Angiogenesis in Inflammatory Arthritis

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ABSTRACT: Angiogenesis is the outgrowth of new blood vessels from existing ones and is an early occurrence in inflamed joint tissue. It is governed by a tightly controlled balance of pro- and anti-angiogenic stimuli, which promote or inhibit generation and proliferation of new endothelial cells, vascular morphogenesis, and vessel remodeling. At the beginning, capillary formation is crucial in maintaining the supply of various nutrients as well as oxygen to the inflamed tissue. Local and systemic expression of angiogenic factors may indicate a constant remodeling of synovial vasculature. Redox signaling is closely related to angiogenesis and can alter angiogenic responses of synovial cells. In this review we discuss key issues about the endothelial pathology in inflammatory arthritis followed by a review of angiogenic processes and main angiogenic mediators. We discuss the hypoxia-vascular endothelial growth factor (VEGF)-Ang/Tie2 system and its related therapeutic implications in detail with further review of various mediator protein targets and intracellular regulatory pathway targets with their current and potential future role in preclinical or clinical setting whilst ameliorating inflammation.

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Angiogenesis is the formation of new capillaries from pre-existing blood vessels, which is differentiated from another neovascular process – vasculogenesis – where capillary sprouting originates from pre-existing precursors (endothelial precursor cells [EPCs]) and finally forms a primitive vascular network [1-3]. Numerous physiological processes involve angiogenesis, such as reproduction, development, and repair, but pathological implications include cancer development, psoriasis, vasculitis, atherosclerosis, and inflammatory arthritis [3,4]. Inflammatory arthritis is one of the prototypic diseases in which angiogenesis plays an important role in the morphological alterations of the vascular endothelial integrity. This process is characterized by a cascade of multiple events where existing blood vessels grow new capillary sprouts in a dysregulated fashion. The imbalance between positive and negative angiogenic regulators drives the process with constant remodeling in a tightly controlled step by step manner [2,3,5,6]. Angiogenic mediators activate endothelial cells (EC) through their receptors switching various signal transduction pathways on.

Proteases are produced by activated cells and degrade the basement membrane of the endothelium and the interstitial resulting in a leaky endothelium. Critical changes such as vasodilation and increased permeability promote vascular injury and regeneration [7-9]. ECs migrate to form new capillary sprouts. Some of them proliferate and undergo intensive mitosis in the midsection of the sprout, whereas others at the tip of the sprout only migrate. A lumen forms when two sprouts anastomose with each other into a capillary loop. Tube formation is finalized by new basement membrane synthesis and vessel stabilization through pericyte recruitment [Figure 1] [1,7,9]. Efficiency of oxygen supply to the synovium is weak due to the highly dysregulated synovial microvasculature. This occurrence, along with the increased energy demands of activated infiltrating immune cells and inflamed resident cells, leads to an hypoxic microenvironment as well as mitochondrial dysfunction [Figure 1] [10-12].

In the review, we present angiogenic mediators that are most relevant for arthritis. We also discuss regulatory networks in angiogenesis. Finally, with regard to the presentation of the most important angiostatic compounds, we elaborate on the possible strategies of angiogenesis targeting in inflammatory arthritis.

ANGIOGENIC MEDIATORS

Several inflammatory cytokines, chemokines, chemokine receptors, proteases, matrix molecules, growth factors, and cell adhesion molecules have been recognized in the neovascularization of the inflamed tissue [Table 1] [2,4,6,13-16]. In this section, we discuss mediators that are involved in the perpetuation and maintenance of the angiogenic process. Table 1 demonstrates some angiogenic mediators implicated in synovial angiogenesis and further details are discussed later.

Growth factors

The process of vessel formation starts with the release of growth factors (GF), primarily vascular endothelial growth factor (VEGF) and angiopoetin-2 (Ang2) that interact to control angiogenesis by inducing EC proliferation and sprouting. VEGF is the main regulator of new vessel formation. Several VEGF isoforms exist, with VEGF-A considered the major regulator

Figure 1. The process of inflammatory angiogenesis
In response to inflammatory stimuli, local macrophages and synovial fibroblasts produce proinflammatory cytokines and allow the expression of angiogenic mediators. This process leads to activation of signal transduction pathways regulating the degradation of extracellular matrix, endothelial cell migration, proliferation, adhesion, tube formation, vessel stabilisation, and pericyte recruitment

