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REVIEW ARTICLE

The vasculature in rheumatoid arthritis: cause or consequence?

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Summary

The expansion of the synovial lining of joints in rheumatoid arthritis (RA) necessitates an increase in the vascular supply to the synovium, to cope with the increased requirement for oxygen and nutrients. New blood vessel formation – 'angiogenesis' – is recognized as a key event in the formation and maintenance of the pannus in RA, suggesting that targeting blood vessels in RA may be an effective future therapeutic strategy. Although many pro-angiogenic factors have been demonstrated to be expressed in RA synovium, vascular endothelial growth factor (VEGF) has been demonstrated to a have a central involvement in the angiogenic process in RA. Nevertheless, it is unclear whether angiogenesis – whether driven by VEGF and/or other factors – should be considered as a 'cause' or 'consequence' of disease. This ongoing 'chicken *vs.* egg' debate is difficult, as even the success of angiogenesis inhibition in models of RA does not provide a direct answer to the question. This review will focus on the role of the vasculature in RA, and the contribution of different angiogenic factors in promoting disease. Although no data regarding the effectiveness of anti-angiogenic therapy in RA have been reported to date, the blockade of angiogenesis nevertheless looks to be a promising therapeutic avenue.

Keywords
arthritis, angiogenesis, VEGF

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease, with a prevalence of about 1% in most parts of the world. Patients present with pain and stiffness in multiple joints, although one-third of patients initially experience symptoms at just one location or at a few scattered sites. In the majority of patients, symptoms appear over weeks to months, starting in one joint and often accompanied by prodromal symptoms including anorexia, weakness, or fatigue. The disease course of RA may range from a brief, self-limiting oligoarticular illness with minimal joint damage, to a sustained polyarticular, synovial inflammation resulting in progressive cartilage destruction and erosion of bone. As the loss of bone and cartilage progresses, the joint surface becomes destroyed, impairing range of movement and leading to deformity. In terms of visible effects on hand appearance, the fingers in RA are typically deviated towards the little finger (ulnar deviation). Other common features in RA are Boutonniere deformity (the joint nearest the knuckle is

permanently bent toward the palm while the furthest joint is bent away), swan neck deformity (in which the joint farthest from the knuckle is permanently bent toward the palm while the nearest joint is bent away from it), and the so-called 'Z-thumb' fixed flexion and subluxation at the metacarpophalangeal joint.

The onset age of RA is variable, ranging from children to individuals in their 90s, but the most common onset age is 40–50 years. In a proportion of RA patients, systemic and extra-articular features may be observed in addition to the characteristic joint changes, including anaemia, vasculitis, nodules in subcutaneous, pulmonary and sclera tissues, and interstitial inflammation in lungs as well as in exocrine salivary and lachrymal tissue. Furthermore, approximately half of RA patients have tendon involvement, and dorsal wrist swelling as a result of tenosynovitis can be the first presentation of the disease (Williamson & Feldon 1995). Proliferation of the synovial lining of tendons causes scarring and adhesion formation, and 50% of patients with tendon disease will also show synovial invasion into the tendon substance itself. This invasion is associated with multiple tendon ruptures and a poorer prognosis for long-term hand function (Ertel *et al.* 1988). Importantly, RA is associated with increased mortality, most probably because of the high frequency of cardiovascular disease (Gabriel *et al.* 2003; Kaplan 2006; Van Doornum *et al.* 2006). Recently, it was reported that the odds ratio for the risk of all-category stroke in RA was 1.64, and for the risk of ischaemic stroke was 2.66 (Nadareishvili *et al.* 2008). A study from Finland also reported that RA patients were at increased risk of dying of malignancies, as well as urogenital, gastrointestinal and respiratory diseases (Sihvonen *et al.* 2004). Depressive symptoms are highly associated with RA and may occur in nearly half of patients (Bruce 2008). It is generally considered that interactions between genetic factors, sex hormones, and possibly an infectious agent or other factor, are involved in initiating the autoimmune mechanism in RA. In 8–15% of patients, symptoms commence within a few days of a specific event, such as an infectious illness (Harris 1992). In England and Wales, there are between 250,000 and 500,000 RA patients, and hence the economic burden of musculoskeletal diseases such as RA and osteoarthritis (OA) is quite significant. For example, RA patients are more likely to stop working on health grounds than matched controls.

At the cellular level, RA is characterized by inflammation of the synovial tissue which lines joints and tendons. Normally the synovium is made up of a well-organized matrix containing proteoglycan aggregates. Within this structure are found the synovial cells (fibroblast- and macrophage-like), as well as a network of capillaries and lymphatic vessels. Between the cartilage and synovium is the synovial fluid, which nourishes and lubricates the joint. However, in RA the synovium becomes infiltrated by cells of lympho-haematopoietic origin, chiefly T-helper cells, B cells and macrophages. The synovial fluid increases in volume because of oedema, leading to joint swelling and pain. In addition, the synovium becomes thickened, from a layer of 1–2 cells to approximately 6–8 cells, and becomes locally invasive at the interface with cartilage and bone or tendon.

In the past, the traditional treatment of RA was represented by a pyramidal approach starting with non-steroidal anti-inflammatory drugs at the base of the pyramid and progressing to disease-modifying anti-rheumatic drugs (DMARD) such as gold, sulphasalazine and methotrexate (MTX). Despite such pharmacological interventions, 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-bedside studies of human tissue and animal models of disease, have led to the identification of a number of new molecular targets for intervention. The first of these was tumour necrosis factor-α (TNF-α), which mediates many inflammatory and immunoregulatory activities relevant to the development of RA. TNF-α is present in synovial fluid, and in RA synovial membrane (Brennan *et al.* 1989). The observation of TNF-α expression in RA, inhibition by TNF-α antibody of cytokine production by *ex vivo* synovial cell cultures, and effectiveness of TNF-α blockade in murine arthritis, formed the basis of the hypothesis that TNF-α was a possible therapeutic target in RA (Feldmann & Maini 2002).

To date, three biological inhibitors of this cytokine have been approved for clinical use, and more than 1,500,000 patients have been treated with these inhibitors. Monoclonal antibodies such as infliximab and adalimumab bind to TNF-α with high affinity, while etanercept is a fusion protein containing soluble TNF receptor type II fragments linked to an immunoglobulin fragment. Infliximab was the first biological developed, and from the earliest trials has shown remarkable therapeutic efficacy (Maini *et al.* 1999; Maini & Taylor 2000). Infliximab was first approved in 1999 to be used with MTX for patients with RA who had inadequate response to MTX alone. Subsequently, the US Federal Drugs Administration (FDA) approved an expanded label for infliximab in combination with MTX as a first line regimen to treat patients with moderate to severe RA. This recommendation was based on the ASPIRE (Active Controlled Study of Patients Receiving Infliximab for Treatment of Rheumatoid Arthritis of Early Onset) study, which involved patients with

RA of less than 3 years duration. In this study, patients in the infliximab groups had significantly fewer joints with new erosions after 1 year compared with controls, highlighting the impact of early intervention (Smolen *et al.* 2004).

Other newer generation TNF-α inhibitors are currently under trials. These include certolizumab (CDP870), a PEG-linked Fab fragment of a humanized TNF inhibitor monoclonal antibody, and golimumab (CNTO148), also a fully human anti-TNF-α monoclonal antibody. Golimumab with MTX was recently shown to reduce signs and symptoms of RA, with 61% of patients receiving golimumab plus MTX achieving an American College of Rheumatology (ACR) 20 response (defined as at least 20% improvement in tender joint count and 20% improvement in swollen joint count and at least a 20% improvement in three out of following five endpoints: patient pain assessment, patient global assessment, physician global assessment, patient self-addressed disability, erythrocyte sedimentation rate and C-reactive protein) at week 16 compared with 37% of patients in the placebo plus MTX group (Kay *et al.* 2008). Other cytokine targets include interleukin (IL)-2, with a humanized monoclonal antibody (daclizumab) targeted against the α-chain of the IL-2 receptor (CD25), which reduced disease severity in animal models of arthritis, and IL-6 (Williams *et al.* 2007). A humanized anti-IL-6 receptor antibody, tocilizumab (MRA), was tested in patients with RA, and was shown to reduce disease activity in a dose-dependent manner, also in combination with DMARD (Nishimoto *et al.* 2004; Genovese *et al.* 2008). An alternative approach involves blockade of T cell co-stimulatory pathways using cytotoxic T lymphocyte-associated antigen 4 (CTLA4)-based molecules such as abatacept. Patients with active RA and an inadequate response to TNF-α inhibitors who received abatacept plus at least one DMARD showed a better clinical improvement (ACR 20: 50.4%) compared with a placebo cohort (19.5%) at 6 months, with an improvement in physical function (Genovese *et al.* 2005). Abatacept was approved at the end of 2005 by the FDA for use in adult patients with moderately to severely active RA who have had an inadequate response to one or more DMARD, or TNF-α antagonists.

