Angiogenesis in joint disease: the need for clinical data

Rheumatoid arthritis (RA) is a widely studied inflammatory joint disease which impacts hugely on patients' quality of life. The challenge remains to improve on current treatments, such as TNF inhibitors, which despite their unquestionable clinical success, are associated with increased infection risk and are not effective in all patients. There are certain similarities between RA and solid tumors, such as the presence of inflammatory cells and cytokines, hypoxia, invasiveness and excessive cellular proliferation. Inhibition of VEGF has proved to be a huge step forward in the treatment of many cancers. This successful clinical development of angiogenesis inhibitors for other indications has prompted interest in whether a similar approach might be used for RA. This review discusses why angiogenesis is a feature of RA and the lessons learnt from disease models for future therapeutic approaches, particularly targeting VEGF.

KEYWORDS: angiogenesis ■ arthritis ■ blood vessel ■ VEGF

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Joint diseases such as arthritis (from Greek: *arthro*, joint and the suffix *-itis*, inflammation) are a group of disorders involving joints of the body and include rheumatoid arthritis (RA), osteoarthritis (OA) and related conditions such as lupus, ankylosing spondylitis, gout and psoriatic arthritis. RA, the primary focus of this review, is a chronic inflammatory disease of unknown etiology, affecting approximately 1% of the population worldwide and 1–3% of the population in the Western world. Patients present with painful, stiff and swollen joints, predominantly the small joints of the hands and wrists, as well as the metatarsophalangeal joints, ankles, knees and cervical spine. In most patients, symptoms appear over weeks to months, starting in one joint and often accompanied by prodromal symptoms including anorexia weakness, or fatigue. Peri-articular structures, such as bursae and tendon sheaths, are commonly inflamed. Approximately half of RA patients have tendon involvement, and dorsal wrist swelling due to tenosynovitis is often the first presentation of the disease. Proliferation of the synovial lining of tendons causes scarring and adhesion formation and 50% of patients with tendon disease will also show tenosynovial invasion into the tendon substance itself. This invasion is associated with multiple tendon ruptures and a poorer prognosis for long-term hand function. Direct surgical repair of ruptured tendons is impossible. Reconstruction depends upon the use of complex tendon transfers or grafts, representing a considerable investment of time and effort for patient and surgeon, and the majority of RA patients become clinically disabled within 20 years.

While not as headline grabbing as other diseases, such as breast or colorectal cancer, there is no doubt that RA causes a significant level of disability and distress. The financial impact is magnified by the high level of functional impairment RA causes. Up to 30% of people with RA become permanently work-disabled within 3 years of diagnosis if they do not have medical treatment [1]. For example, it was reported in a recent study that at the time of first symptoms of RA, 86% of men and 64% of women aged under 65 years were working. More than a third (37%) of these patients reported subsequent work disability, with the probabilities of continuing to work being 80 and 68% at 2 and 5 years, respectively [2]. Since RA patients of working age are more likely to stop work on health grounds than controls matched for age, gender and employment status at baseline, RA has a major impact on quality of life, hence making it a focus of considerable research in the field of joint diseases.

Pathogenesis of rheumatoid arthritis

It is generally thought that interplay between environmental and genetic factors, sex hormones and perhaps an infectious agent or other immune-activating factor, initiates an autoimmune pathogenic mechanism that culminates in a disease with inflammatory and destructive features [3]. In monozygotic twins, there is a 12–15% concordance for RA development,

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with 80% of Caucasians with RA expressing HLA-DR1 or HLA-DR4 subtypes [4]. This genetic predisposition has been additionally linked to environmental factors such as smoking and certain autoantibodies. For example, an association between smoking, HLA-DRB1 shared epitope alleles and antibodies to cyclic citrullinated peptides (CCP) has been demonstrated [5]. Furthermore, in anti-CCP-positive individuals, antibodies to the immunodominant citrullinated α-enolase epitope CEP-1 were detected in 43–63% of patients and this was linked to HLA-DRB1*04, suggesting that citrullinated α-enolase may be an autoantigen linking smoking to genetic risk factors in the development of RA [6].

