Vascular endothelium — role in chronic inflammatory disease

ABSTRACT

The vascular endothelial lining of blood vessels plays a key ‘target-effector’ role in vivo, integrating the body’s response to inflammatory cytokines, chemokines and growth factors (derived from both endothelial cells themselves and from other cells such as leukocytes and fibroblasts), to allow leukocyte activation, adhesion and extravasation from the flowing blood into underlying tissue. Endothelial proliferation, through the process of angiogenesis, results in an increased cell surface area for these events to occur, and further functions to deliver oxygen and nutrients, and to remove waste products. In addition to playing an important role in physiology, the endothelium is thus an active participant in inflammatory pathologies. One of the best understood diseases in which inflammation and angiogenesis play a part is rheumatoid arthritis (RA). Blockade of the inflammatory cascade in RA has significant consequences for the vasculature, highlighting the links between reducing endothelial activation and therapeutic benefit in chronic inflammatory disorders.

INTRODUCTION

The human vascular endothelial lining of blood vessels, which covers the body’s network of arteries, veins, capillaries and lymphatics, was in the past thought to be merely an inactive barrier between the circulation and underlying tissues. However, it is now clear that despite its relatively small total mass, endothelium actively participates in physiology and pathology in vivo. Endothelial cells produce mediators regulating blood flow, and influence coagulation and fibrinolysis, usually presenting a non-thrombogenic surface to flowing blood. Furthermore, the endothelium plays a role in the process of cell recruitment, through expression of cytokines and chemokines, thus affecting the activation status of leukocytes. Finally, endothelial cells play a central role in the process of angiogenesis, which is vital for efficient supply of oxygen and nutrients to tissue, and for removal of waste products.

Vascular endothelium thus fulfills a vital homeostatic function and acts as a rapid response facility in situations of inflammation, injury or infection. Indeed endothelium plays an important target-effector role in many diseases associated with inflammation. Such diseases include diabetes type 1, where microvascular and macrovascular complications combine with activation of the immune system and inflammation. In chronic airway disease, inflammation leads to changes in the airways and obstruction of airflow, but other events include vascular remodelling and angiogenesis. Other disorders, such as systemic lupus erythematosus, atherosclerosis and inflammatory bowel diseases, also involve immune system activation and enhanced blood coagulation in association with pro-inflammatory cytokine expression.

Rheumatoid arthritis (RA) is a prototypical inflammatory disease, in which angiogenesis and changes in oxygen tension interact with inflammation to culminate in the features of joint and cartilage destruction. Since the pathogenesis of RA is relatively well understood, RA can serve as a paradigm for understanding the role of the vasculature in inflammation, particularly in the light of observations using therapies targeting aspects of the inflammatory process in RA, such as anti-tumour necrosis factor α (TNFα) biologicals. The involvement of the vasculature in RA pathogenesis will be discussed in detail in the following sections.

RHEUMATOID ARTHRITIS — A PROTOTYPICAL INFLAMMATORY DISEASE WITH VASCULAR INVOLVEMENT

RA is a common human disease, affecting about 1% of the population in most parts of the world, and is characterized by inflammation of the synovial membrane which lines the joint spaces, leading to the localized invasion and
destruction of underlying cartilage and bone. Every year in the United Kingdom there are approximately 20 000 new cases of RA, which is more common in women than men by a factor of 3:1 [1]. The clinical presentation can vary in terms of severity and the age of onset, although the peak of RA onset occurs between the fifth and sixth decades of life. Patients display painful, stiff and swollen joints, and usually present with a symmetrical polyarthritis, predominantly involving the small joints of the hands and wrists, as well as the metatarsophalangeal joints, ankles and knees. RA is associated with a range of non-articular symptoms, including inflammatory nodules, vasculitis and pericarditis, together with involvement of the lungs and nervous system, depression and anaemia. Furthermore, the standardized mortality ratio for patients with RA is more than 1.5–2.5-fold higher than for the general population. The major cause of mortality (more than 40% of deaths) is cardiovascular disease, including ischemic heart disease and heart failure [2]. A total of 10 million working days were lost in 2006–2007 in the UK due to musculoskeletal conditions such as RA, second only to stress, depression and anxiety, representing a significant economic impact due to lost production. RA patients of working age are significantly more likely to stop work on health grounds than matched controls. RA thus imposes a significant social and economic burden, due to loss of earnings and medical expenses, apart from adversely affecting quality of life.