The advent of biological therapies, selectively targeting specific molecules, such as TNF-α, IL-6 or CTLA-4, has been a major advance in the treatment of RA. However, whereas TNF-α inhibitors such as infliximab, etanercept and adalimumab have demonstrated an acceptable safety profile, there is nonetheless an increased risk of minor infection and, more rarely, of serious infection such as tuberculosis (Keane *et al.* 2001; Bongartz *et al.* 2006). Thus, despite the clinical success of anti-cytokine biologicals, further initiatives in drug discovery are highly desirable with a view to achieve further improvements in the pharmacological management of RA. The proliferative and invasive nature of arthritic synovium has frequently led to comparisons with tumour development. Both the arthritic synovium and the growing tumour exhibit the apparently paradoxical features of hypoxia and angiogenesis, and both disease processes revolve around metabolically active cells undergoing uncontrolled proliferation and invasion within an altered pro-inflammatory micro-environment. The aggressive invasion of proliferating synovium causes joint destruction and deformities in RA, and in cancer, results in local spread and distant metastasis. This paradigm shift towards the concept of a 'tumour-like phenotype' in RA has made this disease – just like cancer – a potential target for anti-angiogenic therapy.

Angiogenesis: a central role in RA

Formation of new blood vessels from pre-existing vasculature ('angiogenesis') is important from the earliest stages of embryo development, right through to the adult stage, playing a key role in physiological processes such as healing of wounds or fractures and in the female reproductive cycle. Organization of blood vessels, in terms of time, space, and composition, needs to take into account the requirements of a particular organ or tissue, and appropriately adapt the type of mural cells and composition of sub-endothelial matrix. A range of different factors can promote angiogenesis, including fibroblast growth factor (FGF)-1 and FGF-2, angiopoietins, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) (Ferrara *et al.* 2003; Roy *et al.* 2006).

Probably the first evidence of a pro-angiogenic phenotype in RA was the report in 1980 that synovial fluids from patients with RA contained a low molecular weight angiogenesis factor (Brown *et al.* 1980). This factor was most likely what is now described as endothelial cell-stimulating angiogenesis factor (ESAF), a low molecular weight non-peptide molecule with angiogenic activity. A subsequent review in 1982 suggested that in RA *'microcirculatory compromise, concomitant with an increase in metabolic needs of synovial tissue, may initiate tissue injury via anoxia and acidosis, resulting in hydrolytic enzyme release, increased vascular permeability and acceleration of inflammatory processes'* (Rothschild & Masi 1982).

RA synovial fluids were subsequently shown to induce morphological changes in human vascular endothelial cells, with formation of tubule-like structures and induction of angiogenesis in an *in vitro* assay (Kumar *et al.* 1985; Semble *et al.* 1985). Changes in synovial blood vessel density and alterations in endothelial proliferative responses in RA have

been demonstrated. For example, the number of synovial blood vessels has been found to correlate with synovial cell hyperplasia, mononuclear cell infiltration, and indices of joint tenderness (Rooney *et al.* 1988). A morphometric study has suggested that capillaries are distributed more deeply in RA synovium, compared with normal tissue, although the blood volume fraction was greater in normal knees relative to knees affected with RA (Stevens *et al.* 1991a,b). Another group noted that although perivascular mononuclear cell infiltration and increased thickness of the synovial lining layer were observed in tissue from both inflamed and non-inflamed joints of RA patients, vascular proliferation was seen only in tissues from inflamed joints (FitzGerald *et al.* 1991). Endothelial cells lining blood vessels within RA synovium have been shown to express cell cycle-associated antigens such as proliferating cell nuclear antigen (PCNA) and Ki67, and integrin αvβ3, which is associated with vascular proliferation (Ceponis *et al.* 1998). Endothelial proliferation and cell death indices were shown to be increased in synovium from patients with RA compared with controls or individuals with OA (Walsh *et al.* 1998).

Taken together, these data suggested that alterations in the density and/or function of the blood vessels present in RA synovial tissue are a cause – or maybe consequence – of RA (Figure 1).

Role of VEGF and other growth factors in regulating angiogenesis in rheumatic arthritis

The molecular characterization of key pro-angiogenic factors such as VEGF heralded the description of this and many other angiogenic growth factors in RA. The dual activities of VEGF – first as an endothelial cell mitogen and second, as a modulator of changes in vascular permeability – are both of relevance in the pathogenesis of RA. More than 10 years ago, the groups of Koch and Fava almost simultaneously reported VEGF expression in RA synovial fluids and tissue (Fava *et al.* 1994; Koch *et al.* 1994). In addition to synovial expression of VEGF, circulating (serum) levels of VEGF are increased, and correlate with inflammatory response markers such as C-reactive protein and swollen joint counts (Harada *et al.* 1998; Kikuchi *et al.* 1998; Paleolog *et al.* 1998; Lee *et al.* 2001; Sone *et al.* 2001b). VEGF levels are increased even in RA patients with disease duration of less than 2 years, and predict subsequent joint destruction, suggesting that angiogenesis may be an early event in RA progression

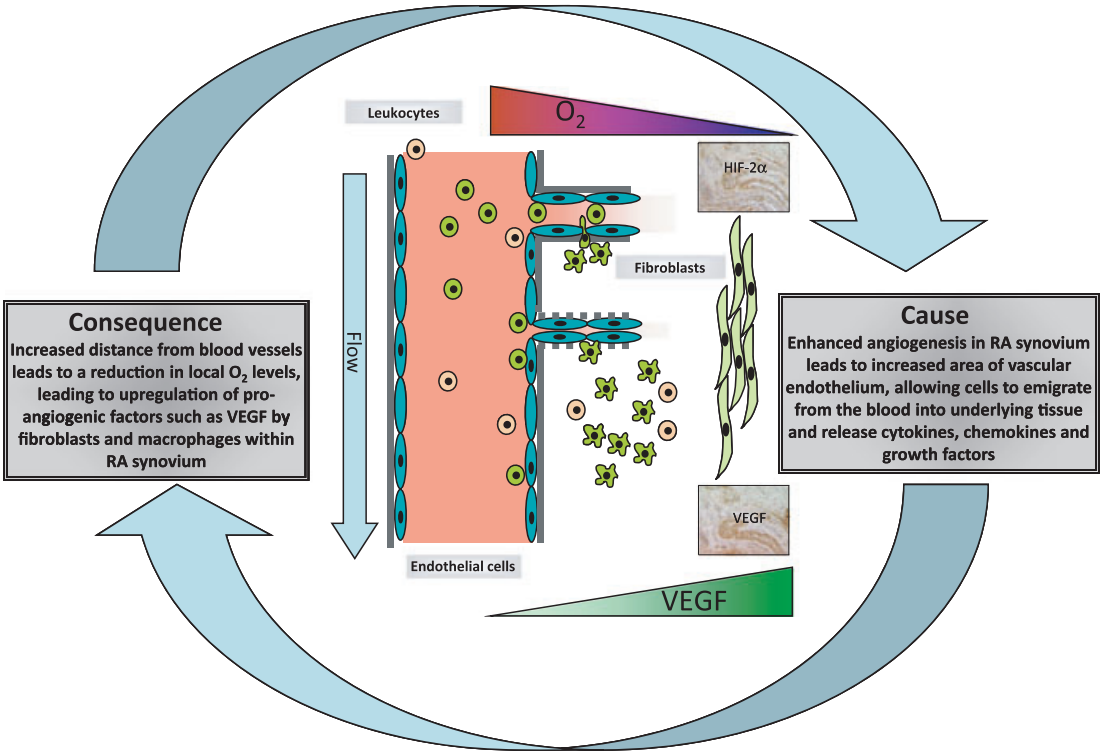

Figure 1 The role of blood vessels in RA pathogenesis: cause and/or consequence. Figure shows infiltration of RA synovium by blood-derived cells, and the relationship between oxygen tension and VEGF release (by macrophage- and fibroblast like synovial cells). Typical expression patterns of VEGF and HIF-Zalpha (by immunohistochemistry) in RA synovium are illustrated.