Mortality rates in patients with RA are approximately 1.5-fold higher than in the general population, most probably due to the high frequency of cardiovascular disease, although infections and pulmonary and renal disease are also contributory factors [7,8]. Obesity is a recognized risk factor and it has been reported that having a body mass index of 30 or more is associated with an adjusted odds ratio of 3.74 for developing RA [9]. More than 40% of deaths in RA have been reported to be due to cardiovascular diseases, including ischemic heart disease and heart failure. The standardized mortality ratio for the RA cohort was 2.64 relative to the general population, compared with 0.98 for the control group [10]. A recent study reported that the odds ratio for the risk of all-category stroke in RA was 1.64, and 2.66 for the risk of ischemic stroke [11]. In another prospective cohort study, which comprised more than 100,000 women free of RA and cardiovascular disease at baseline, the adjusted relative risks of myocardial infarction and stroke in women subsequently diagnosed with RA were 2.00 and 1.48, respectively, when compared with women without RA [12]. A high 10-year risk of cardiovascular disease in newly diagnosed RA patients has been described, with the absolute cardiovascular risk in RA patients similar to that in non-RA subjects who were 5–10 years older [13]. Young adults with RA may have a particular propensity for increased cardiovascular disease, compared with individuals without RA. RA patients also have an increased risk of fatality following myocardial infarction (assessed as the 30-day mortality rates following a first acute cardiovascular event) [14]. A very recent study reported arterial stiffness to be strongly associated with endothelial dysfunction and overt atherosclerosis in patients with autoimmune diseases such as RA [15]. Furthermore, RA is associated with a range of other symptoms, including fatigue, anemia, weight loss and vasculitis, nodules in subcutaneous, pulmonary and sclera tissues and interstitial inflammation in lungs as well as in exocrine salivary and lachrymal tissue. A significant proportion of RA patients have tendon involvement, and dorsal wrist swelling due to tenosynovitis can be the first presentation of the disease. RA patients may also be at increased risk of dying of urogenital, gastrointestinal, respiratory infections and cancers [10], and depressive symptoms occur in many patients [16].

Development of cytokines as therapeutic targets

In RA, the synovial tissue that lines joints and tendons becomes inflamed. Normally one to two cell layers thick, the synovium in RA increases to a thickness of several cell layers, due to a combination of cellular hyperproliferation and infiltration by cells derived from the circulation. The cells infiltrating the synovium are predominantly CD4-positive T-cells, with high expression of memory CD45RO antigens and activation markers such as HLA-DR and CD69. The synovial fluid becomes rich in polymorphonuclear leukocytes and increases in volume due to edema, leading to joint swelling and pain. This synovium invades cartilage and bone and the progressive destruction of articular cartilage, subchondral bone and peri-articular soft tissues eventually results in functional deterioration and profound disability.

In the past, the traditional treatment of RA was represented by a pyramidal approach targeting the inflammatory and immune events underlying RA pathogenesis and starting with nonsteroidal anti-inflammatory drugs at the base of the pyramid, progressing to disease-modifying antirheumatic drugs such as gold, sulphasalazine and methotrexate (MTX). Despite these treatments, up to 90% of patients with aggressive synovitis exhibited radiological evidence of bone erosion within 2 years of diagnosis, despite treatment. However, over the last 20 years, major advances in the understanding of the pathogenesis of RA, based on bench-to-bedside studies of patients, human tissue and animal models of disease, have led to the identification of a number of new molecular targets for intervention. Specifically, the advent of biological therapies to target specific events in the disease process has been a major advance in the treatment of RA. The first of these targets was TNF-α, which mediates many inflammatory and immunoregulatory

activities relevant in RA, and a number of inhibitors of this cytokine have been approved for use in RA [17–19].

At the time of writing, five TNF-α inhibitors have been approved in the USA and Europe for treatment of RA. Infliximab, a chimeric mouse Fv, human IgG1κ anti-TNF-α antibody, was first approved for use with MTX for the treatment of patients with RA who had inadequate response to MTX alone. Infliximab has shown good therapeutic efficacy from the earliest clinical trials. For example, in a multicenter, placebo-controlled, randomized, double-blind trial, a single infusion of infliximab led to a marked improvement in signs and symptoms of RA [18]. In addition to infliximab, the dimeric soluble TNF receptor type II Fc fusion protein etanercept has been approved by regulatory authorities in the USA and Europe for treating RA, as well as psoriasis, ankylosing spondylitis, psoriatic arthritis and juvenile RA. The first fully human anti-TNF-α antibody approved for RA was adalimumab, created using phage display to produce an antibody with human-derived heavy and light chain variable regions and human lgG1κ constant regions. Recently, two new agents have been approved, namely golimumab and certolizumab. Anti-TNF antibody golimumab is fully human and is used to treat patients with moderate-to-severe RA, as well as active psoriatic arthritis and ankylosing spondylitis. A combination of golimumab plus MTX effectively reduced the signs and symptoms of RA and was generally well-tolerated in patients with an inadequate response to MTX [20,21]. Certolizumab, by comparison, is a Fab′ fragment of a humanized anti-TNF monoclonal antibody covalently linked to polyethylene glycol to prolong its plasma half-life [22].

