

Antiangiogenesis for Rheumatoid Arthritis

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Abstract: Angiogenesis, i.e., the induction of new blood vessels from existing vasculature, is a crucial event in the formation and maintenance of the pannus in rheumatoid arthritis (RA). The arthritis is characterized by destruction of peripheral joints in which the cartilage and bone are destroyed by proliferative synovitis. This is characterized by infiltration of inflammatory cells and formation of new blood vessels. Angiogenesis occurs since the early stage of the disease, and supports progression of the arthritis. It has been demonstrated in animal models of arthritis that inhibition of angiogenesis reduces of the arthritis. This suggests that pharmacological inhibition of angiogenesis may play an important role in the treatment of RA. In particular, disruption of new blood vessels can not only prevent delivery of nutrients to the inflammatory site, but can also lead to vessel regression, hence reversal of disease.

To sum up, since angiogenesis is central in maintaining of synovitis in RA, antiangiogenesis probably represents a therapeutic tool. This view is supported by recent studies in animal models of arthritis where antiangiogenic drugs deliver a therapeutic benefit.

Keywords: Angiogenesis; rheumatoid arthritis; antiangiogenesis.

ANGIOGENESIS IN RA

Since 1982 it has been suggested that the metabolic need of RA synovial tissue is increased and may initiate tissue injury via anoxia and acidosis, and subsequent release of hydrolytic enzymes, enhancement of vascular permeability, and acceleration of inflammation [1].

Also, the synovial tissue displays an increase in the density of blood vessels and alterations in endothelial cell proliferative responses: the more hyperplasia of synovial cells and infiltration of mononuclear cells the higher vessel density and endothelial cell proliferation [2]. A morphometric study showed that capillaries localize more deeply in RA than normal synovium, although the blood-volume fraction is greater in normal than in RA knees [3]. Although perivascular mononuclear cell infiltration and thickness of synovium were increased in both inflamed and noninflamed joints, vascular proliferation occurred only in tissues from inflamed joints [4]. Endothelial cells of these proliferating vessels were shown to express cell-cycle-associated antigens, such as PCNA and Ki67, and integrin $\alpha v \beta 3$, which is actually associated with vascular proliferation [5,6]. Indices of endothelial cell proliferation and death were shown to be higher in the synovium from RA patients than in patients with osteoarthritis (OA) and healthy subjects [6]. Overall data suggested that in RA synovium a rapid endothelial proliferation takes place, and led to hypothesize that angiogenesis may contribute to the disease maintenance.

A wide array of growth factors, cytokines, and chemokines governs angiogenesis in the RA synovium: fibroblast growth factors (FGFs), such as FGF-1 (acidic FGF) and FGF-2 (basic FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), IL-1, IL-8, TGF- α , and TNF- α . All these factors induce proliferation, migration, and differentiation of macrophages, lining synovial cells, and endothelial cells. Platelet-derived growth factor (PDGF), which is a potent mitogen for many cell types, including fibroblasts and smooth muscle cells, is strongly expressed in RA synovium. Hepatocyte growth factor/scatter factor (HGF/SC) is also highly expressed in the RA synovium and synovial fluids [7,8]. It promotes migration and proliferation of endothelial cells.

Other potential angiogenic stimuli expressed in RA include some soluble adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and E-selectin [9], that are able to induce endothelial cell chemotaxis. Serum levels of both molecules are elevated in the RA patients, and chemotactic activity of RA synovial fluid is blocked by antibodies to these molecules. Among the different angiogenic factors, special emphasis has been focused on FGF-2, VEGF, angiopoietin and fraktalkine.

ROLE OF FGF-2

FGF-2 belongs to heparin-binding growth factors, and shows specifically enhanced expression in RA synovial fluid as compared to OA

and healthy subjects [10]. It stimulates angiogenesis directly and by up-regulating VEGF [11]. It induces osteoclastogenesis in murine bone marrow cultures and accelerates osteoclast differentiation [12-15], thus contributing to bone resorption in RA. FGF-2 levels increase in rat arthritis induced by adjuvant administration, and in RA joint fluids [16]. Also FGF-2 gene transfer enhances VEGF expression in synovial extracts from the rat arthritis, and administration of anti-FGF-2-neutralizing Ab lowers disease severity, suggesting that inhibition of FGF-2 is a plausible therapeutic approach.