(Ballara *et al.* 2001). We have reported a significant correlation between serum VEGF levels at presentation in untreated RA patients, and the change in hand and feet radiographs, taken at initial presentation and at 1 year follow-up and scored according to the van der Heijde modification of Sharp's method (Ballara *et al.* 2001). Treatment of RA with TNF-α inhibitors (alone or with MTX), DMARD or anti-IL-6 receptor antibody significantly reduced serum VEGF concentrations (Paleolog *et al.* 1998; Nagashima *et al.* 2000; Ballara *et al.* 2001; Nakahara *et al.* 2003; Aggarwal *et al.* 2004; Macias *et al.* 2005).

It is especially relevant in the context of RA that VEGF is so powerfully up-regulated by hypoxia. In the context of tumours, hypoxia is a well-described phenomenon, arising from a hyperplastic response by the tumour cells, leading to an increased distance from pre-existing blood vessels. As arthritic synovium is also characterized by an altered proliferative response, it is not surprising that hypoxia is also thought to contribute to RA development. Around 30 years ago, Lund-Olesen demonstrated that mean synovial fluid oxygen tension in RA knee joints was as low as 27 mmHg, compared with 43 mmHg in osteoarthritis and 63 mmHg in traumatic effusions of otherwise healthy individuals (Lund-Olesen 1970). Subsequent studies supported these findings and even recorded oxygen tension values below 15 mmHg in humans (Treuhaft & McCarty 1971). Using silver microelectrodes, we recorded mean intra-articular oxygen tension values of 13 mmHg in mice with established arthritis (Etherington *et al.* 2002). More recently, our group has confirmed using a sensitive microelectrode technique that synovium in RA patients is more hypoxic than normal synovium (Sivakumar *et al.* 2008). We observed that median synovial oxygen tension in patients with RA was 6% (46 mmHg), compared with 10% (74 mmHg) in patients without RA. Furthermore, we studied patients with RA hand disease, and documented that invasive tenosynovium was significantly more hypoxic (median oxygen tension 3%, 26 mmHg) than either non-invasive tenosynovium or joint synovium in the same RA patients, suggesting that hypoxia might be driving invasion of tendon by the synovial tissue, and hence potentially promoting tendon rupture (Sivakumar *et al.* 2008). Contributory factors to hypoxia in RA joints include the high metabolic demands of inflamed synovial tissue and the rapid rate of synovial proliferation, so that cells become more distant from the closest blood vessels, compounding the hypoxic state. Other factors promoting raised intra-articular pressures as high as 300 mmHg include movement and accumulation of synovial fluid in involved joints (Jawed *et al.* 1997). This further compromises the vasculature and thus exacerbates hypoxia in an already ischaemic environment.

Tissue hypoxia has marked effects on genes involved in angiogenesis, apoptosis, vasomotor control, erythropoiesis and energy metabolism. A key regulator of the cellular response to oxygen is a transcription factor family known as hypoxia-inducible factor (HIF) (Semenza 2001). Approximately 1% of all human genes are regulated by HIF, including genes involved in angiogenesis, in VEGF, as well as apoptosis, vasomotor control, erythropoiesis and energy metabolism. HIF is a heterodimeric transcription factor, composed of two different subunits, HIF-α, which is oxygen-regulated, and HIF-β, which is expressed constitutively in the nucleus (Wang *et al.* 1995). There at least 2 α-subunits, termed HIF-1α and HIF-2α. Regulation of HIF-dependent gene expression requires α-subunit accumulation in the cytoplasm and translocation into the nucleus, which enables it to dimerize with β-subunits of HIF. HIF heterodimers are then recognized by co-activators and bind to the hypoxia-response elements in the target gene to initiate transcription. A growing body of evidence implicates aberrant HIF regulation in angiogenesis as part of rheumatoid joint disease (Gaber *et al.* 2005), and indeed VEGF is a well-documented HIF-regulated gene. Several studies have shown that hypoxia is a potent stimulus for VEGF induction in *ex vivo* cultures of RA synovial membrane cells (Paleolog *et al.* 1998; Jain *et al.* 2006; Sivakumar *et al.* 2008). Two, closely related HIF-α isoforms (HIF-1α, HIF-2α) as well as VEGF, are expressed in human RA synovium (Hollander *et al.* 2001; Hitchon *et al.* 2002; Giatromanolaki *et al.* 2003), and also in experimental rat arthritis (Peters *et al.* 2004), suggesting that synovial hypoxia leads to up-regulation of HIF in the joint, accumulation of VEGF and induction of synovial angiogenesis. Targeted deletion of HIF-1α in myeloid cells impairs arthritis in an *in vivo* model of the disease (Cramer *et al.* 2003), further underlining the links between hypoxia and angiogenesis in RA. Hypoxia has been reported to cause a general down-regulation of gene expression in micro-array studies in murine fibroblasts. In human hepatoma cells, a significant up- or down regulation of 159 genes by hypoxia has been reported; of these 45 were upregulated and 112 were downregulated. Using HIF-1a null mouse fibroblasts, Greijer *et al.* were able to establish that of the genes that were upregulated in their study, 89% were dependent on HIF-1, as opposed to only 17% of the downregulated genes, supporting a role for HIF-1 in upregulating genes necessary for cell survival and adaptation to stress. On a larger scale using mouse fibroblasts and micro-array analysis, Greijer *et al.* were able to group hypoxia up-regulated genes into genes involved in glycolysis, and other metabolic genes, genes involved in cell mobility, and genes involved in apoptosis (Greijer *et al.* 2005). Recently, it has become apparent

that HIF-1 and HIF-2 may exert different effects on cellular function. Despite high similarity with respect to protein sequence and activation pathway, a growing number of physiological and mechanistic differences between HIF-1 and HIF-2 are being reported. It has been shown that HIF-2, but not HIF-1, was responsible for renal cell carcinoma (RCC) growth in animals (Maranchie *et al.* 2002; Kondo *et al.* 2003). Blocking HIF-1 did not shrink tumours in that model. A panel of hypoxia-inducible genes is dependent on HIF-1α but not HIF-2α in endothelial and breast cancer cells (Sowter *et al.* 2003). It has been shown that in some cases RCC over-expression of HIF-2α accelerated tumour growth, whereas HIF-1α reduced tumour growth, suggesting that HIF-1α acts as a tumour suppressor, with HIF-2α being a tumour promoter (Raval *et al.* 2005). Similarly, hypoxia has been reported to promote cartilage matrix synthesis through HIF-2α-mediated induction of the transcription factor SOX9 and hence of cartilage genes (*COL2A1* and *COL9A1*) (Lafont *et al.* 2007). It is thus clear that by having contrasting effects on regulation of HIF-target genes, HIF-1α and HIF-2α may differently contribute to RA.

In addition to VEGF, expression of angiopoietin (Ang)-1 and Ang-2 (Scott *et al.* 2002; Gravallese *et al.* 2003) and cognate receptors Tie-1 and Tie-2 (Uchida *et al.* 2000; Shahrara *et al.* 2002; DeBusk *et al.* 2003) in RA synovial tissue has been described. An interesting study documented 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 exhibit different features in terms of vascular morphology, in that blood vessels in psoriatic synovium were highly tortuous in appearance, compared with the predominantly 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 (Fearon *et al.* 2003). Fibroblasts from late stage RA have been reported to express elevated levels of Ang-1 and Ang-2 (Scott *et al.* 2002). Ang-1 is chemotactic and weakly mitogenic for endothelial cells (Davis *et al.* 1996; Witzenbichler *et al.* 1998), promotes formation of endothelial sprouts (DeBusk *et al.* 2004) and has been proposed to act in concert with VEGF to promote vascular network maturation (Asahara *et al.* 1998; Koblizek *et al.* 1998). Furthermore, Ang-1 was found to be a survival factor for endothelium, protecting endothelial cells from apoptosis induced by serum withdrawal, whereas Ang-2 is thought to result in vessel destabilization and regression (Papapetropoulos *et al.* 1999), suggesting that the Ang-Tie axis may play a multifaceted role in regulating angiogenesis in RA. Additionally, FGF-1 and FGF-2 have been detected in RA synovial tissue (Sano *et al.* 1990; Nakashima *et al.* 1994), together with PDGF (Remmers *et al.* 1991; Sano *et al.* 1993) and hepatocyte growth factor (HGF) (Koch *et al.* 1996). HGF may contribute to endothelial migration and angiogenesis in RA, as anti-HGF partially neutralized the chemotactic activity for endothelial cells found in RA synovial fluids (Koch *et al.* 1996). Patients with RA have elevated levels of HGF in the synovial fluid and serum, and these correlate with disease activity (Yukioka *et al.* 1994; Feuerherm *et al.* 2001).