The advent of anti-TNF-α biologicals has without doubt revolutionized the treatment of RA. However, increasing usage of anti-TNF-α biologicals has led to reports of infections, including tuberculosis [23–26], as a consequence of their mechanism of action in blocking the action of TNF-α. Although patients with RA are at increased risk of certain types of bacterial infection compared with the general population, it is nonetheless generally accepted that TNF inhibitors are associated with an approximately two- to four-fold increased risk of serious bacterial infections. The risk of nonserious infections is also increased, with the most common associated infections including urinary tract infections, pneumonia and other upper respiratory infections. There is therefore room for improvement and biological response modifiers acting on targets other than TNF-α have been trialed.

In the context of this review, angiogenesis has been postulated as a potential target for new therapeutic approaches in RA. Angiogenesis involves a succession of events controlled by receptors and signaling molecules, many of which are also important in vasculogenesis, with VEGF playing a crucial role. Endothelial cells normally form a tight barrier between the flowing blood and underlying tissue, as a consequence of interactions between extracellular matrix proteins and adhesion molecules. Such interactions need to be overcome prior to endothelial migration and new vessel formation. Sprouting angiogenesis thus involves retraction of pericytes from the abluminal surface of capillaries, protease secretion by activated endothelial cells to disrupt these cell–matrix interactions, degradation of the extracellular matrix surrounding the pre-existing vessels and then endothelial migration and proliferation towards the angiogenic stimulus. A provisional matrix is laid down, consisting of molecules such as fibrin and fibronectin. Cell adhesion molecules, such as integrins αvβ3 and αvβ5, mediate interactions of the endothelial cells in the newly formed sprout, as they adhere to one another and to the matrix. Subsequently, these endothelial sprouts differentiate into mature vessels, which become stabilized and surrounded by pericytes. Finally, lumen and capillary loop formation and stabilization of the mature vessels occur. Endothelial cells orientate themselves so that the lumenal surfaces are aligned, creating vessels that then branch to form the network of capillary loops. Nonsprouting angiogenesis or intussusception allows pre-existing capillaries to merge, or to increase in length and diameter, by fusion with additional endothelial cells.

Angiogenesis: a key role in RA

The vascular endothelial lining of blood vessels plays a key gatekeeper role in RA pathogenesis. Changes in venules and capillaries are thought to occur early on in RA and include acquisition of cuboidal morphology resembling that of high endothelial venules in lymphoid tissue. Microvascular and macrovascular involvement in RA includes altered permeability and vasoconstriction/vasodilatation, secretion of a host of cytokines, chemokines and inflammatory mediators, changes in matrix components and stimulation of angiogenesis/vasculogenesis. Considerable evidence has now converged to support a key role in RA for vascular endothelium. For example, documenting the responsiveness of endothelium to cytokines expressed in RA (e.g., TNF-α);

the presence on endothelial cells of receptors for these cytokines; and expression by endothelium of adhesion molecules and chemoattractants. All of these events are intimately interlinked and it is hard to distinguish between cause and effect. For example, the increase in synovial tissue mass, due to enhanced proliferation of synovial fibroblasts, is likely to result in local hypoxia and impaired perfusion, driving the need for a compensatory neovascularization, to increase the supply of nutrients and oxygen [27]. Hypoxia is a powerful stimulus for angiogenesis, through the induction of molecules such as VEGF, which is a potent stimulator of the angiogenic process. The increased vascular supply will provide an increased surface for leukocyte adhesion and extravasation. However, mediators that promote fibroblast proliferation are likely to be produced by infiltrating leukocytes and therefore it is difficult to ascertain whether infiltration precedes angiogenesis, *vice versa* or indeed whether several events are occurring synchronously.