In spite of many years of intensive investigation, the cause of RA remains unknown, although current thinking favours the concept of a multi-factorial disease, in which contributory genetic factors, including shared epitope alleles of the human leukocyte antigen and a polymorphism of protein tyrosine phosphatase N22 [3,4], combine with environmental factors (such as smoking), sex hormones, and perhaps an infectious agent or other immune-activating factor, to initiate an autoimmune response that culminates in a disease with inflammatory and destructive features [5]. As mentioned, the primary site of inflammation is the synovial lining of the closed spaces of articular joints. The normal synovium is generally 1–3 cell layers thick and is composed of loosely associated macrophage- and fibroblast-like cells, as well as vascular endothelial cells. In RA, the synovium is altered to a thickened tissue several cell layers thick, and becomes infiltrated by blood-derived cells, including T cells, B cells and macrophages. Subsequently the synovium becomes locally invasive at the synovial interface with cartilage and bone. Progressive destruction of cartilage and bone eventually combine to produce deformities and functional deterioration and profound disability in the long term [6].

Of relevance to this review, the term “vascular rheumatology” has been coined, to emphasise the importance of microvascular and macrovascular endothelium in RA and in other rheumatic disorders.

ROLE OF ENDOTHELium IN INFLAMMATORY DISEASE

RA, as an autoimmune disease, is characterized by the presence of circulating auto-antibodies, including some that bind with high affinity to human endothelial cells [7]. Integration of antigen-presentation, amplification of lymphocytes and generation of mediators of humoral and cellular immunity needs to occur in the peripheral lymphoid organs, primarily lymph nodes and spleen. T lymphocytes circulate between non-lymphoid tissues and the lymph nodes, entering through the afferent lymphatic blood vessels and across the high endothelial venules, and exiting via efferent lymphatic vessels. This continuous lymphocyte trafficking across endothelium enables the antigen-sensitive cells to be exposed to their specific antigen, prompting clonal expansion. Blood vessels thus allow recruitment of the activated lymphocytes to specific sites, which is promoted further by vasodilation. An increase in vessel density through angiogenesis further increases the endothelial surface area available for the ingress of cells and molecules to the site of inflammation, amplifying and maintaining the immune response.

The involvement of endothelium in the pathogenesis of RA can also be inferred from observations that RA is associated with vascular and haematological abnormalities (such as anaemia). The swollen joints which occur in RA patients suggest altered vascular permeability, with increased plasma extravasation and oedema formation. Interestingly, a recent study has documented the presence of immature blood vessels in RA synovium. Comparison of the staining patterns for CD31 and the pericyte marker α-smooth muscle cell actin revealed a significant fraction of CD31-positive but α-smooth muscle cell actin-negative cells in RA tissue when compared to osteoarthritis or control tissue [8]. This altered vascular signal in RA synovium was also detected using Doppler ultrasound [9,10]. Studies using mouse models of RA have also shown vascularised synovium in arthritic mice [11] (Fig. 1).

Therefore, activation of an effective immune response, enhanced leukocyte activation and extravasation, expression of chemokines, and increased angiogenesis, possibly leading to formation of immature and highly permeable

Figure 1. Synovial vascularity in an in vivo model of arthritis. Arthritis was induced in mice using bovine collagen and arthritis development and severity were monitored daily [11]. Serial sections of paw tissue from healthy (A, C) and severely arthritic (B, D) mice were stained with either (A, B) haematoxylin and eosin or (C, D) with anti-CD31 antibody and counter-stained with haematoxylin. Images show the metatarsal joint of mouse paws. The healthy section demonstrates a normal joint architecture without signs of inflammation or bone destruction. In the arthritic joint, synovial hyperplasia, blood vessels (CD31-positive), inflammatory cell infiltration, and loss of cartilage and bone are evident. Abbreviations: B – bone; C – cartilage; S – synovium; JS – joint space.
blood vessels, all point to the underlying role of inflammation-mediated vascular endothelial activation in RA, which will be discussed in more detail in subsequent sections.