Taken together, these observations led to the hypothesis that the vasculature may be a therapeutic target in RA.

Lessons from angiogenesis inhibition in cancer for future treatment of RA

The involvement of angiogenesis has made both cancer and RA a potential target for anti-angiogenic therapy. In particular, studies on the molecular and cellular mechanisms underlying cancer of the colon and rectum have made this type of cancer the first to be treated with angiogenesis inhibitors.

In 1971, Judah Folkman described the critical role of tumour angiogenesis to potentiate tumour growth and metastasis, and as a result angiogenesis has been a putative target for anti-cancer therapy since the 1970s (Folkman 1971). In particular, expression of VEGF is up-regulated in numerous solid malignancies, and interrupting the VEGF pathway has become a major focus of oncological research (Ferrara *et al.* 2003). The most successful anti-angiogenic approach is bevacizumab, a humanized IgG_1 monoclonal anti-VEGF antibody, approved in 2004 as a combination with intra-venous 5-fluorouracil-based (5-FU) chemotherapy as a treatment for patients with previously untreated metastatic cancer of the colon or rectum. Colorectal cancer is the third most common cancer worldwide, with 307,432 new cases diagnosed in 2006 in the European Union alone. In the UK, colorectal cancer is the second leading cause of all cancer related deaths. In the original phase III clinical trial of 813 patients with untreated metastatic colorectal cancer, patients were randomized to receive irinotecan, 5-FU and leucovorin (IFL) alone, or in combination with bevacizumab at 5 mg/kg every 2 weeks. The group receiving additional bevacizumab had a longer median duration of survival, namely 20.3 months *vs.* 15.6 months i.e. a median survival benefit of 4.7 months, which while at first glance unremarkable, was in fact as large as or larger than that observed in any other phase III trial for the treatment of (metastatic) colorectal cancer. The progression-free survival was also increased (10.6 months *vs.* 6.2 months) (Hurwitz *et al.* 2004; Kabbinavar *et al.* 2005). More recently, bevacizumab was approved in combination with

FOLFOX4 (5-FU, leucovorin, and oxaliplatin) for the second-line treatment of metastatic carcinoma of the colon or rectum. This was the result of a phase III trial using newer chemotherapeutic regimes, which confirmed improved overall survival in patients receiving FOLFOX-4 in combination with bevacizumab (12.5 months), relative to FOLFOX-4 alone (10.7 months) (Cohen *et al.* 2007).

Bevacizumab is now also approved in combination with carboplatin and paclitaxel, for first-line treatment of patients with unresectable, locally advanced, recurrent, or metastatic non-squamous non-small cell lung cancer (NSCLC). Moreover, in February 2008, accelerated approval was granted for bevacizumab in combination with paclitaxel, for the treatment of patients with metastatic HER2-negative breast cancer who have not received chemotherapy. The approval for breast cancer came after a phase III study that showed that bevacizumab in combination with paclitaxel chemotherapy resulted in a 52% reduction in the risk of disease progression or death compared with those treated with paclitaxel alone and a doubling in progression-free survival. Results from ongoing trials in patients with previously untreated (RiBBON 1) and treated (RiBBON 2) metastatic breast cancer are awaited for the accelerated approval to be converted into full approval.

While bevacizumab has been a major step forward in the treatment of some cancers, VEGF has a critical physiological role *in vivo*. This is highlighted by the heterozygous lethality of VEGF knock-out mice (Carmeliet *et al.* 1996; Ferrara *et al.* 1996). In colorectal patients receiving bevacizumab, out of the 402 patients assigned to receive IFL and bevacizumab, six patients (1.5%) developed perforation of the gastrointestinal tract as opposed to none in the IFL-alone control arm. Similar results were also observed in the First BEAT trial (Berry *et al.* 2006), established to evaluate the safety profile of bevacizumab, in which gastrointestinal perforations were reported in 1.2% of patients receiving bevacizumab. These events are for the most part mild-moderate in severity and clinically manageable. Increased thromboembolic events have been reported, as has hypertension, although the latter is generally manageable using standard anti-hypertensive approaches, as have. For example, in a clinical trial in patients with NSCLC, comparison of bevacizumab plus carboplatin and paclitaxel *vs.* carboplatin and paclitaxel alone, severe and life-threatening adverse events occurred more frequently in patients receiving bevacizumab, namely, hypertension (8% *vs.* 0.7%) and thrombosis/embolism (5% *vs.* 3%). Fatal, treatment-related adverse events in patients receiving bevacizumab included pulmonary haemorrhage, gastrointestinal haemorrhage and myocardial infarction (Cohen *et al.* 2007). The induction by VEGF of the vasodilatior nitric oxide (NO), through the activation of endothelial NO synthase, may underlie the apparent hypertension and increased risk of thromboembolic events associated with bevacizumab. Nevertheless, even patients over 65 years of age with a high likelihood of thromboembolic events may benefit from bevacizumab, and clearly in such situations the patient risk-benefit ratio needs to be weighed up. The importance of angiogenesis in wound-healing suggests that bevacizumab might impair this process, which would have significant implications for patients undergoing surgery. While slower wound-healing is listed as a possible side-effect, a study found that bevacizumab administered in combination with 5-FU/leucovorin-based chemotherapy 28–60 days after primary cancer surgery caused no increased risk of wound-healing complications compared with chemotherapy alone. Nonetheless, wound-healing complications were increased in patients who had major surgery during bevacizumab therapy (13% bevacizumab-treated patients *vs.* 3.4% control patients) (Scappaticci *et al.* 2007). Proteinuria has also been reported, suggesting possible renal side-effects, and discontinuation of bevacizumab is recommended if marked proteinuria persists. Finally, VEGF has important neuroprotective effects, which were highlighted by a study showing that a targeted reduction in VEGF expression in the mouse spinal cord was associated with adult-onset progressive motor neuron degeneration, reminiscent of amyotrophic lateral sclerosis (Oosthuyse *et al.* 2001).

The first generation of angiogenesis inhibitors has clearly revolutionized our approach in the management of advanced colorectal cancer. The success story of bevacizumab provides direction and encouragement for the potential use of angiogenesis blockade in other diseases.

Potential for anti-angiogenic therapy in RA

The relative success of VEGF blockade in cancer prompted much speculation that RA might also be a potential target for angiogenesis inhibitors. Rodent models have been used extensively to study the mechanisms underlying the angiogenic process in arthritic diseases and to develop new therapeutic interventions, including those based on inhibition of VEGF. Arthritis can be induced in genetic susceptible mouse strains by immunization with type II collagen, resulting in an autoimmune response against autoantigens in the articular cartilage and eventually leading to a destructive polyarthritis. Heterologous collagen-induced arthritis (CIA) in mice shares many features with RA, including linkage to the major histocompatibility region, infiltration of synovium by blood-derived cells, synovial hyperplasia, pannus formation,

angiogenesis, as well as destruction of cartilage and bone. This model has been widely used to study mechanisms involved in the arthritic process and to identify new strategies for RA treatment, such as TNF-α inhibitors. While no animal model of disease is ideal, heterologous CIA has been used extensively to investigate new therapeutic targets, in part attributable to the success of this model in predicting the success of TNF-α blockade (Williams *et al.* 1994, 1995, 2000). Moreover, we and others have shown that inhibition of angiogenesis ameliorates disease (Kim *et al.* 2002; Sumariwalla *et al.* 2003; Bainbridge *et al.* 2007).

