [–] CXC chemokines
Extracellular matrix components	Collagen, laminin, fibronectin, vitronectin tenascin	Thrombospondin 1, thrombospondin 2
Cell adhesion molecules	$\alpha_v\beta_3$ integrin, E-selectin, VCAM-1, endoglin, MUC18, Lewis ^x /H, PECAM-1	–
Proteases	MMPs, plasminogen activators	TIMPs, PAIs
Environmental factors	Hypoxia	
Vessel stabilizers, apoptosis inhibitors	Ang1-Tie2, survivin	
Others	Prostaglandin E2, angiogenin, angiotropin, PAF, prolactin, substance P, etc.	DMARDs, antibiotics, angiostatin, endostatin, paclitaxel, troponin, osteonectin, chondromodulin, etc.

See text for abbreviations.

Table 2

Anti-angiogenic targets.

Endogenous inhibitors	Angiostatin and related agents
	Endostatin
	Thrombospondin 2
	IL-4, IL-13
	PF4/CXCL4
Exogenous inhibitors	Classical DMARDs
	Anti-TNF agents
	Thalidomide/fumagillin analogues
	VEGF inhibitors
	HIF inhibitors
	Ang1/Tie2 inhibitors
	$\alpha_v\beta_3$ integrin inhibitors
	Microtubule destabilizers (e.g. paclitaxel)
	Others (soluble FasL, PPAR γ agonists)

See text for abbreviations.