LEUKOCYTE-ENDOTHELIAL INTERACTIONS IN RA

The cellular infiltration which is a characteristic of RA synovium suggests that activation of endothelium, together with expression of leukocyte adhesion molecules, as well as of cytokines and chemokines, is likely to be involved in RA pathogenesis. Adhesion of leukocytes to vascular endothelium \textit{in vivo} must overcome the normal vascular mobility of circulating cells and result in a localized arrest of leukocytes at relevant sites. Endothelial cells resembling high endothelium of circulating cells and result in a localized arrest of leukocytes at relevant sites. Endothelial cells resembling high endothelial venules, which control lymphocyte migration into organized lymphoid tissues, have been observed in RA synovium. These synovial microvascular endothelial cells acquire a cuboidal morphology, and become fenestrated [12], suggesting specialised mechanism(s) regulating leukocyte extravasation into joint tissue.

Inflammatory cytokines such as TNFα and interleukin (IL)-1, which play a central role in RA pathogenesis, have the potential capacity to regulate many of the events occurring in the RA microvasculature, including leukocyte extravasation and chemotaxis [13]. TNFα is a fundamental inducer of endothelial cell responses, and both TNFα receptors CD120a/TNF-R1 and CD120b/TNF-R2 have been detected on RA synovial endothelial cells. In RA, endothelial cells express numerous cytokine-inducible adhesion molecules, including E-selectin [14], vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1. For example, scintigraphy utilizing a $^{99m}$Tc-anti-E-selectin-Fab was used to image synovitis in RA, and demonstrated improved specificity compared to a conventional tracer for bone and joint, and specificity for targeting active joint inflammation [15]. In our laboratory, we recently demonstrated that imaging of anti-E-selectin \textit{in vivo} could detect endothelial activation in models of arthritis and could be applied to quantify disease and investigate the effects of novel therapies [16]. Elevated levels in RA of soluble forms of E-selectin, ICAM-1 and VCAM-1 have also been described. Synovial membrane and synovial fluid T cells display an enhanced capacity to interact with purified E-selectin and VCAM-1, relative to peripheral blood lymphocytes from either the same patients or from healthy donors, due to increased levels of VLA-4α, the counter-ligand for VCAM-1. In addition, synovial fluid lymphocytes show higher expression of other integrins such as CD29, VLA-1α, VLA-5α and VLA-6α [17,18]. Accumulation of T cells in RA synovium thus appears to result from elevated expression of adhesion receptors on synovial microvascular endothelium, leading to the selective emigration of memory T lymphocytes, which may bear enhanced levels of ligands for these adhesion molecules as a result of a previous activation step. Other adhesion molecules present on synovial endothelial cells include CD31, vascular adhesion proteins (VAP)-1 and VAP-2 and CD146 [19].

Moreover, endothelial cells are a source of a range of pro-inflammatory cytokines, including IL-1, IL-6 and granulocyte macrophage colony-stimulating factor. Many of the features of the rheumatoid synovial environment suggest possible roles for chemoattractant cytokines, in that the large number of infiltrating leukocytes, especially the accumulation of monocyte/macrophages and lymphocytes, could in part be a response to the elaboration of chemokines. Endothelial cells secrete and present on cell surface proteoglycans chemokines of both CC and CXC sub-sets, in particular IL-8/CXCL8, monocyte chemoattractant protein-1/CCL2, RANTES/CCL5 and Groα/CXCL1 [20]. The ability of endothelium to capture chemokines ensures that mediators become anchored on the endothelial surface, to enable a relatively high concentration of chemoattractants close to the vessel wall, and hence to temporally and spatially regulate activation of circulating cells (Fig. 2).