Nonetheless, there is significant evidence that altered vascularity is a feature of RA. The synovium in RA is characterized by an abundance of blood vessels (of different sizes) both in areas of diffuse synovitis and in regions of large leukocytic infiltrates with germinal center-like structures [28]. Synovial fluids from RA patients have been shown to induce morphological changes in human vascular endothelial cells, with formation of tubule-like structures and induction of angiogenesis *in vitro* [29,30]. The number of synovial blood vessels has been found to correlate with synovial cell hyperplasia and indices of joint tenderness [31]. Endothelial cells lining blood vessels within RA synovium express cell cycle-associated antigens, such as PCNA and Ki67 [32], and endothelial proliferation was shown to be increased in synovium from patients with RA [33]. A recent study has documented the presence of immature blood vessels in rheumatoid synovium. Comparison of the staining patterns for CD31 and the pericyte/smooth muscle cell marker α-smooth muscle cell actin revealed a significant fraction of CD31-positive, but α-smooth muscle cell actin-negative, cells in RA tissue. The proportion of RA tissues with CD31-positive/α-smooth muscle cell actin-negative vessels was 80%, compared with just 21% OA tissue and none in the control tissue. In patients who responded to TNF inhibitors, these immature blood vessels were selectively depleted [34]. This intriguing observation suggests that, in RA, increased blood vessel formation may not necessarily result in improved perfusion/oxygenation and might explain the apparent paradox that while blood vessel density is increased, hypoxia is also a feature of RA. We have demonstrated that synovial tissue in RA patients is hypoxic, as demonstrated by direct measurements of O_2 in synovial tissue. The median O_2 in patients with RA was equivalent to 2–4%, compared with 9–12% patients without RA [35]. These findings complement historical data obtained more than 30 years ago, revealing synovial fluid hypoxia in RA patients [36,37]. A number of factors are believed to interplay to produce the hypoxic environment. The oxygen consumption of the RA synovium is elevated, quite likely as a consequence of the hyperplasia that characterizes RA [38]. A recent study assessed whether synovial proliferation differentially affects hypoxia in patients with different joint diseases. OA patients characterized by synovial proliferation (assessed by ultrasonography as visible synovial thickening and nodular or villous appearance) had higher synovial fluid O_2 levels than RA patients, suggesting that the proliferative response may impact differentially in RA and OA on synovial oxygenation [39]. These findings of an anaerobic and acidic microenvironment have been supported by magnetic resonance spectroscopy, confirming the presence of low-molecular-weight metabolites, consistent with hypoxia [38,40]. An inverse correlation between synovial oxygen tension and macroscopic synovitis, sub-lining $CD3^+$ cells and sub-lining $CD68^+$ cells, with resultant oxidative damage, has recently been described [41,42].

Finally, as has been briefly mentioned, there are a significant proportion of immature blood vessels in RA synovium, particularly in areas of low oxygen tension [43]. Therefore, perfusion might also be impaired in RA, compounding the hypoxic milieu. The regulators of the adaptive response to alterations in oxygen tension are members of the family of transcription factors termed hypoxia-inducible factors (HIFs), which are exquisitely sensitive to changes in oxygen tension [44]. Activation of HIF signaling leads to extensive changes in expression of genes involved in cell metabolism, changes in pH, angiogenesis (including VEGF) and apoptosis, which allow cells, tissues and organisms to adapt to reduced oxygenation. HIF-α isoforms (HIF-1α, HIF-2α) are expressed in human RA synovium [45–47] and also in experimental arthritis models [48], and correlate with indices of angiogenesis [49,50].

There is also ample evidence documenting expression of factors that would regulate angiogenesis in RA. These can be broadly categorized into growth factors, chemokines, cytokines and molecules involved in cell–cell and cell–matrix interactions. The best characterized growth factor expressed in RA is VEGF, a potent and relatively selective inducer of endothelial cell migration and proliferation [51,52]. The primary activity of VEGF is to promote proliferation of endothelial cells *in vitro* and to induce angiogenesis *in vivo* [53–55]. Many studies have also implicated VEGF in the protection of endothelial cells from apoptosis, acting as a survival factor through the induction of inhibitors of apoptosis, such as Bcl-2, XIAP and survivin [56]. VEGF also increases vascular permeability (hence the alternative name of 'vascular permeability factor') and this activity of VEGF could also be relevant to the joint edema, which is observed in RA [57]. VEGF expression in RA is upregulated by both cytokines and hypoxia/HIFs, thus making VEGF a pivotal regulator of angiogenesis in RA and a potential meeting point for inflammatory and oxygen-regulated signaling pathways [35,58,59]. In addition to synovial expression of VEGF, circulating (serum) levels of VEGF are increased and correlate with disease markers in RA such as C-reactive protein [59–62]. Treatment of RA with TNF-α inhibitors (alone or with MTX), disease-modifying anti-rheumatic drug or anti-IL-6 receptor antibody significantly reduced serum VEGF concentrations [59,63–67]. The biological significance of VEGF expression in RA synovium was confirmed by the observations that VEGFR1 and VEGFR2 mRNA were expressed by RA synovial microvascular endothelial cells of nearby blood vessels [52], and that conditioned medium from synovial tissue explants promotes angiogenesis [51,68]. Recently, a correlation between serum VEGF and synovial blood flow has been reported [69,70].