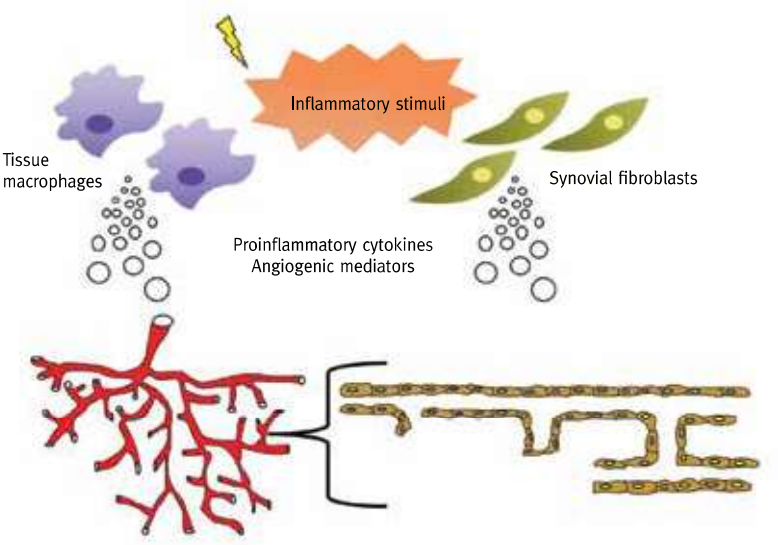


Table 1. Some angiogenic mediators implicated in inflammatory arthritis

Chemokines and their receptors	CXCL1, CXCL5, CXCL7, CXCL8, CXCL12, CXCL13, CXCL16, CCL2, CCL3, CCL5, CCL20, CCL21, CCL23, CCL28. CX3CL, CCR7, CXCR4, CXCR5, CCR10
Matrix molecules	Type I collagen, fibronectin, vitronectin, laminin, tenascin, proteoglycans, heparin, heparan sulphate, CD147, ADAM, ADAMTS
Cell adhesion molecules	β_1 and β_3 integrins, E-selectin, P-selectin, CD34, VCAM-1, endoglin, PECAM-1, VE-cadherin, Lev/H, MUC18, JAM
Growth factors	VEGF, Ang2, Tie2, Ang1, bFGF, aFGF, PDGF, EGF, IGF-I, HIF-1, TGF-β, HGF, MIF, PlGF, FGF-1. FGF-2, HGF, HB-EGF, KGF, CTGF
Cytokines	TNF-α, IL-6, IL-11, IL-15, IL-18, IL-17A, IL-11
Proteases	MMPs, plasminogen activators
Others	angiogenin, angiotropin, pleiotrophin, PAF, substance P, prolactin, SAA, ET-1, COX2, EPO, adenosin, histamin, thrombin, S1P, TLR, leptin, resistin

in angiogenesis. Ang2 acts as a partial agonist to the receptor tyrosine kinase-2 (Tie2) and by competing with angiopoetin-1 (Ang1) for Tie2 binding subsequently replaces the full agonist activity of Ang1 with a much weaker activity.

The relative balance of Ang2–Ang1 determines the activation state of Tie2 and Ang2, which eventually acts toward the inhibition of vessel maturation. As the vessel maturates, it becomes less VEGF-dependent and primarily Ang1. PDGF and TGF-β promote maturation [15,17,18]. Other regulators that act through either VEGF-dependent or independent pathways are interleukins (IL), such as IL-6, IL-17, IL-18, nitric-oxide (NO), endothelin-1, monocyte/macrophage migration inhibitory factor (MIF), placenta GF (PlGF), fibroblast GF (FGF-1 and FGF-2), epidermal GF (EGF), hepatocyte GF (HGF), heparin-binding endothelial growth factor (HB-EGF), keratinocyte GF (KGF), insulin-like GF (IGF-1), connective tissue GF (CTGF), platelet-derived GF (PDGF), and transforming GF-b (TGF-b) [2,3,15,19-21].

Pro-inflammatory cytokines

The main pro-angiogenic cytokines are tumor necrosis factor α (TNF-α), IL-1α and β, IL-6, IL-8, IL-15, IL-17, IL-18, G-CSF, GM-CSF, and oncostatin M. These cytokines have been implicated in synovial angiogenesis [2,3,19]. They may exert direct effect on the endothelium or act indirectly via promoting secretion of pro-angiogenic molecules from inflammatory cell types [3,14,16,19,20].

Chemokines and chemokine receptors

Chemokines are cytokines that induce leukocyte transmigration into the tissues, they also modulate inflammatory angiogenesis and many has been implicated from the CXC, CC, C, and CX3C families [13,22]. The pro-angiogenic nature of most CXC chemokines has been associated with the glutamyl-leukyl-arginyl (ELR) amino acid motif within their structure, and many CXC chemokines induce angiogenesis in rheumatoid arthritis (RA) [13,23]. ELR-containing CXC chemokines with angiogenic nature such as CXCL1, CXCL5, CXCL7, CXCL8, CXCL10, CXCL13, and CXCL16 have been frequently described in inflammatory synovial tissue [13,23]. Those CXC chemokines without ELR motif appear to be angiostatic in nature, with the exception of CXCL12, which exerts angiogenic effects when binding to its receptor CXCR4 [13]. The CXCL12/CXCR4 axis is fundamental in vasculogenesis as it attracts EPCs to line the newly formed blood vessels. The CXCL12/CXCR4 axis has also been implicated in RA [24].