In terms of specific targeting VEGF in disease models, Lu *et al.* showed that VEGF and its receptors are expressed during the development of murine CIA, and the level of VEGF expression correlated with disease severity and the degree of neovascularization. Neutralization of VEGF activity by administration of anti-VEGF antibody delayed disease onset, but appeared less effective when administered during the chronic phase of disease (Lu *et al.* 2000). In another study, application of anti-VEGF treatment in established bovine CIA inhibited synovitis, as indicated by a reduction in clinical scores and paw-swelling relative to untreated mice (Sone *et al.* 2001a). A soluble form of VEGF receptor (VEGFR)-1, a naturally occurring antagonist of VEGF, has been shown to significantly suppress established arthritis. Mice that received adenoviral vectors expressing human soluble VEGFR-1 after the onset of arthritis showed reductions in the extent and severity of disease (assessed as the clinical score), and decreased paw-swelling and joint destruction, when compared with control animals. Furthermore, decreased levels of VEGF were observed in ankle lysates of these animals (Miotla *et al.* 2000; Afuwape *et al.* 2003; Jin *et al.* 2008). 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-VEGFR-1 (but not anti-VEGFR-2) antibody abrogated bone and cartilage destruction. The antibody delayed the onset of arthritis and attenuated the severity of disease (de Bandt *et al.* 2003). The group of Carmeliet also compared different approaches targeting VEGF (using anti-VEGFR-1 and anti-VEGFR-2 antibodies) in chicken CIA in mice. Treatment with anti-VEGFR-1 reduced the incidence of joint disease by 60%, and suppressed the development of clinical symptoms by 85%, whereas anti-VEGFR-2 appeared ineffective (Luttun *et al.* 2002). The reason underlying the effectiveness of VEGFR-1 blockade as compared with VEGFR-2-targeted approaches is unclear, although one possible explanation put forward is that VEGF, via VEGFR-1, also promotes monocyte- and neutrophil trafficking (Clauss *et al.* 1990, 1996). Thus, inhibition of VEGFR-1 would, in addition to affecting vessel growth, reduce cell trafficking to the RA synovium. The VEGFR tyrosine kinase inhibitor PTK787/ZK222584 has also been shown to be effective in arthritis models (Grosios *et al.* 2004). Knockout of the VEGF-B gene also reduced angiogenesis in *Vegf-B*$^{-/-}$ mice, and attenuated both collagen-induced and adjuvant-induced arthritis, suggesting that VEGF-B contributes to the angiogenic process in RA (Mould *et al.* 2003). However, while targeting VEGF in models of disease has been effective, the side-effects of therapies such as bevacizumab include increased risk of thromboembolic events. It has already been highlighted that RA is associated with increased frequency of cardiovascular disease (Gabriel *et al.* 2003; Kaplan 2006; Van Doornum *et al.* 2006). The complex nature of RA makes it difficult to predict the consequences of VEGF inhibition *in vivo*, although in the future combining animal models such as CIA with atherosclerosis models such as apoE$^{-/-}$ mice should yield some information on whether this might be a useful therapeutic approach in human disease.

In terms of other angiogenic targets for RA therapy, we have recently reported that a splice variant of Tie-1 markedly reduced disease severity in murine CIA. Angiopoietin signalling was until recently considered to be mediated via Tie-2. However, the embryonic lethality of Tie-1 knockout mice suggested that Tie-1 signalling is important in vascular network formation. It is now thought that Tie-1 may modulate signalling through Tie-2 (Kontos *et al.* 2002; Saharinen *et al.* 2005; Yuan *et al.* 2007). It was reported that activation of Tie-1 ectodomain cleavage increased activation of Tie-2, which could potentially control signalling via Tie-2 (Marron *et al.* 2007). It is therefore of interest that Tie-1 may modulate CIA, and we believe that ours is the first demonstration that inhibition of the Ang-Tie axis can markedly reduce arthritis severity. (Jin *et al.* 2008). In addition, a competitive HGF antagonist, NK4, which binds to the c-Met receptor, but does not induce tyrosine phosphorylation of c-Met, has been described (Date *et al.* 1997). Interestingly, NK4 is able to inhibit angiogenesis not only induced by HGF, but also by other pro-angiogenic factors like FGF-2 and VEGF (Kuba *et al.* 2000; Nakabayashi *et al.* 2003; Matsumoto & Nakamura 2005), but its efficacy in RA has not been assessed. However, the role of HGF in RA may be complex, as evidenced by the report that HGF suppresses CIA in mice (Okunishi *et al.* 2007).

Conclusions

There has been a phenomenal interest in the development of inhibitors of angiogenesis, which represent a major suc-

cess in the fight against cancer. However while the spotlight has been on malignancies, the many parallels between tumour growth and RA reinforce the hypothesis that inhibition of angiogenesis might also potentially be effective in RA. The question of 'cause or consequence' – that is, whether angiogenesis drives RA or whether the enhanced synovial proliferation promotes angiogenesis – is a difficult one, to say the least, as angiogenesis, hypoxia and synovial expansion are so intricately linked (Figure 1). As briefly mentioned, the thromboembolic complications of bevacizumab in cancer patients raise the question of the side-effects of VEGF inhibition in RA, and more sophisticated animal model studies are needed before such concerns are addressed. Anti-angiogenic therapy is not likely to ever be a replacement for treatments such as TNF inhibitors, rituximab, abatacept, tocilizumab, daclizumab or others. Instead, use of anti-angiogenic therapy is likely as an adjunct, thus allowing concomitant targeting of the inflammatory cascade (using cytokine- or T-cell/B-cell-targeted approaches) and blood vessel formation, hence 'starving' the synovium of nutrients and oxygen. Unfortunately, only one clinical trial has been initiated so far in RA using involving anti-$\alpha v\beta 3$ antibody, which closed early because of lack of benefit. In terms of RA patients, patients with RA of less than 3-year duration show marked synovial thickening and vascular signal, suggesting these may go hand-in-hand (Taylor *et al.* 2006). It is possible to use *in vivo* models to address this question, in that animals can be followed from the induction of disease. In an interesting study in murine CIA, *in vivo* fluorescence microscopy of knee using labelled-dextran joints revealed marked leucocyte activation and interaction with synovial microvessels before onset of clinical symptoms of arthritis (Gierer *et al.* 2005). We and others are currently using more sophisticated and specific *in vivo* imaging approaches to determine whether the alterations in the vasculature precede synovial thickening in CIA. Either way, however, the unique role of angiogenesis in RA supports the premise that this process is an exciting target for therapy in RA, whether using bevacizumab, or newer generation angiogenesis inhibitors.

References

Afuwape A.O., Feldmann M., Paleolog E.M. (2003) Adenoviral delivery of soluble VEGF receptor 1 (sFlt-1) abrogates disease activity in murine collagen-induced arthritis. *Gene Ther.* **10**, 1950–1960.

Aggarwal A., Panda S., Misra R. (2004) Effect of etanercept on matrix metalloproteinases and angiogenic vascular endothelial growth factor: a time kinetic study. *Ann. Rheum. Dis.* **63**, 891–892.

Asahara T., Chen D., Takahashi T. *et al.* (1998) Tie2 receptor ligands, angiopoietin-1 and angiopoietin-2, modulate VEGF-induced postnatal neovascularization. *Circ. Res.* **83**, 233–240.

Bainbridge J., Madden L., Essex D., Binks M., Malhotra R., Paleolog E.M. (2007) Methionine aminopeptidase-2 blockade reduces chronic collagen-induced arthritis: potential role for angiogenesis inhibition. *Arthritis Res. Ther.* **9**, R127.

Ballara S.C., Taylor P.C., Reusch P. *et al.* (2001) Raised serum vascular endothelial growth factor levels are associated with destructive change in inflammatory arthritis. *Arthritis Rheum.* **44**, 2055–2064.

de Bandt M., Ben Mahdi M.H., Ollivier V. *et al.* (2003) Blockade of vascular endothelial growth factor receptor I (VEGF-RI), but not VEGF-RII, suppresses joint destruction in the K/BxN model of rheumatoid arthritis. *J. Immunol.* **171**, 4853–4859.

Berry S., Cunningham D., Michael M. *et al.* (2006) Preliminary safety of bevacizumab with first-line Folfox, Capox, Folfiri and capecitabine for mCRC–First B.E.A.Trial. *J. Clin. Oncol.* ASCO Annual Meeting Proceedings Part I. **24**, 3534.

Bongartz T., Sutton A.J., Sweeting M.J., Buchan I., Matteson E.L., Montori V. (2006) Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* **295**, 2275–2285.