ENDOTHELIUM AND ANGIOGENESIS IN RA

Another feature of the synovium in RA is altered density of sub-lining capillaries and post-capillary venules, supporting an important role for angiogenesis. Endothelial cells, exposed to inflammatory cytokines and growth factors, respond by altering their proliferation rate and cellular metabolism, to form new
blood vessels. Blood vessels therefore fulfill an important role in RA, fuelling synovial expansion and infiltration by inflammatory cells from the blood, by supplying oxygen and nutrients necessary for cell metabolism and division, as well as by bringing in leukocytes and signalling mediators such as cytokines and growth factors [21-25]. The number of synovial blood vessels has been found to correlate with synovial cell hyperplasia and indices of joint tenderness [26]. Endothelial cells lining blood vessels within RA synovium express cell cycle-associated antigens, and indices of endothelial turnover are increased in synovia from patients with RA compared with non-inflamed controls. A morphometric study also suggested that capillaries are distributed more deeply in RA synovium [27], and endothelial proliferation was shown to be increased in synovium from patients with RA [28].

Many of the cytokines and growth factors expressed in RA have the potential to stimulate endothelial proliferation [21]. For example, serum levels of vascular endothelial growth factor (VEGF) are markedly elevated in RA, relative to either patients with osteoarthritis or normal controls, and correlate with levels of inflammatory markers such as C-reactive protein (CRP). Serum VEGF levels have also been shown to correlate with blood flow in wrist synovium of patients with RA [29]. Expression of VEGF by RA lining layer cells has been reported, and microvascular endothelial cells in the vicinity of VEGF-positive cells express VEGF receptors [30]. Conditioned medium from synovial tissue explants was shown to be mitogenic for endothelial cells, an activity reduced by anti-VEGF antibody [30], further supporting the concept of an important role for endothelium and VEGF in RA. In addition to synovial expression of VEGF, circulating (serum) levels of VEGF are increased, and correlate with inflammatory response markers [31,32]. Treatment of murine arthritis using anti-VEGF antibody delayed disease onset and reduced symptoms of arthritis [33,34]. Targeting VEGF receptors, specifically VEGF receptor 1, also resulted in disease amelioration [35-37]. In summary, the invasive synovium in RA is highly vascularised, and molecules such as VEGF are expressed, and are thus likely to significantly modulate endothelial activation (Fig. 2).

CARDIOVASCULAR DISEASE IN RA — ROLE OF ENDOTHELIAL DYSFUNCTION

The mortality of patients with severe RA is equivalent to that of individuals with triple vessel coronary artery disease, with the major cause of mortality (more than 40% of deaths) being cardiovascular disease, including ischemic heart disease and heart failure [38]. Endothelial dysfunction is known to occur in RA, providing a possible link between these seemingly disparate pathologies. The endothelial lining of blood vessels has a critical function in atherosclerosis, serving as the site of initial injury and leukocyte adhesion/migration. Maintenance of an intact vascular lining and an uninterrupted vascular supply is thus critical in the prevention of the cascade of events which trigger acute coronary syndromes such as myocardial infarction. Many studies have reported an association between RA and traditional cardiovascular risk factors such as cholesterol and low density lipoprotein levels. The acute phase response inflammatory marker CRP is a risk factor for atherosclerosis, and CRP levels are markedly elevated in RA, as part of the ongoing systemic inflammatory processes, suggesting that such an augmented inflammatory burden may be linked to the increased cardiovascular risk in RA [39,40].

Interestingly, the process of vasodilation is altered in RA. Herbrig et al., who studied blood flow in the forearm following infusion of acetylcholine, showed that vasodilatation was significantly reduced in RA patients [41]. A more recent study examined the relationship between flow-mediated endothelium-dependent vasodilatation and carotid artery intima-media wall in RA patients without clinically evident cardiovascular disease, and found that carotid intima-media thickness values were higher and flow-mediated vasodilatation were lower in individuals with long-standing RA compared to those with shorter disease duration [42]. Another recent study reported arterial stiffness to be associated with endothelial dysfunction and atherosclerosis in patients with autoimmune diseases such as RA [43].