Chemokines may also act indirectly to promote angiogenesis through the attraction of immune cells to the site of inflammation. The CC family includes chemoattractants, such as CCL2, CCL3, and CCL5, which are chemotactic for monocytes, lymphocytes, and macrophages. They further induce angiogenesis. CCL21 promotes neovascularisation indirectly via other pro-inflammatory cytokine secretions in synovial tissue, but CCL20 and CCL28 are also implicated in angiogenesis with an effect on B-cell and endothelial cell migration [13,22,25].

CX3C chemokines have three amino acids between two cysteine residues. CX3CL1 (fractalkine) has a role in leukocyte recruitment and can also promote vessel formation in inflammatory angiogenesis and atherosclerosis [26].

Extracellular matrix components, cell adhesion molecules, and matrix-degrading proteases

Among constituents of the synovial extracellular matrix (ECM), type I collagen, fibronectin, laminin, vitronectin, tenascin, and proteoglycans are ECM components that mediate, while thrombospondin 1 (TSP-1) is a glycoprotein that

inhibits EC adhesion and neovascularization. As described earlier, some growth factors bind to ECM proteoglycans during angiogenesis [2,3,9].

Among cellular adhesion molecules (CAM), most β_1 and β_3 integrins, E-selectin, the L-selectin ligand CD34, selectin-related glycoconjugates including Lewis^y/H and MUC18, intercellular adhesion molecule 1 (ICAM-1), ICAM-3, vascular CAM 1 (VCAM-1), platelet-endothelial CAM 1 (PECAM-1), endoglin, and junctional adhesion molecules (JAMs) are expressed on the EC surface and promote neovascularization [3,27,28]. Several CAMs are abundantly expressed in the synovium, with elevated serum and synovial fluid levels of various CAMs detected in RA [27-29]. The $\alpha_v\beta_3$ integrin has significant importance as this CAM is involved in osteoclast activation leading to erosions, as well as synovial neovascularization in RA [30]. Focal adhesion kinases (FAK) modulate $\alpha_v\beta_3$ integrin signaling. FAKs is expressed in the RA synovium, suggesting their role in synovial inflammatory angiogenesis [31]. Other angiogenic factors, such as chemokines, may also act via integrin-dependent pathways [13].

Matrix metalloproteinases (MMP), ADAM, and ADAMTS proteases digest the ECM, release growth factors and other angiogenic mediators and thus promote inflammatory angiogenesis [3,9].

Other mediators

Further important angiogenic factors implicated in inflammatory angiogenesis are serum amyloid A (SAA), endothelin 1 (ET-1), members of the cyclooxygenase-2 (COX-2)-prostaglandin E_2 network, angiogenin, angiotropin, pleiotrophin, platelet-activating factor (PAF), substance P, erythropoietin, adenosine, histamine, prolactin, thrombin, sphingosine-1-phosphate (S1P), toll-like receptors (TLR), and others [3,16,32].

Regulatory networks in angiogenesis

The hypoxia-HIF-VEGF-Ang/Tie system is of critical importance in inflammatory arthritis-associated angiogenesis. Hypoxia has been detected in the RA joint [11,12,33]. Hypoxia is defined as cellular demand for molecular oxygen that exceeds the vascular supply leading to a bioenergetic crisis.

Explanatory mechanisms for hypoxia in the RA synovium were postulated by three hypotheses:

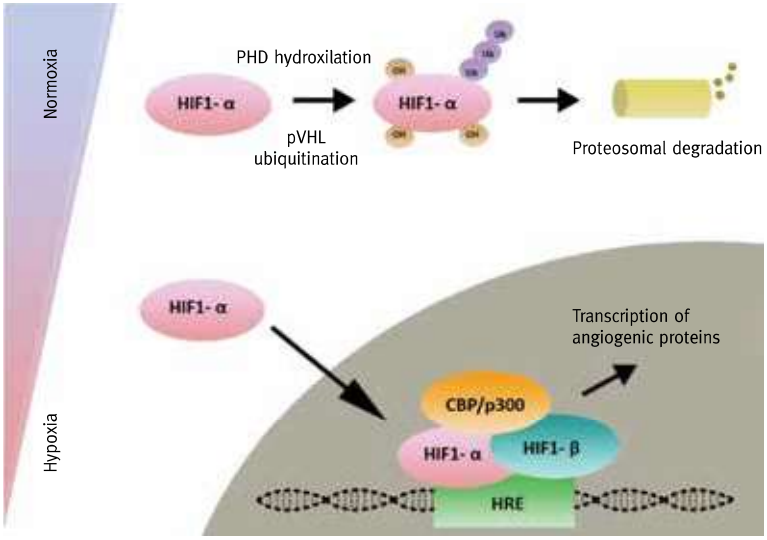
- Capillary closure is mediated due to synovial hyperplasia, synovial effusion, and joint movements within a rigid capsule
- Metabolic demand increases due to migration, proliferation, and distance increment between proliferating cells and nearby blood vessels
- Expression of angiotensin converting enzyme (ACE) induces the formation of angiotensin II, which is responsible for vasoconstriction and enhanced hypoxia [10,12]

The angiogenic neovascular network is dysfunctional in inflammatory arthritis and fails to restore tissue oxygen homeostasis, which leaves the inflamed synovial tissue and synovial fluid markedly hypoxic [10,12]. This result serves an angiogenic driver in the inflammatory tissue and has been associated with disease activity and increased expression of angiogenic VEGF.