Brennan F.M., Chantry D., Jackson A., Maini R., Feldmann M. (1989) Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet* **2**, 244–247.

Brown R.A., Weiss J.B., Tomlinson I.W., Phillips P., Kumar S. (1980) Angiogenic factor from synovial fluid resembling that from tumours. *Lancet* **1**, 682–685.

Bruce T.O. (2008) Comorbid depression in rheumatoid arthritis: pathophysiology and clinical implications. *Curr. Psychiatry Rep.* **10**, 258–264.

Carmeliet P., Ferreira V., Breier G. *et al.* (1996) Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* **380**, 435–439.

Ceponis A., Konttinen Y.T., Imai S. *et al.* (1998) Synovial lining, endothelial and inflammatory mononuclear cell proliferation in synovial membranes in psoriatic and reactive arthritis: a comparative quantitative morphometric study. *Br. J. Rheumatol.* **37**, 170–178.

Clauss M., Gerlach M., Gerlach H. *et al.* (1990) Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J. Exp. Med.* **172**, 1535–1545.

Clauss M., Weich H., Breier G. *et al.* (1996) The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth

factor in monocyte activation and chemotaxis. *J. Biol. Chem.* **271**, 17629–17634.

Cohen M.H., Gootenberg J., Keegan P., Pazdur R. (2007) FDA drug approval summary: bevacizumab (Avastin) plus Carboplatin and Paclitaxel as first-line treatment of advanced/metastatic recurrent nonsquamous non-small cell lung cancer. *Oncologist* **12**, 713–718.

Cramer T., Yamanishi Y., Clausen B.E. *et al.* (2003) HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* **112**, 645–657.

Date K., Matsumoto K., Shimura H., Tanaka M., Nakamura T. (1997) HGF/NK4 is a specific antagonist for pleiotrophic actions of hepatocyte growth factor. *FEBS Lett.* **420**, 1–6.

Davis S., Aldrich T.H., Jones P.F. *et al.* (1996) Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. *Cell* **87**, 1161–1169.

DeBusk L.M., Chen Y., Nishishita T., Chen J., Thomas J.W., Lin P.C. (2003) Tie2 receptor tyrosine kinase, a major mediator of tumor necrosis factor alpha-induced angiogenesis in rheumatoid arthritis. *Arthritis Rheum.* **48**, 2461–2471.

DeBusk L.M., Hallahan D.E., Lin P.C. (2004) Akt is a major angiogenic mediator downstream of the Ang1/Tie2 signaling pathway. *Exp. Cell Res.* **298**, 167–177.

Ertel A.N., Millender L.H., Nalebuff E., McKay D., Leslie B. (1988) Flexor tendon ruptures in patients with rheumatoid arthritis. *J. Hand Surg. [Am.]* **13**, 860–866.

Etherington P.J., Winlove P., Taylor P., Paleolog E., Miotla J.M. (2002) VEGF release is associated with reduced oxygen tensions in experimental inflammatory arthritis. *Clin. Exp. Rheumatol.* **20**, 799–805.

Fava R.A., Olsen N.J., Spencer-Green G. *et al.* (1994) Vascular permeability factor/endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue. *J. Exp. Med.* **180**, 341–346.

Fearon U., Griosios K., Fraser A. *et al.* (2003) Angiopoietins, growth factors, and vascular morphology in early arthritis. *J. Rheumatol.* **30**, 260–268.

Feldmann M. & Maini R.N. (2002) Discovery of TNF-alpha as a therapeutic target in rheumatoid arthritis: preclinical and clinical studies. *Joint Bone Spine* **69**, 12–18.

Ferrara N., Carver-Moore K., Chen H. *et al.* (1996) Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* **380**, 439–442.

Ferrara N., Gerber H.P., LeCouter J. (2003) The biology of VEGF and its receptors. *Nat. Med.* **9**, 669–676.

Feuerherm A.J., Borset M., Seidel C. *et al.* (2001) Elevated levels of osteoprotegerin (OPG) and hepatocyte growth factor (HGF) in rheumatoid arthritis. *Scand. J. Rheumatol.* **30**, 229–234.

FitzGerald O., Soden M., Yanni G., Robinson R., Bresnihan B. (1991) Morphometric analysis of blood vessels in synovial membranes obtained from clinically affected and unaffected knee joints of patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **50**, 792–796.

Folkman J. (1971) Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* **285**, 1182–1186.

Gaber T., Dziurla R., Tripmacher R., Burmester G.R., Buttgereit F. (2005) Hypoxia inducible factor (HIF) in rheumatology: low O2! See what HIF can do!. *Ann. Rheum. Dis.* **64**, 971–980.

Gabriel S.E., Crowson C.S., Kremers H.M. *et al.* (2003) Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years. *Arthritis Rheum.* **48**, 54–58.

Genovese M.C., Becker J.C., Schiff M. *et al.* (2005) Abatacept for rheumatoid arthritis refractory to tumor necrosis factor alpha inhibition. *N. Engl. J. Med.* **353**, 1114–1123.

Genovese M.C., McKay J.D., Nasonov E.L. *et al.* (2008) Interleukin-6 receptor inhibition with tocilizumab reduces disease activity in rheumatoid arthritis with inadequate response to disease-modifying antirheumatic drugs: the tocilizumab in combination with traditional disease-modifying antirheumatic drug therapy study. *Arthritis Rheum.* **58**, 2968–2980.

Giatromanolaki A., Sivridis E., Maltezos E. *et al.* (2003) Upregulated hypoxia inducible factor-1alpha and -2alpha pathway in rheumatoid arthritis and osteoarthritis. *Arthritis Res. Ther.* **5**, R193–R201.

Gierer P., Ibrahim S., Mittlmeier T. *et al.* (2005) Gene expression profile and synovial microcirculation at early stages of collagen-induced arthritis. *Arthritis Res. Ther.* **7**, R868–R876.

Gravallese E.M., Pettit A.R., Lee R. *et al.* (2003) Angiopoietin-1 is expressed in the synovium of patients with rheumatoid arthritis and is induced by tumour necrosis factor alpha. *Ann. Rheum. Dis.* **62**, 100–107.

Greijer A.E., van der Groep P., Kemming D. *et al.* (2005) Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). *J. Pathol.* **206**, 291–304.

Grosios K., Wood J., Esser R., Raychaudhuri A., Dawson J. (2004) Angiogenesis inhibition by the novel VEGF receptor tyrosine kinase inhibitor, PTK787/ZK222584, causes significant anti-arthritic effects in models of rheumatoid arthritis. *Inflamm. Res.* **53**, 133–142.

Harada M., Mitsuyama K., Yoshida H. *et al.* (1998) Vascular endothelial growth factor in patients with rheumatoid arthritis. *Scand. J. Rheumatol.* **27**, 377–380.

Harris E.D. Jr, (1992) Excitement in synovium: the rapid evolution of understanding of rheumatoid arthritis and expectations for therapy. *J. Rheumatol. Suppl.* **32**, 3–5.

Hitchon C., Wong K., Ma G., Reed J., Lyttle D., El-Gabalawy H. (2002) Hypoxia-induced production of stromal cell-derived factor 1 (CXCL12) and vascular endothelial growth factor by synovial fibroblasts. *Arthritis Rheum.* **46**, 2587–2597.

Hollander A.P., Corke K.P., Freemont A.J., Lewis C.E. (2001) Expression of hypoxia-inducible factor 1alpha by macrophages in the rheumatoid synovium: implications for targeting of therapeutic genes to the inflamed joint. *Arthritis Rheum.* **44**, 1540–1544.

Hurwitz H., Fehrenbacher L., Novotny W. *et al.* (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med.* **350**, 2335–2342.

Jain A., Kiriakidis S., Brennan F., Sandison A., Paleolog E., Nanchahal J. (2006) Targeting rheumatoid tenosynovial angiogenesis with cytokine inhibitors. *Clin. Orthop. Relat. Res.* **446**, 268–277.

Jawed S., Gaffney K., Blake D.R. (1997) Intra-articular pressure profile of the knee joint in a spectrum of inflammatory arthropathies. *Ann. Rheum. Dis.* **56**, 686–689.

Jin P., Zhang J., Sumariwalla P.S. *et al.* (2008) Novel splice variants derived from the receptor tyrosine kinase superfamily are potential therapeutics for rheumatoid arthritis. *Arthritis Res. Ther.* **10**, R73.