ROLE OF VEGF

A great deal of evidence suggests that VEGF plays an important role in the pathogenesis of RA. In RA patients, VEGF is produced by macrophages, fibroblasts surrounding microvessels, vascular smooth muscle cells, synovial lining cells [17], neutrophils of synovial fluid [18], and peripheral blood mononuclear cells [19]. Its levels are significantly higher in synovial fluids from RA as compared with OA [20-22]. In addition, serum VEGF levels of RA patients correlate with scores of disease activity and signs of radiologic progression [23]. In early RA, serum VEGF at presentation correlates significantly with the magnitude of radiologic deterioration of hands and feet within the first year. This suggests that high serum VEGF levels at an early stage may predict the size of subsequent damage of joints.

The mechanism of VEGF production in RA is not completely known. VEGF is up-regulated by hypoxia, as shown in dissociated cells from the synovial membrane (Fig. 1) [24]. It was selectively up-regulated following 24-h hypoxia at variance with IL-1 β and IL-8 which were unaffected. In patients undergoing knee arthroscopy, the synovial pO₂ levels were significantly lower during active RA compared to nonactive disease or OA, and release of VEGF from synovial cells was greater, suggesting that reduced intra-articular pO₂ triggers for local VEGF production [25].

Tissue hypoxia in the RA joint results in increased VEGF mRNA stability [26], and enhances VEGF gene transcription through the binding of hypoxia-inducible transcriptional factors, such as hypoxia-inducible factor-1 (HIF-1) and HIF-2. Both of these factors are degraded within minutes from exposure to an oxygen tension >3-5%, but are stabilised under conditions of hypoxia (<3% oxygen), then translocated to the nucleus, where they bind to hypoxia-responsive elements of hypoxia-inducible genes to upregulate their expression [27]. Thus, it may be well that the hypoxic environment in the RA joint which is compounded of high metabolic demands for synovial inflammation promotes transcriptional allowing permissive to perpetuation of synovitis.

ROLE OF ANGIOPOIETINS

It is generally accepted that there is not a single event that increases synovial microvasculature: angiopoietins (Angs) actually add to other growth factors involved in the RA-associated angiogenesis. Angs together with their receptors (Tie-1 and Tie-2) play an important role in the development of neovasculature, and have been implicated in the control of vessel stabilisation and regression. Ang-1 acts as stabilizer of new vessels elicited by VEGF, while Ang-2 destabilises these vessels, leading to new

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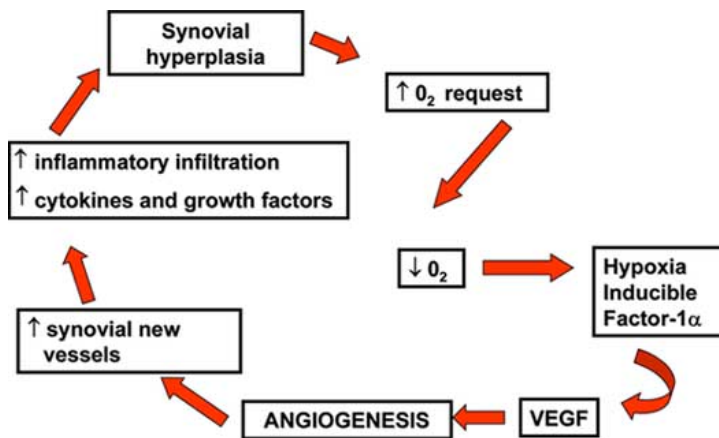


Fig. (1). Hypoxia as an inducer of VEGF in RA.

vessel sprouts in the presence of VEGF, or to vessel regression in the absence of VEGF. Detectable levels of Ang-1 mRNA and its receptors have been demonstrated in the RA synovial tissue. The expression of Ang-1 and its receptor was significantly higher than in tissues from OA patients and healthy controls [28].

FRAKTALKINE

It is well established the proinflammatory role of fractalkine (CX3CL1) in RA. It is the sole member of the so-called CX3C chemokines, and favours monocyte chemotaxis and angiogenesis in the RA synovium. Blaschke and co-workers analyzed 17 patients for the expression of fractalkine within different circulating T-cell subsets by flow cytometry, and expression of its receptor CX3CR1 within the RA synovium by immunohistochemistry and laser capture microdissection microscopy [29]. T cell subsets revealed a low proportion of fractalkine-expressing CD4+ and CD8+ T cells in both RA patients and controls. In RA, fractalkine was predominantly expressed by CD4+ T cells with a Th1-type cytokine expression profile, and was detected in synovial macrophages, dendritic cells, endothelial cells, and a small proportion of T cells. The expression of CX3CR1 was shown in synovial macrophages, dendritic cells, and T cells, as well as in fibroblasts. Also, stimulation of cultured synovial fibroblasts with fractalkine resulted in a marked up-regulation of matrix metalloproteinase-2 (MMP-2) production. Overall data support the hypothesis that fractalkine has a proinflammatory role in RA.