Furthermore, although blood vessel density is altered in RA, and angiogenesis has generally been thought to underlie these changes, endothelial progenitor cells may also play a role. Endothelial progenitor cells have been found to differentiate into endothelial cells, express classic endothelial cell markers, including CD31, CD34 and VEGF receptor 2 and to exhibit endothelial cell properties, such as expression of the endothelial-specific isoform of nitric oxide synthase (eNOS) and the adhesion molecule E-selektin. The endothelial cells present in the circulation are capable of integrating into vessel walls, and it is these endothelial progenitor cells which may link RA with increased cardiovascular morbidity and mortality. In RA synovium, CD34/VEGF receptor 2-positive cells have been described found in apposition to synovial blood vessels [44]. Bone marrow-derived CD34-positive cells, which can expand into CD34- and von Willebrand factor-expressing cells, have been reported to be generated at a higher rate from bone marrow samples taken from RA patients, compared to normal subjects. Furthermore, the capacity of bone marrow-derived cells from RA patients to progress into endothelial cells correlated with synovial microvessel density [45]. In a parallel to the situation seen with coronary artery disease and ischemic heart disease patients, endothelial progenitor cells numbers are decreased in the peripheral blood of RA patients compared with healthy individuals. Circulating endothelial progenitor cells (CD34/VEGF receptor 2-positive) were lower in patients with active RA than in individuals with inactive disease or healthy controls [46]. The observation of reduced circulating endothelial progenitor cells in RA patients was confirmed in another study, which demonstrated reduced migration of endothelial progenitor cells from RA patients in response to VEGF, suggesting that the functional capacity of these cells may be attenuated in RA. Endothelial progenitor cells from RA patients exhibited only modest adhesion to endothelial cells stimulated with TNFα, compared with cells from healthy subjects, despite comparable levels of adhe-
sion to unstimulated endothelial cells or matrix proteins such as fibronectin or laminin [41].

The above data suggest enhanced recruitment from peripheral blood of endothelial progenitor cells to RA synovium. This might then lead to increased RA synovial blood vessel formation, perpetuating disease. Furthermore, increased endothelial progenitor cell trafficking to the synovium would be paralleled by reduced peripheral blood endothelial progenitor cells in RA, which could be a significant factor in the increased cardiovascular morbidity and mortality seen in RA.

INSIGHTS FROM STUDIES USING TNFα INHIBITORS

Over the last 25 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. TNFα mediates many inflammatory and immunoregulatory activities relevant in RA. The concept of TNFα as a therapeutic target was put forward by Feldmann and Maini in the late 1980s, and to date several biological inhibitors of this cytokine have been approved for use in RA.

Clinical trials of these inhibitors, which commenced in the late 1990s, have shed considerable light on the role of the endothelium in RA. The first Phase I/II study was an open-label trial of a single intra-venous infusion of infliximab (Remicade™), a chimeric mouse Fv-human IgG1k antibody that binds both soluble and membrane-bound TNFα with high affinity, in long-standing active RA patients who had failed all prior therapy. The results were striking, showing reductions in pain and morning stiffness, swollen and tender joint counts, and CRP levels. Since trafficking into the synovium of blood-borne cells is a feature of RA, and since TNFα is one of the most potent regulators of leukocyte trafficking, it seemed reasonable to hypothesize that anti-TNFα antibody treatment might regulate synovial infiltration. This question has been addressed over the years with increasingly sophisticated studies, which started with the measurement of soluble adhesion molecules, which could be quantified in serially acquired serum samples. Levels of serum E-selectin and ICAM-1 were decreased after infusion of anti-TNFα antibody, and the extent of the decrease was significantly higher in patients in whom a clinical benefit of anti-TNFα was observed. Moreover, a significant reduction was observed in CD3- and CD68-positive cells in the synovium, as well as a decrease in synovial expression of VCAM-1 and E-selectin [47,48]. Later studies showed that synovial and serum chemokines (IL-8/CXCL8 and monocyte chemoattractant protein-1/CCL2) were decreased [47]. These reductions correlated with a rapid and sustained increase in blood lymphocyte counts [48].