Hypoxia enables the outgrowth of immature, unstable microvasculature. Angiogenic factors such as VEGF are induced by hypoxia and hypoxia-inducible factors (HIF-1 and HIF-2) in RA. HIF is a heterodimeric transcription factor and acts as a key regulator in the induction of the angiogenetic process. HIF is composed of HIF-α and HIF-β subunits. Among the three isoforms of HIF-α subunits, HIF-1α and HIF-2α share structural and functional similarities and in hypoxic conditions they are able to translocate to the nucleus and dimerize with HIF-1β. This heterodimer binds to hypoxia-response elements (HRE) enabling the transcription of HIF-dependent genes.

One of the most important target genes containing HREs is VEGF. Under normoxic conditions, prolyl-hydroxylases hydroxylate HIF-α later undergoes proteasomal degradation. Hypoxia is an inhibitor of HIF hydroxylation allowing HIF-1α to stabilize and dimerize with HIF-1β, thus initiating the transcription of genes containing HRE [Figure 2]. Both HIF-1α and HIF-2α are

Figure 2. Regulation of HIF1-α activity by normoxia and hypoxia
Under normoxic conditions HIF1-α is hydroxilated by prolyl-4-hydroxilase enzimes (PHD) followed by ubiquitination involving the von Hippel-Lindau protein (pVHL). This process targets the complex for further proteosomal degradation. Under hypoxia, with a lack of co-factors such as oxygen and iron, an attenuated degradation and subsequent stabilisation of HIF1-α is observed. HIF1-α is able to transfer into the nucleus and it dimerizes with HIF1-β. In the presence of co-activators, such as CBP/p300, it induces the transcription of hypoxia responsive element genes (HRE), which leads to the transcription of proteins (most importantly VEGF) implicated in angiogenesis in inflammatory arthritis



CBP = CREB1-binding protein, HIF1 = hypoxia-inducible factors, OH = hydroxylation, Ub = ubiquitin, VEGF = vascular endothelial growth factor

strongly expressed in the RA synovium. However, hypoxia may also act via HIF-independent regulatory pathways including the peroxisome-proliferator-activated receptors (PPAR), as well as NFκB-, Notch-, and JAK/STAT-mediated mechanisms. The Ang1/Tie2 complex interacts with VEGF during vessel stabilization in the neoangiogenesis. In contrast, Ang2, an antagonist of Ang1, inhibits vessel maturation. Both Ang1 and Tie2, as well as VEGF, have been detected in the RA synovium even in very early phases of the disease. Hypoxia also stimulates the production of CXCL12, a major angiogenic chemokine, by RA synovial fibroblasts [3,10,11,15,18,33].

TARGETING ANGIOGENIC MEDIATORS: ANGIOSTATIC COMPOUNDS AND STRATEGIES

Targeting the HIF-VEGF-Ang/Tie axis

The VEGF-dependent pathway we described has been extensively targeted. VEGF or VEGF receptor (VEGFR) inhibitors have been targeted mainly in cancer studies revolutionizing anti-angiogenesis therapies. Antibodies to VEGF or VEGFRs, as well as soluble VEGFR constructs, have been examined in malignancies. A limited number of pre-clinical studies has also been conducted in arthritis [15,17,34].

The most well-known VEGF inhibitor, the anti-VEGF-A antibody bevacizumab, has been approved for treatment in various forms of metastatic cancer including colorectal, lung, metastatic renal cell carcinoma, glioblastoma, and ovarian and breast malignancies. The anti-VEGFR2 tyrosine kinase inhibitor, ramucirumab, showed potent anti-tumor activity in clinical trials. It gained U.S. Food and Drug Administration (FDA) approval for the treatment of advanced gastric, gastro-esophageal cancer and non-small cell lung cancer. Other novel anti-angiogenic molecules, such as regorafenib (anti-VEGFR-2) and aflibercept (VEGF inhibitor), have been approved for metastatic colorectal cancers, gastrointestinal stromal tumors, and hepatocellular carcinoma treatment following promising results with several phase 3 randomized trials. Pazopanib, a multi-tyrosine kinase inhibitor of VEGFR and PDGFR α and β, was also effective in the amelioration of angiogenesis in vitro and in vivo in phase 2 clinical trials for renal cell carcinoma and other solid tumors. Pazopanib has been approved by the FDA for the treatment of advanced renal cell carcinoma and soft tissue sarcoma. These agents have not yet been tested in arthritis [15,17,34].