ANGIOGENESIS BLOCKADE IN ANIMAL MODELS OF ARTHRITIS

A number of broadly-acting angiogenesis inhibitors are able to modulate RA in animal models (Fig. 2). TNP-470 and thalidomide have been shown to efficiently inhibit pannus formation and neovascularisation [30,31].

In vivo administration of TNP-470 suppresses arthritis and protects from bone destruction. Assessment of clinical and histologic features of

angiogenesis inhibition in a transgenic mouse model that closely mimics RA showed that TNP-470 delays the appearance of clinical signs of arthritis, or even abolishes cartilage and bone destruction [32].

Studies of thalidomide in a rat model of immune-mediated inflammation demonstrated little or no suppression of disease activity [31]. However, the thalidomide analogue CC1069 efficiently inhibited production of both TNF-α and IL-2 by rat cells *in vitro* and suppressed disease development [33]. Actually, TNF-α production plays a central role in induction of angiogenesis and inflammation of RA [34].

The mechanism of action of CC1069 differs from that of thalidomide. It inhibits phosphodiesterase-4 (PDE-4), a property not shared with thalidomide [35]. Elevation of intracellular cAMP levels by PDE-4 inhibition resulted in suppression of inflammatory responses, especially the TNF-α production by monocytes [36]. *In vitro* studies have also shown that PDE-4 inhibition reduces the IL-2 synthesis [37].

ANTIANGIOGENIC THERAPIES IN RA

Inhibition of angiogenesis has been proposed as a new therapeutic option in RA and different studies on angiogenesis inhibitors have focused on changes in vascular density [7,38,39].

Antirheumatic drugs (DMARDs) such as methotrexate (MTX) [40], sulphasalazine (SASP), and penicillamine have been shown to inhibit angiogenesis in experimental systems. Combinations of these drugs affect production of VEGF by synovial cells *in vitro*. Bucillamine (BUC) and gold sodium thiomalate (GST) inhibit VEGF production, in much the same way as a combination of BUC, GST, and MTX with dexamethasone (DEX) [41]. Others demonstrated the importance of infliximab, cyclosporin and endostatin.

DMARDs

MTX inhibits both basal and stimulated endothelial cell proliferation *in vivo* [42]. DMARDs have been used to control the RA progression. Most of them act as immunomodulatory drugs [42,43], the remaining do this

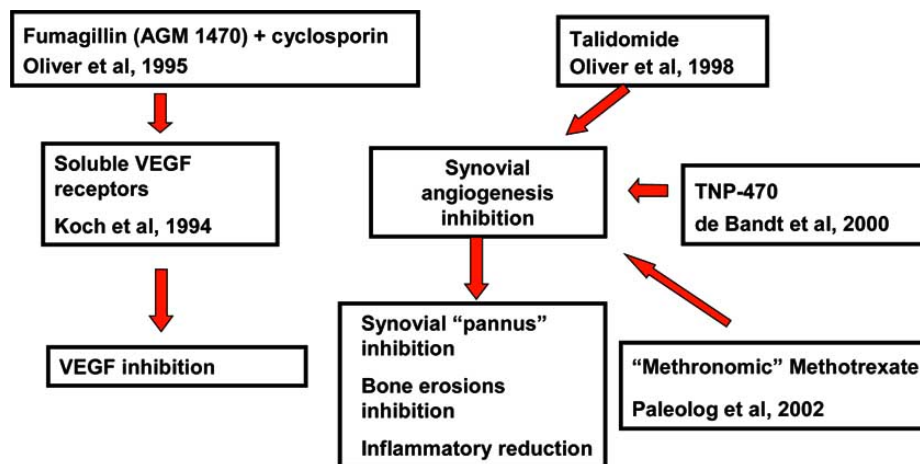


Fig. (2). Antiangiogenic therapy in RA (murine models).

together with inhibition of cytokines and endothelial cell proliferation [40,44,45]. Various combinations of BUC, GST, MTX, SASP and DEX inhibited the VEGF and bFGF production in cultured synovocytes. However, BUC and GST gave the inhibitory effect even when given alone. BUC and GST act as inhibitors because of their suppressive effect on the transcription of VEGF mRNA. The combinations of BUC, GST, SASP, MTX and DEX, two by two, except the combination MTX plus SASP, also have inhibitory effects. These *in vitro* results were supported by similar observations in the peripheral blood of RA patients.