Kabbinavar F.F., Hambleton J., Mass R.D., Hurwitz H.I., Bergsland E., Sarkar S. (2005) Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. *J. Clin. Oncol.* **23**, 3706–3712.

Kaplan M.J. (2006) Cardiovascular disease in rheumatoid arthritis. *Curr. Opin. Rheumatol.* **18**, 289–297.

Kay J., Matteson E.L., Dasgupta B. *et al.* (2008) Golimumab in patients with active rheumatoid arthritis despite treatment with methotrexate: a randomized, double-blind, placebo-controlled, dose-ranging study. *Arthritis Rheum.* **58**, 964–975.

Keane J., Gershon S., Wise R.P. *et al.* (2001) Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N. Engl. J. Med.* **345**, 1098–1104.

Kikuchi K., Kubo M., Kadono T., Yazawa N., Ihn H., Tamaki K. (1998) Serum concentrations of vascular endothelial growth factor in collagen diseases. *Br. J. Dermatol.* **139**, 1049–1051.

Kim J.M., Ho S.H., Park E.J. *et al.* (2002) Angiostatin gene transfer as an effective treatment strategy in murine collagen-induced arthritis. *Arthritis Rheum.* **46**, 793–801.

Koblizek T.I., Weiss C., Yancopoulos G.D., Deutsch U., Risau W. (1998) Angiopoietin-1 induces sprouting angiogenesis in vitro. *Curr. Biol.* **8**, 529–532.

Koch A.E., Harlow L.A., Haines G.K. *et al.* (1994) Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. *J. Immunol.* **152**, 4149–4156.

Koch A.E., Halloran M.M., Hosaka S. *et al.* (1996) Hepatocyte growth factor. A cytokine mediating endothelial migration in inflammatory arthritis. *Arthritis Rheum.* **39**, 1566–1575.

Kondo K., Kim W.Y., Lechpammer M., Kaelin W.G. Jr, (2003) Inhibition of HIF2alpha is sufficient to suppress pVHL-defective tumor growth. *PLoS Biol.* **1**, E83.

Kontos C.D., Cha E.H., York J.D., Peters K.G. (2002) The endothelial receptor tyrosine kinase Tie1 activates phosphatidylinositol 3-kinase and Akt to inhibit apoptosis. *Mol. Cell. Biol.* **22**, 1704–1713.

Kuba K., Matsumoto K., Date K., Shimura H., Tanaka M., Nakamura T. (2000) HGF/NK4, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppresses tumor growth and metastasis in mice. *Cancer Res.* **60**, 6737–6743.

Kumar P., Erroi A., Sattar A., Kumar S. (1985) Weibel-Palade bodies as a marker for neovascularization induced by tumor and rheumatoid angiogenesis factors. *Cancer Res.* **45**, 4339–4348.

Lafont J.E., Talma S., Murphy C.L. (2007) Hypoxia-inducible factor 2alpha is essential for hypoxic induction of the human articular chondrocyte phenotype. *Arthritis Rheum.* **56**, 3297–3306.

Lee S.S., Joo Y.S., Kim W.U. *et al.* (2001) Vascular endothelial growth factor levels in the serum and synovial fluid of patients with rheumatoid arthritis. *Clin. Exp. Rheumatol.* **19**, 321–324.

Lu J., Kasama T., Kobayashi K. *et al.* (2000) Vascular endothelial growth factor expression and regulation of murine collagen-induced arthritis. *J. Immunol.* **164**, 5922–5927.

Lund-Olesen K. (1970) Oxygen tension in synovial fluids. *Arthritis Rheum.* **13**, 769–776.

Luttun A., Tjwa M., Moons L. *et al.* (2002) Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat. Med.* **8**, 831–840.

Macias I., Garcia-Perez S., Ruiz-Tudela M., Medina F., Chozas N., Giron-Gonzalez J.A. (2005) Modification of pro- and antiinflammatory cytokines and vascular-related molecules by tumor necrosis factor-a blockade in patients with rheumatoid arthritis. *J. Rheumatol.* **32**, 2102–2108.

Maini R.N. & Taylor P.C. (2000) Anti-cytokine therapy for rheumatoid arthritis. *Annu. Rev. Med.* **51**, 207–229.

Maini R., St Clair E.W., Breedveld F. *et al.* (1999) Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* **354**, 1932–1939.

Maranchie J.K., Vasselli J.R., Riss J., Bonifacino J.S., Linehan W.M., Klausner R.D. (2002) The contribution of VHL substrate binding and HIF1-alpha to the phenotype of VHL loss in renal cell carcinoma. *Cancer Cell* **1**, 247–255.

Marron M.B., Singh H., Tahir T.A. *et al.* (2007) Regulated proteolytic processing of Tie1 modulates ligand responsiveness of

the receptor-tyrosine kinase Tie2. *J. Biol. Chem.* **282**, 30509–30517.

Matsumoto K. & Nakamura T. (2005) Mechanisms and significance of bifunctional NK4 in cancer treatment. *Biochem. Biophys. Res. Commun.* **333**, 316–327.

Miotla J., Maciewicz R., Kendrew J., Feldmann M., Paleolog E. (2000) Treatment with soluble VEGF receptor reduces disease severity in murine collagen-induced arthritis. *Lab. Invest.* **80**, 1195–1205.

Mould A.W., Tonks I.D., Cahill M.M. *et al.* (2003) Vegfb gene knockout mice display reduced pathology and synovial angiogenesis in both antigen-induced and collagen-induced models of arthritis. *Arthritis Rheum.* **48**, 2660–2669.

Nadareishvili Z., Michaud K., Hallenbeck J.M., Wolfe F. (2008) Cardiovascular, rheumatologic, and pharmacologic predictors of stroke in patients with rheumatoid arthritis: a nested, case-control study. *Arthritis Rheum.* **59**, 1090–1096.

Nagashima M., Wauke K., Hirano D. *et al.* (2000) Effects of combinations of anti-rheumatic drugs on the production of vascular endothelial growth factor and basic fibroblast growth factor in cultured synoviocytes and patients with rheumatoid arthritis. *Rheumatology (Oxford)* **39**, 1255–1262.

Nakabayashi M., Morishita R., Nakagami H. *et al.* (2003) HGF/NK4 inhibited VEGF-induced angiogenesis in in vitro cultured endothelial cells and in vivo rabbit model. *Diabetologia* **46**, 115–123.

Nakahara H., Song J., Sugimoto M. *et al.* (2003) Anti-interleukin-6 receptor antibody therapy reduces vascular endothelial growth factor production in rheumatoid arthritis. *Arthritis Rheum.* **48**, 1521–1529.

Nakashima M., Eguchi K., Aoyagi T. *et al.* (1994) Expression of basic fibroblast growth factor in synovial tissues from patients with rheumatoid arthritis: detection by immunohistological staining and in situ hybridisation. *Ann. Rheum. Dis.* **53**, 45–50.

Nishimoto N., Yoshizaki K., Miyasaka N. *et al.* (2004) Treatment of rheumatoid arthritis with humanized anti-interleukin-6 receptor antibody: a multicenter, double-blind, placebo-controlled trial. *Arthritis Rheum.* **50**, 1761–1769.

Okunishi K., Dohi M., Fujio K. *et al.* (2007) Hepatocyte growth factor significantly suppresses collagen-induced arthritis in mice. *J. Immunol.* **179**, 5504–5513.

Oosthuyse B., Moons L., Storkebaum E. *et al.* (2001) Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nat. Genet.* **28**, 131–138.

Paleolog E.M., Young S., Stark A.C., McCloskey R.V., Feldmann M., Maini R.N. (1998) Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. *Arthritis Rheum.* **41**, 1258–1265.

Papapetropoulos A., Garcia-Cardena G., Dengler T.J., Maisonpierre P.C., Yancopoulos G.D., Sessa W.C. (1999) Direct actions of angiopoietin-1 on human endothelium: evidence for network stabilization, cell survival, and interaction with other angiogenic growth factors. *Lab. Invest.* **79**, 213–223.

Peters C.L., Morris C.J., Mapp P.I., Blake D.R., Lewis C.E., Winrow V.R. (2004) The transcription factors hypoxia-inducible factor 1alpha and Ets-1 colocalize in the hypoxic synovium of inflamed joints in adjuvant-induced arthritis. *Arthritis Rheum.* **50**, 291–296.