Angiogenesis is the formation of new blood vessels, which plays a major role in the development of arthritis

With respect to arthritis, vatalanib (PTK787) and an anti-VEGFR1 antibody-exerted significant angiostatic and antiarthritic effects in animal models of arthritis, whereas a soluble VEGFR1 chimeric protein also inhibited synovial endothelial proliferation in arthritic models. Soluble Fas ligand (sFasL, CD178) is a functional inhibitor of the 165-amino acid form of VEGF (VEGF165), and was shown to inhibit angiogenesis in arthritis. Studies in the collagen-induced arthritis (CIA) model revealed the significance of early VEGF inhibition in arthritis showing major reduction in disease severity with anti-VEGF sera treatment initiated before the onset of arthritis compared to treatment started after arthritis development. Peroxisome proliferator-activated receptor γ (PPARγ) ligands rosiglitazone and pioglitazone inhibited VEGF-induced angiogenesis. A soluble Tie2 receptor transcript delivered via an adenoviral vector to mice attenuated the incidence and severity of CIA. A bispecific antibody containing Ang2 targeting peptide genetically fused to adalimumab enhanced anti-TNF efficacy [15,17,18,34].

HIF-1 may also be targeted in arthritis, as well as in cancer. No advanced human clinical trials have been conducted yet. A pilot trial of the oral HIF-1α inhibitor topotecan significantly decreased histological HIF-1α and VEGF expression. A decrease in tumor blood flow and vessel permeability in vivo also assessed by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). The benzophenone analogue HIF-1α inhibitor, BP-1, ameliorated adjuvant-induced arthritis (AIA) in rats. The proteasome inhibitor bortezomib in combination with bevacizumab was investigated in a phase 1 clinical trial in advanced refractory malignancies and results suggested the inhibition of HIF-1 related angiogenic pathway. YC-1, a superoxide-sensitive stimulator of soluble guanylate cyclase and an HIF-1 inhibitor, may potentially be used to suppress inflammatory angiogenesis [10,14,34].

Indirect targeting of angiogenesis via conventional and targeted anti-rheumatic drugs

Antirheumatic agents in current use include rofecoxib, dexamethasone, chloroquine, sulfasalazine, methotrexate, azathioprine, cyclophosphamide, leflunomide, thalidomide, tacrolimus, minocycline. Anti-TNF and other biologics non-specifically suppress angiogenesis. For example, TNF-α blockade by infliximab was shown to reduce VEGF, Ang1, and Tie2 expression as well as vascularity within the RA synovium. Among non-TNF biologics, tocilizumab also decreased serum levels of VEGF in RA. The JAK inhibitor tofacitinib demonstrated inhibitory effects on migration, invasion, and pro-angiogenic cytokine secretion in psoriatic arthritis in vitro. IL-17 has been implicated in inflammatory angiogenesis; therefore, anti-IL-17 blockade may also be feasible in this respect [14,34].

Chemokine and chemokine receptor targeting

Direct or indirect approaches may aim to target chemokines and their receptors. Some nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, as well as traditional and biologic DMARDs, in addition to their other anti-inflammatory effects, inhibit chemokines and chemokine receptors. Briefly antioxidants, such as N-acetyl-L-cysteine and 2-oxothiazolidine-4-carboxylate, the bioflavonoid quercetin, the lipid-lowering

simvastatin, and green tea extracts (epigallocatechin gallate) inhibited the expression of various chemokines in vitro. PPARγ agonists, such as glitazones, and oriental medicines, such as triptolide, lingzhi, curcumin, tongbiling, honokiol, and cool-cool, also exert anti-arthritic effects, which may in part, be explained by chemokine and chemokine receptor inhibition. With respect to specific molecular targeting with neutralizing antibodies to various chemokine ligands, inhibition of arthritis both therapeutically and preventatively was observed in various RA animal models. Due to the complexity of the several regulatory loops in the chemokine network, most of these approaches failed in human RA. There has only been a very limited number of human anti-chemokine trials and therefore we do not aim to discuss those in detail. Without being exhaustive few examples are given from recent chemokine and chemokine receptor targeting research. An anti-CCL2 antibody, ABN912, was evaluated in a randomized, placebo-controlled study, but there was no detectable clinical benefit that would suggest that targeting of a single chemokine may not be effective in arthritis. Some CXCR antagonists have been used in animal models and show antitumor activity in human cancer. From CCR antagonists the ones inhibiting CCR1 (CP-481,715 and MLN3897), CCR2 (MLN1202) and CCR5 (AZD5672, SCH351125, maraviroc) reached the stage of phase 1 and 2 clinical trials in RA with disappointing results [13,19,22].