Infliximab

Since the earliest trials in 1992 Infliximab (anti-TNF- α MoAb) has shown remarkable therapeutic efficacy, due to reduction of both clinical and laboratory indices of disease activity [46,47]. TNF- α has been reported to induce release of VEGF [48]. To test this hypothesis, serum VEGF levels were measured in RA patients treated with Infliximab. Patients receiving 10 mg Infliximab per kg body weight gave more than 40% reduction in VEGF levels which persisted even 4 weeks. In addition, the treatment with multiple infusions of Infliximab and MTX resulted in a more prolonged decrease in VEGF levels than in patients treated with Infliximab alone. Infliximab alone reduced the VEGF levels but these rapidly recovered to initial values after the final infusion. In contrast, the Infliximab-MTX treatment gave reduced VEGF up to the end of the trial [49]. Data suggested that TNF- α triggers angiogenic VEGF *in vivo*, and that the beneficial effect of Infliximab may be attributable to a down-modulation of VEGF and RA-associated angiogenesis.

In another study, patients with active RA received Infliximab 10 mg per kg body weight as a single dose [50]. This treatment reduced the synovial vascularity, as assessed by immunostaining with endothelial cell markers, such as CD31 and factor VIII-related antigen (FVIII-RA). A significant reduction in the number of $\alpha_v\beta_3$ -integrin-positive vessels was also found. The reduced expression of CD31, FVIII-RA, and $\alpha_v\beta_3$ integrin after TNF- α blockade agrees with the hypothesis that the balance of new vessel growth and regression leaned towards regression. Since the endothelial surface plays a key role in mediating cell traffic and delivery of nutrients, the vessel regression was thought to deliver therapeutic efficacy.

Cyclosporin-A (Cy-A)

We showed that Cy-A inhibits angiogenesis and reduces inflammatory cells in the chick embryo chorioallantoic membrane (CAM) implanted with RA synovium [51]. The Cy-A antiangiogenic activity was due to inhibition of certain functions of cultured endothelial cells needed for angiogenesis to develop, namely cell proliferation, chemotaxis, morphogenesis on Matrigel, secretion of MMP-2, as well as of angiogenesis in the *in vivo* CAM. Furthermore, Cy-A inhibited chemotaxis and proliferation of mononuclear cells and antigen/mitogen-induced secretion of lymphokines, such as IL-2, IL-6, IL-10, TNF- α , transforming growth factor- β (TGF- β) and IFN- γ by clonal T cells in inflammation sites [35,36]. We demonstrated that Cy-A is able to reduce the perilesional inflammatory infiltrate in RA. Several angiogenic factors released by the mononuclear cells, including lymphocytes, scattered throughout the CAM mesenchyme may contribute to RA-induced angiogenesis. In fact, these cells secrete FGF-2, VEGF, TGF- β , TNF- α , IL-1, IL-6 and IL-8 [39].

Endostatin

The effects of human recombinant endostatin were investigated in a novel model of RA, in which human RA synovium was grafted into SCID mice (SCID-HuRag) [52]. The synovial volume of SCID-HuRag mice was significantly decreased by endostatin administration, and the number of inflammatory macrophages and lymphocytes was also significantly reduced in a dose-dependent manner. In addition, the number of vessels stained by FVIII-RA and type IV collagen was decreased, although apoptotic cells were increased in the RA synovium. These data suggest that antiangiogenesis treatment through endostatin represents a potential new therapeutic strategy for RA.

CONCLUSIONS

Angiogenesis is essential during the RA development: the formation of new blood vessels from existing vasculature is essential in maintaining the invasive pannus, suggesting that targeting blood vessels in arthritis may represent a new tool for future therapeutic strategies. Disrupting new blood vessel formation can prevent delivery of nutrients into the inflammatory site, and can also contribute to vessel regression and disease

reversal. Some of the antiangiogenic therapies used now for their antitumor effect (such as inhibition of VEGF-receptor tyrosine kinases) may be also proven therapeutically effective in RA.

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