In addition to VEGF, other growth factors are also expressed in RA, such as EGF and EGFR such as HER-2/ErbB2 [71,72]. Other EGFR ligands have also been detected, namely TGF-α and amphiregulin [73,74]. Additional growth factors expressed in RA include FGF-1 and FGF-2 [75,76], PDGF [77,78] and HGF [79]. The potential role of TGFβ during the course of RA and in synovial angiogenesis is unclear. TGFβ was shown to induce VEGF expression in human synovial fibroblasts [80]. Indeed, TGFβ is by far the most powerful inducer of VEGF secretion by human synovial fibroblasts, when compared with other cytokines associated with the pathogenesis of RA, such as IL-1 or PDGF. Thus, it seems likely that in RA, TGFβ exerts its angiogenic effects predominantly through the induction of VEGF secretion by fibroblasts. Expression of angiopoietins (Ang)-1 and Ang-2 [81,82], and Ang receptors Tie-1 and Tie-2 [83–85] in RA synovial tissue has been described. Numerous chemokines and chemokines receptors, which have been implicated in angiogenesis, are also expressed in RA. These include stromal cell-derived factor (SDF)-1/CXCL12, IL-8/CXCL8, ENA-78/CXCL5 and MCP-1/CCL2, as well as their cognate receptors, such as CCR2 and CXCR4.

While the driving force behind the altered synovial vascularity in RA is generally thought to be enhanced angiogenesis, vasculogenesis may also play a role. Angiogenesis, the process of formation of new blood vessels from pre-existing vessels, differs from vasculogenesis, namely the coalescence of endothelial cells, which was originally described in the embryo as underlying formation of the primordial vascular. Vasculogenesis has now been shown to be recapitulated in adults, with endothelial progenitor cells incorporating into sites of angiogenesis *in vivo* [86]. In patients with active RA, circulating endothelial progenitor cell counts are lower than in individuals with inactive disease or healthy controls. Moreover, treatment of patients with TNF-α inhibitors restores circulating endothelial progenitor cell levels to those seen in healthy control subjects [87]. VEGF is also a key regulator of vasculogenesis and markers characteristic of endothelial progenitor cells, namely CD34 and VEGFR2, have been found in the vicinity of RA synovial blood vessels [88]. Expansion of bone marrow-derived CD34-positive cells into CD31- and von Willebrand factor-expressing cells occurs at a higher rate from bone marrow samples taken from RA patients, compared with normal subjects [89]. Endothelial progenitor cells are a potential link to the increased risk of cardiovascular disease in RA, since administration of these cells into models of hind limb ischemia resulted in incorporation into capillary vessel wall in the revascularized hind limb [86]. Both VEGF and SDF-1 promote endothelial progenitor cell mobilization, thus playing roles in both angiogenesis and vasculogenesis. The inference is that reduced circulating endothelial progenitor cells in patients with active RA would result in a poorer response to ischemia and thereby cardiovascular events such as stroke or myocardial infarction. Concomitantly, increased endothelial progenitor cells in the RA synovium would promote further synovial blood vessel formation.

Finally, there is evidence that angiogenesis may be a feature of other joint diseases, such as OA. Expression of VEGF and HIFs has been reported, together with chemokines, HGF and members of the Ang–Tie family [90–93]. An interesting study by Veale *et al.* reported that expression of Ang-2 and VEGF was higher in synovium of patients with psoriatic arthritis, relative to RA, whereas Ang-1 levels were more comparable. Psoriatic arthritis and RA exhibited different features in terms of vascular morphology, in that blood vessels in psoriatic synovium were highly tortuous in appearance, compared with the straight and branching vessels seen in RA, suggesting that the balance between Ang-1, Ang-2 and VEGF may affect vessel growth and maturation in arthritic synovium [28].