Other potentially angiostatic compounds

Among antibiotic derivatives, minocyclin, fumagillin analogues, deoxyspergualin, roxithromycin, and clarithromycin also inhibit angiogenesis and VEGF release. Synthetic fumagillin derivatives, TNP-470, and PPI-2458 inhibit VEGF and VEGF-dependent angiogenesis. Minocycline, roxithromycin, and clarithromycin exerted moderate but significant clinical effects [3,14,15,34].

The hypoxia-vascular endothelial growth factor-angiopoietin system plays a central role in inflammatory angiogenesis

Traditional herbal compounds have also been implicated in angiogenesis. Scopolin, a coumarin derivative found in Erycibe obtusifolia Benth; celastrol, an active ingredient of Tripterygium wilfordii, also known as "Thunder God Vine;" or fisetin, the active ingredient of Rhus verniciflua Stokes may also have angiostatic effects in arthritis. Resveratrol, curcumin and tetramethylpyrazine in combination also appeared to inhibit the production of angiogenic cytokines in a murine arthritis model [3, 14, 34].

INTRACELLULAR PATHWAYS AS POTENTIAL THERAPEUTIC TARGETS

Nuclear Factor-κB

One of the most recognized signal transduction pathways modulating inflammation is the nuclear factor-κB pathway. NF-κB signaling follows a canonical and a non-canonical route. Canonical NF-κB signaling is fast acting mainly via the actions of RelA/p50 DNA binding whereas the non-canonical pathway is slower and uses RelB/p52 heterodimerisation for gene transcription. Both pathways have potent pro-angiogenic potential in pathologies including cancer and autoimmune rheumatic diseases and they mediate the production of various pro-angiogenic molecules such as TNF-α, IL-1β, IL-6, CCL2 and IL-8/CXCL8, COX-2, iNOS, MMPs, and the adhesion molecules ICAM-1 and VCAM-1 [6,35,36].

Numerous compounds with reported inhibitory effects on NF-κB signaling have been developed, but larger clinical trials are lacking, possibly due to concerns about toxicity associated with global NF-κB inhibition or off-target effects. NF-κB targeting selective for a specific cell type could offer a solution. For example, a specific recombinant protein – sneaking ligand construct – has been developed that binds to cytokine-activated endothelial cells and appears to be effective in inflammatory states in vivo. Both the canonical and non-canonical pathways can be triggered by activation of LT β receptor (LTβ-R), CD40, the B cell activating factor (BAFF) receptor, and receptor activator of NF-κB (RANK). Direct inhibition of NIK kinases or IKKα homodimers of the non-canonical pathway may serve a potential therapeutic target. In RA synovial inflammation, NIK can be targeted using small molecule inhibitors. BAY32-5915, a specific IKKα inhibitor has been reported in melanoma treatment [6,35,36].

Mitogen-activated protein kinases

Three pathways are involved in this signaling mediated by the extracellular signaling kinases (ERK): the c-Jun N-terminal kinases (JNK) and the p38 mitogen activated kinases. All these kinases have been detected in RA synovial tissues with peak expression of ERK on synovial blood vessels. One transcription factor, known to be activated by the MAPK family of proteins, is Ets-1. Ets-1 promotes angiogenesis through the p38 MAPK in endothelial cells with downstream effects of VEGF synthesis. It also regulates the production of proangiogenic molecules (TNF-α and CCL2) by lymphocytes [6,34,35].

Targeting of all three kinases have been attempted. Small molecule p38 inhibitors appeared effective in animal studies, but showed lack of efficacy or failed due to toxicity in clinical trials, possibly due to the fact that p38 also exerts anti-inflammatory effects. Moreover, blocking one kinase may lead to the compensatory activation of others that regulating the same genes. Clinical trials using semapimod in inflammatory bowel disease were promising, and further studies reflected a mild beneficial effect in some patients. Blocking kinases that are located more upstream (such as MKK3/6) may serve as better therapeutic potential. Among ERK inhibitors, FR180204 significantly ameliorated arthritis in CIA mice. Examining non-competitive inhibitors of MEK1/2 (PD184352 and PD0325901) have also reached clinical trial stage as potential anticancer

agents. However, no clinical trials in chronic inflammatory diseases have been performed so far. The granzyme B gene silencing in the rat CIA model revealed suppressed activation of MAPK pathway by reduced phosphorylation of ERK and MEK. A direct inhibitor of JNK activity, SP600125 decreased paw swelling in the rat AIA model with associated inhibition of radiographic damage. Later this JNK inhibitor was replaced by more selective compounds. At present, several companies are developing such JNK inhibitors, but no data have yet been reported in arthritis or other inflammatory conditions [6,35].