Raval R.R., Lau K.W., Tran M.G. *et al.* (2005) Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol. Cell. Biol.* **25**, 5675–5686.

Remmers E.F., Sano H., Lafyatis R. *et al.* (1991) Production of platelet derived growth factor B chain (PDGF-B/c-sis) mRNA and immunoreactive PDGF B-like polypeptide by rheumatoid synovium: coexpression with heparin binding acidic fibroblast growth factor-1. *J. Rheumatol.* **18**, 7–13.

Rooney M., Condell D., Quinlan W. *et al.* (1988) Analysis of the histologic variation of synovitis in rheumatoid arthritis. *Arthritis Rheum.* **31**, 956–963.

Rothschild B.M. & Masi A.T. (1982) Pathogenesis of rheumatoid arthritis: a vascular hypothesis. *Semin. Arthritis Rheum.* **12**, 11–31.

Roy H., Bhardwaj S., Yla-Herttuala S. (2006) Biology of vascular endothelial growth factors. *FEBS Lett.* **580**, 2879–2887.

Saharinen P., Kerkela K., Ekman N. *et al.* (2005) Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2. *J. Cell Biol.* **169**, 239–243.

Sano H., Forough R., Maier J.A. *et al.* (1990) Detection of high levels of heparin binding growth factor-1 (acidic fibroblast growth factor) in inflammatory arthritic joints. *J. Cell Biol.* **110**, 1417–1426.

Sano H., Engleka K., Mathern P. *et al.* (1993) Coexpression of phosphotyrosine-containing proteins, platelet-derived growth factor-B, and fibroblast growth factor-1 *in situ* in synovial tissues of patients with rheumatoid arthritis and Lewis rats with adjuvant or streptococcal cell wall arthritis. *J. Clin. Invest.* **91**, 553–565.

Scappaticci F.A., Skillings J.R., Holden S.N. *et al.* (2007) Arterial thromboembolic events in patients with metastatic carcinoma treated with chemotherapy and bevacizumab. *J. Natl. Cancer Inst.* **99**, 1232–1239.

Scott B.B., Zaratin P.F., Colombo A., Hansbury M.J., Winkler J.D., Jackson J.R. (2002) Constitutive expression of angiopoietin-1 and -2 and modulation of their expression by inflammatory cytokines in rheumatoid arthritis synovial fibroblasts. *J. Rheumatol.* **29**, 230–239.

Semble E.L., Turner R.A., McCrickard E.L. (1985) Rheumatoid arthritis and osteoarthritis synovial fluid effects on primary human endothelial cell cultures. *J. Rheumatol.* **12**, 237–241.

Semenza G.L. (2001) HIF-1, O_2, and the 3 PHDs: how animal cells signal hypoxia to the nucleus. *Cell* **107**, 1–3.

Shahrara S., Volin M.V., Connors M.A., Haines G.K., Koch A.E. (2002) Differential expression of the angiogenic Tie receptor family in arthritic and normal synovial tissue. *Arthritis Res.* **4**, 201–208.

Sihvonen S., Korpela M., Laippala P., Mustonen J., Pasternack A. (2004) Death rates and causes of death in patients with rheumatoid arthritis: a population-based study. *Scand. J. Rheumatol.* **33**, 221–227.

Sivakumar B., Akhavani M.A., Winlove C.P., Taylor P.C., Paleolog E.M., Kang N. (2008) Synovial hypoxia as a cause of tendon rupture in rheumatoid arthritis. *J. Hand Surg. [Am.]* **33**, 49–58.

Smolen J., van der Heijde D., Devogelaer J.-P. *et al.* (2004) The Effect of Infliximab Therapy on the Prevention of New Erosions in Patients with Early Rheumatoid Arthritis. *Arthritis Rheum.* **50**(Suppl. 9), Abstract 357.

Sone H., Kawakami Y., Sakauchi M. *et al.* (2001a) Neutralization of vascular endothelial growth factor prevents collagen-induced arthritis and ameliorates established disease in mice. *Biochem. Biophys. Res. Commun.* **281**, 562–568.

Sone H., Sakauchi M., Takahashi A. *et al.* (2001b) Elevated levels of vascular endothelial growth factor in the sera of patients with rheumatoid arthritis correlation with disease activity. *Life Sci.* **69**, 1861–1869.

Sowter H.M., Raval R.R., Moore J.W., Ratcliffe P.J., Harris A.L. (2003) Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. *Cancer Res.* **63**, 6130–6134.

Stevens C.R., Blake D.R., Merry P., Revell P.A., Levick J.R. (1991a) A comparative study by morphometry of the microvasculature in normal and rheumatoid synovium. *Arthritis Rheum.* **34**, 1508–1513.

Stevens C.R., Williams R.B., Farrell A.J., Blake D.R. (1991b) Hypoxia and inflammatory synovitis: observations and speculation. *Ann. Rheum. Dis.* **50**, 124–132.

Sumariwalla P., Cao Y., Wu H., Feldmann M., Paleolog E. (2003) The angiogenesis inhibitor protease-activated kringles 1-5 reduces the severity of murine collagen-induced arthritis. *Arthritis Res. Ther.* **5**, R32–R39.

Taylor P.C., Steuer A., Gruber J. *et al.* (2006) Ultrasonographic and radiographic results from a two-year controlled trial of immediate or one-year-delayed addition of infliximab to ongoing methotrexate therapy in patients with erosive early rheumatoid arthritis. *Arthritis Rheum.* **54**, 47–53.

Treuhaft P.S. & McCarty D. (1971) Synovial fluid pH, lactate, oxygen and carbon dioxide partial pressure in various joint diseases. *Arthritis Rheum.* **14**, 475–484.

Uchida T., Nakashima M., Hirota Y., Miyazaki Y., Tsukazaki T., Shindo H. (2000) Immunohistochemical localisation of protein tyrosine kinase receptors Tie-1 and Tie-2 in synovial tissue of rheumatoid arthritis: correlation with angiogenesis and synovial proliferation. *Ann. Rheum. Dis.* **59**, 607–614.

Van Doornum S., Brand C., King B., Sundararajan V. (2006) Increased case fatality rates following a first acute cardiovascular event in patients with rheumatoid arthritis. *Arthritis Rheum.* **54**, 2061–2068.

Walsh D.A., Wade M., Mapp P.I., Blake D.R. (1998) Focally regulated endothelial proliferation and cell death in human synovium. *Am. J. Pathol.* **152**, 691–702.

Wang G.L., Jiang B.H., Rue E.A., Semenza G.L. (1995) Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5510–5514.

Williams R.O., Mason L.J., Feldmann M., Maini R.N. (1994) Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2762–2766.

Williams R.O., Ghrayeb J., Feldmann M., Maini R.N. (1995) Successful therapy of collagen-induced arthritis with TNF receptor-IgG fusion protein and combination with anti-CD4. *Immunology* **84**, 433–439.

Williams R.O., Marinova-Mutafchieva L., Feldmann M., Maini R.N. (2000) Evaluation of TNF-alpha and IL-1 blockade in collagen-induced arthritis and comparison with combined anti-TNF-alpha/anti-CD4 therapy. *J. Immunol.* **165**, 7240–7245.

Williams R.O., Paleolog E., Feldmann M. (2007) Cytokine inhibitors in rheumatoid arthritis and other autoimmune diseases. *Curr. Opin. Pharmacol.* **7**, 1–6.

Williamson S.C. & Feldon P. (1995) Extensor tendon ruptures in rheumatoid arthritis. *Hand Clin.* **11**, 449–459.

Witzenbichler B., Maisonpierre P.C., Jones P., Yancopoulos G.D., Isner J.M. (1998) Chemotactic properties of angiopoietin-1 and -2, ligands for the endothelial-specific receptor tyrosine kinase Tie2. *J. Biol. Chem.* **273**, 18514–18521.

Yuan H.T., Venkatesha S., Chan B. *et al.* (2007) Activation of the orphan endothelial receptor Tie1 modifies Tie2-mediated intracellular signaling and cell survival. *FASEB J.* **21**, 3171–3183.

Yukioka K., Inaba M., Furumitsu Y. *et al.* (1994) Levels of hepatocyte growth factor in synovial fluid and serum of patients with rheumatoid arthritis and release of hepatocyte growth factor by rheumatoid synovial fluid cells. *J. Rheumatol.* **21**, 2184–2189.