Therefore, there is considerable evidence demonstrating the expression of growth factors such as VEGF, members of the Ang family and other growth-promoting cytokines and factors in inflammatory joint disease. As briefly alluded to, the question is frequently asked as to whether angiogenesis drives the inflammatory events, which ultimately culminate in synovial inflammation and invasion, or whether blood vessel formation is a response to hypoxia and hypoperfusion as a consequence of inflammation. At present it is difficult to speculate on this, in particular because clinical specimens always show evidence of both angiogenesis and inflammation. The presence of immature blood vessels in inflamed synovium has recently been shown [34,43], perhaps suggesting that angiogenesis (or specifically, an inappropriate angiogenic response, leading to immature vessels with impaired functionality) is a response to the production of growth factors, such as VEGF, by infiltrating monocyte/macrophages. However, the use of angiogenesis inhibitors in disease-relevant models of joint disease, coupled ideally with inhibitors of inflammation, such as anti-TNF-α antibody, could provide some pointers as to the sequence of events that underlies RA. The current state-of-the-art regarding angiogenesis blockade *in vivo* is reviewed in the next section.

Angiogenesis inhibition in rheumatoid arthritis

While TNF-α blockade has been a success and a therapeutic benchmark in RA, the increased rate of infection, coupled with the lack of responsiveness in some patients, has driven the search for alternative therapeutic targets. The central role of angiogenesis in RA suggests that suppression of blood vessel formation should slow arthritis progression. Therapeutic agents and strategies are being devised to either interrupt or inhibit one or more of the pathogenic steps involved in angiogenesis.

Angiogenesis can be targeted at several different stages, including inhibition of production of stimuli such as VEGF, binding of pro-angiogenic factors (using antibodies or soluble receptors), interruption of downstream signaling, blockade of matrix degradation or even using anti-angiogenic stimuli. In particular, inhibition of VEGF/VEGFR has been a popular approach. In our laboratory, we have used murine collagen-induced arthritis (CIA), a model widely used for the testing of potential therapeutics for RA, such as TNF-α inhibitors. We observed that blockade of VEGF using soluble VEGFR1 significantly suppressed established arthritis [94–96]. The effectiveness of VEGF blockade was subsequently confirmed using anti-VEGF antibody [97,98]. A different strategy to limit angiogenesis via the VEGF pathway was to directly target VEGFR. In a spontaneous model of arthritis in KRN/NOD mice, de Bandt *et al.* observed that treatment with anti-VEGFR1 (but not anti-VEGFR2) antibody abrogated bone and cartilage destruction. The antibody delayed the onset of arthritis and attenuated the severity of disease [99]. The group of Carmeliet *et al.* also compared different approaches targeting VEGF (using anti-VEGFR1 and anti-VEGFR2 antibodies) in CIA. Treatment of mice with anti-VEGFR1 reduced the incidence of joint disease, whereas anti-VEGFR2 appeared ineffective [100]. More recently, neuropilin (NRP)-1 has been targeted. The semaphorin receptors NRP-1 and NRP-2 modulate VEGF signaling through the two receptor tyrosine kinases, VEGFR1 and VEGFR2. In particular, NRP-1 has been shown to bind the VEGF isoform $VEGF_{165}$, thereby enhancing VEGFR2-mediated signal transduction. Indeed for RA cells, NRP-1 has been suggested to be the primary binding site for $VEGF_{165}$, the major human VEGF splice variant [101]. Use of a truncated $VEGF_{165}$ peptide, which specifically blocks binding of native $VEGF_{165}$ to NRP-1, suppressed arthritis severity *in vivo* [102]. In addition to VEGF, expression of Ang-1, Ang-2 and cognate Tie receptors has been described in RA. Blocking Tie-2 has been shown to be effective in CIA [103], and we have recently reported significant findings using Tie-1 inhibition [96,104], suggesting that blockade of the Ang–Tie axis could be beneficial.