Phospho inositide 3-kinase /Akt

The phosphoinositide 3-kinase (PI3K) pathway has also been implicated in regulation of cellular processes such as cell growth, survival, migration, proliferation, and differentiation. The PI3K family of kinases (known as protein kinase B) signals through Akt. Akt is essential for angiogenic processes both in physiological and pathological conditions and once activated can initiate the transcription of VEGF, NO synthase (eNOS), HIF-1α and HIF-2α, E-selectin modulating neovascularisation and chemotaxis [3,6,35].

Targeting angiogenesis by non-specific or specific compounds may attenuate arthritis

First generation broad spectrum PI3K inhibitors have entered preclinical studies mainly targeting cancer. Wortmannin and LY294002 have set safety concerns, but newer agents have been introduced into phase 1 clinical trials (BEZ235, BGT226, BKM120, XL765, XL147, GDC0941, GSK1059615, SF1126, PX-866, CAL-101) offering innovative targeted treatment for the future. Lately idelalisib has shown efficacy in the treatment of chronic lymphocytic leukemia and non-Hodgkin's lymphoma [6,35].

Inducible T-cell costimulator (ICOS) signaling

The costimulatory receptor ICOS facilitates the differentiation and function of follicular helper T cells and inflammatory T cells. ICOS has been shown to be a key co-stimulatory pathway, which controls induction and maintenance of murine CIA. Elevated levels of $ICOS^+$ T cells has been found in the synovial fluid of patients with RA [6,35].

Janus tyrosine kinases and signal transducers and activators of transcription pathways

Signaling by Janus tyrosine kinase (JAK) is a novel pathway that gained great interest in treatment of inflammatory arthritis. In the process of signaling throughout the activation of JAK subunits the cross-linked receptor recruits signal transducers and activators of transcription (STAT) proteins, which then become phosphorylated and are able to translocate to the nucleus to activate gene transcription. STATs are principle regulators of many pro-inflammatory genes including IL-17 and ICAM-1. In RA, expression of activated STAT proteins has been visualized through immunohistochemistry. Several activators of the JAK/STAT pathway have been implicated in angiogenesis such as IL-6 activating STAT1 and STAT3, or GM-CSF activating JAK2/STAT3 leading to neovascularization in the CAM assay. Hypoxia-STAT3 interactions are involved in inflammatory angiogenesis [6,33,35,37,38].

The JAK1/JAK3 inhibitor tofacitinib and the JAK1/JAK2 inhibitor baricitinib have been approved for the treatment of RA (both agents) and psoriatic arthritis (tofacitinib). JAK inhibition may result in the suppression of angiogenesis. The angiostatic effects of the JAK inhibitor AZD1480 have been demonstrated in malignancies [6,35,37,38].

Focal adhesion kinase

The FAK pathway is essential in physiological signaling and contributes to angiogenesis in chronic inflammatory diseases and cancer. Growth factors, integrin, and cytokine receptors can activate FAK and this pathway is very important in angiogenesis. FAK expression is known to be higher in patients with rheumatoid arthritis, osteoarthritis, and several types of cancer compared to healthy subjects. FAK deletion in mice injected with lung carcinoma cells leads to a significant decrease in angiogenic responses to VEGF and the EC isolated from these mice exhibit decreased proliferation and increased apoptosis [6,31,35].

Targeting FAK directly via FAK-silenced RNA or with combined PP2 inhibition with its downstream Src pathway showed some angiostatic effect. Phase 1 and 2 clinical trials using retroviruses expressing p53 indirectly into cancer cells (AD5CMV-p53) or direct FAK phosphorylation with small molecules (TAE226, PF-562,271, PF-04554878, GSK2256098) has been tried [3,6,34,35].

Src kinase

Src kinase signaling is involved in crucial biological processes leading to angiogenesis, such as cell cycle control, cell adhesion, migration and proliferation. Src kinases are activated by various growth factors and cytokines. VEGF and bFGF mediated activation is well recognized in ECs, however, it seems to be dispensable for bFGF-mediated cell growth. Due to the ability of cross-linking with various other pathways the Src family of kinases modulate several cellular processes. In combination with JNK activation Src plays a central role in IL-18-induced angiogenesis in RA. It also acts parallel with the PI3K pathway during soluble E-selectin-induced angiogenesis [6,27,35].

The proto-oncogen Src is another one of the multiple therapeutic targets of angiogenic pathways. Inhibition of the Src gene was investigated in many early phase clinical trials through targeting Src protein, a non-receptor tyrosine kinase from the Src family kinases (SFK). Its complex function partly remains unclear and limitations as a single agent make clinical applications challenging. Inhibition of Src kinases targeted through their small-molecule inhibitors has lately attracted

attention, mainly in cancer treatments. Dasatinib is the first FDA-approved prototype as a SFK/ABL dual inhibitor for the treatment of chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia. Later bosutinib and ponatinib both gained approval for leukemia and several other agents are in clinical trials. Periploca forrestii, a classical traditional Chinese medicine, also appeared to reduce paw swelling of rats in a CIA model with associated downregulation of Src activity [3,6,34,35,39].

Sphingosine kinase-1

Sphingosine-1-phosphate (S1P) and related pathological angiogenesis recently gained considerable interest. This bioactive lipid molecule has been shown to play a role in physiological processes such as cell growth, differentiation, migration, survival and angiogenesis. It is generated by the sphingosine kinase-1 (Spk-1), which has been implicated in the regulation of TNF-α-dependent release of IL-1β and IL-6. S1P promotes the persistence of activated $CD4^+$ T cells in inflamed sites hence perpetuating the inflammatory process. Synovial fluid levels of S1P were found to be higher in patients with RA as compared to osteoarthritis. Inhibiting the production of S1P leads to attenuated expression of TNF-α, IL-6, IL-1β, CCL2/MCP-1 and MMP-9 in RA mononuclear cells in vitro [6,32,35].

Safingol (l-threo-dihydrosphingosine), a putative inhibitor of Spk-1 entered phase 1 clinical trials and showed downregulation of S1P. A Spk-1 inhibitor, ginsenoside compound K (CK) has been studied in the amelioration of cell migration and proliferation and offers a new therapeutic target in cancer treatment. Data on an anti-S1P antibody also suggest that it has a role in the suppression of tumor angiogenesis and proliferation in mouse xenograft models *in vivo* [6,32,35].

Notch signaling

Notch signaling is a highly conserved pathway that has been implicated in EC interactions, proliferation, differentiation, and angiogenesis. Notch canonical ligands (Jagged 1 and 2 [JAG1, JAG2], and Delta-like 1, 3, and 4) binding to their Notch receptors (Notch 1-4) induce γ-secretase mediated cleavage and release of the intracellular receptor domain. The nuclear translocation of Notch activates target genes of Hes-1 and Hrt1 promoting the transcription of angiogenic proteins. High expression of Notch ligands and receptors was confirmed on the endothelium and actual tumor cells of different malignancies playing a role in vessel maturation signals [6,35,40].

Targeting the Notch pathway serves as a as a potential therapeutic target in inflammatory arthritis. However a previous phase 2 trial using RO4929097 compound demonstrated the side effects and safety in tumor angiogenesis inhibition in metastatic melanoma, probably due to inadequate therapeutic drug levels further investigations were not performed. Several other Notch inhibitors (MK-0752, BMS-906024, PF-03084014, LY900009) have been clinically evaluated mainly in the field of cancer therapy. Monoclonal antibodies against Notch receptor and ligand competitors, such as OMP-59R5 (tarextumab), OMP-21M18, A5226A are under development. The Notch receptor domain γ-secretase provides a promising target for receptor inhibitors and several products (z-Ile-Leu-CHO, MRK-003, PF-03084014, BMS-708163) have been investigated in the preclinical setting with anti-angiogenic or anti-proliferative effects and are currently in clinical trials in malignancies. Finally, nanoparticle delivery of Notch-1 siRNA significantly decreased paw swelling and arthritis scores in an animal study of RA highlighting that nanomedicine delivery of small molecules could be a promising future therapeutic approach [6,35,40].

CONCLUSIONS

In this review we discussed the putative role of angiogenesis in inflammatory arthritis. The impaired endothelial biology, the activation of numerous soluble or cell-bound angiogenic mediators, and the dysregulated angiogenesis all contribute to a defective, destructive, and highly active synovial inflammation and pannus formation in vivo. Except for the most relevant angiogenic mediators and intracellular pathways implicated in pathologic synovial angiogenesis, we highlighted current and future therapeutic strategies that may be promising in the treatment of inflammatory arthritis.

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Capsule

A new genomic blueprint of the human gut microbiota

The composition of the human gut microbiota is linked to health and disease, but knowledge of individual microbial species is needed to decipher their biological roles. Despite extensive culturing and sequencing efforts, the complete bacterial repertoire of the human gut microbiota remains undefined. **Almeida** and co-authors identified 1952 uncultured candidate bacterial species by reconstructing 92,143 metagenome-assembled genomes from 11,850 human gut microbiomes. These uncultured genomes substantially expand the known species repertoire of the collective human gut microbiota, with a 281% increase in phylogenetic diversity. Although the newly identified species are less prevalent in well-studied populations compared to reference isolate genomes, they improve classification of understudied African and South American samples by more than 200%. These candidate species encode hundreds of newly identified biosynthetic gene clusters and possess a distinctive functional capacity that might explain their elusive nature. This work expands the known diversity of uncultured gut bacteria, which provides unprecedented resolution for taxonomic and functional characterization of the intestinal microbiota.

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Eitan Israeli

"Bad weather always looks worse through a window"

Tom Lehrer (born 1928), American musician, singer-songwriter, satirist, and mathematician