An additional approach to suppress angiogenesis in arthritis is to use physiologically occurring angiostatic mediators, such as angiostatin, which has been shown to be effective in murine arthritis [105]. We have assessed the effect in CIA

of protease-activated kringles 1–5 (K1–5), an angiogenesis inhibitor that is related to angiostatin. Treatment with K1–5 reduced paw swelling and clinical score compared with untreated animals, and histological assessment of arthritic paws revealed a reduction in joint inflammation and destruction [106]. Treatment of arthritis in rodents with broadly acting angiogenesis inhibitors, such as with AGM-1470 or Taxol (which disrupts microtubule formation) prevented disease, significantly suppressed established arthritis and inhibited neovascularization [107,108]. TNP-470 (AGM-1470) is a synthetic analog of fumagillin, which selectively and irreversibly inhibits the enzyme methionine aminopeptidase type 2 isoform (MetAP-2), involved in protein maturation. An irreversible inhibitor of MetAP-2, PPI-2458, not only inhibited angiogenesis, but also showed significant *in vivo* activity in rodent models of arthritis, reducing established disease [109,110]. Interestingly, a recent study applied a rat model, in which injection of carrageenan with FGF-2 induced persistent synovitis together with inflammation, which was prevented with PPI-2458. This study hypothesized that angiogenesis was important in the transition from acute to chronic inflammation, as is seen in RA, and that inhibition of angiogenesis during the acute phase of synovitis might therefore prevent progression to chronic disease [111].

Future perspective: the need for clinical data

There is a substantial body of evidence documenting the importance of angiogenesis in RA, from seminal studies showing altered synovial vessels density, reports of the expression of angiogenic mediators such as VEGF, to studies demonstrating the effectiveness of VEGF blockade in models of disease. Just in the past 2 years, a significant number of publications have suggested that angiogenesis could be an interesting target for therapy in joint diseases such as RA or OA [50,112–118]. However, the benchmark set by TNF-α inhibitors and subsequent biological therapies, such as rituximab (anti-CD20), anakinra (IL-1 receptor antagonist), abatacept (CTLA4-Ig) or tocilizumab (anti-IL-6 receptor antibody), has apparently discouraged the translation of angiogenesis inhibitors into clinical trials in RA.

This is despite the fact that angiogenesis inhibitors have been approved for clinical use for a number of years. The most successful anti-angiogenic approach is monoclonal anti-VEGF antibody bevacizumab, approved in 2004 as a combination with intravenous 5-fluorouracil-based chemotherapy for patients with previously untreated metastatic cancer of the colon or rectum. Bevacizumab is now also approved in combination with carboplatin and paclitaxel for first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic nonsquamous, non-small cell lung cancer, as well as in combination with paclitaxel for treatment of metastatic HER2-negative breast cancer, progressive glioblastoma following prior therapy and metastatic renal cell carcinoma. Furthermore, ranibizumab, a Fab′ fragment derived from the same parent murine antibody as bevacizumab, is approved for another angiogenesis-dependent disease, age-related macular degeneration, as is the VEGF aptamer pegaptanib sodium. Moreover, targeting such separate mechanistic pathways (i.e., inflammation and angiogenesis) could be beneficial, unlike the combination of targeting IL-1 and TNF-α, which was shown to be pro-infective. A study of combination therapy with etanercept and anakinra in RA patients who had been treated unsuccessfully with MTX demonstrated an increased incidence of serious infections (0% for etanercept alone, 3.7–7.4% for the combination therapy depending on the dose of etanercept) [119]. By contrast, targeting the TNF pathway together with angiogenesis inhibitors should not result in increased infection rates. However, this hypothesis requires rigorous testing in appropriate preclinical models, such as CIA.

The angiogenesis inhibitor to be used is unknown at present. While VEGF is an attractive target, side-effects may complicate this approach. The cautious approach to the use of angiogenesis inhibitors in RA may result from the observation that side-effects of therapies such as bevacizumab include increased risk of thromboembolic events and RA is associated with increased frequency of cardiovascular disease. However, it is to be hoped that a clinical trial combing angiogenesis and TNF-α blockade will be initiated in the not-too-distant future, and provide the scientific and clinical community with the long-awaited answer concerning this possible therapeutic approach for inflammatory joint disease.

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Executive summary

- Rheumatoid arthritis (RA) is driven by inflammation and activation of the immune response.
- Formation of blood vessels contributes to the development and maintenance of inflammatory joint diseases, including RA.
- Numerous growth factor are expressed in RA, including VEGF.
- The approval of VEGF inhibitors, such as anti-VEGF monoclonal antibody, for other indications, such as colorectal cancer, has led to suggestions that VEGF blockade may also be beneficial in RA.

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