Direct evidence of an effect on cell trafficking came from elegant studies using gamma camera imaging of radioactively labelled polymorphonuclear cells. Patients with long-standing RA received a single infusion of anti-TNFα antibody, and the articular localization of autologous polymorphonuclear cells, separated in vitro and labelled with 111In, was studied before and 2 weeks after treatment. Anti-TNFα therapy in RA significantly reduced 111In-labelled granulocyte migration into affected joints. There was a simultaneous reduction in the numbers of infiltrating synovial CD3-positive T cells, CD22-positive B cells, and CD68-positive macrophages [47].

In later studies, ultrasonography has been used to measure synovial inflammation and vascularity. These investigations have shown that assessment of synovial thickening and vascularity at baseline was an early and sensitive measure of response to treatment and radiological changes to anti-TNFα antibody [10]. Indeed ultrasonographic measures of synovial thickening and vascularity were able to discriminate between RA patients receiving anti-TNFα or placebo with greater sensitivity than conventionally used outcome measures of change in disease activity that depend on the use of clinical evaluation, such as the numbers of tender and swollen joints.

In the context of effects on endothelial progenitor cells, RA patients with active disease treated with TNFα inhibitors showed a restoration of circulating endothelial progenitor cells levels to those seen in healthy control subjects. This effect was not seen in patients with active RA but receiving conventional disease-modifying drugs [46]. A more recent study directly examined the effect of anti-TNFα antibody on endothelial progenitor cell numbers and function. A significant increase in endothelial progenitor cell levels and adhesion was seen after 2 weeks of anti-TNFα treatment, together with an improvement in the disease activity score. Interestingly, a significant correlation was seen between the extent of clinical response and the degree of increase in endothelial progenitor cell numbers [49]. The cardiovascular risk profile is also altered after TNFα blockade. Treatment with anti-TNFα antibody significantly increased concentrations of fibrinogen and HDL-cholesterol, whereas LDL and triglyceride levels were unchanged, and no changes in lipid profile were seen in the placebo group [50]. Similarly, treatment with TNFα inhibitors has been reported to reduce the incidence of first cardiovascular events in patients with RA [51].

Given that serum VEGF levels were elevated in patients with RA, it seemed reasonable to suppose that treatment of RA with anti-cytokine biologics might modulate VEGF expression. To examine this hypothesis, we measured serum VEGF levels in RA patients treated with anti-TNFα antibody. In patients receiving a single infusion of anti-TNFα, serum VEGF levels were markedly reduced. Treatment of RA patients with a combination of multiple infusions of anti-TNFα and methotrexate resulted in a more prolonged decrease in serum VEGF levels relative to patients who received anti-TNFα antibody alone [32]. As discussed, the presence or density of immature vessels is significantly increased in RA patients, and interestingly, immature vessels were depleted in response to anti-TNFα therapy, highlighting the co-dependency of angiogenesis and inflammation [8]. Furthermore, as mentioned earlier, endothelial dysfunction is a feature of RA. Impaired flow-mediated vasodilation in RA patients was reversed following TNFα inhibition [52,53].
Cellular responses in situations of reduced availability of oxygen are coordinated by the hypoxia-inducible factor (HIF) transcription factor family. Activation of HIF signalling leads to extensive changes in gene expression, to allow adaption of cells and tissues to reduced oxygenation. The HIF complex consists of a constitutively expressed β subunit, and an oxygen-responsive α subunit. Regulation of HIF- dependent gene expression requires α-subunit accumulation in the cytoplasm and translocation into the nucleus, which enables HIF-α to dimerise with HIF-β and bind HIF co-activators, prior to binding hypoxia-response elements in the target gene to initiate transcription. Hydroxylation by FIH-1 of asparagine residues in HIF-α prevents recruitment of co-activators p300/CBP and thereby HIF-mediated gene transcription. In contrast, prolyl hydroxylase domain (PHD) enzymes (PHD1-3) modify HIF-α by hydroxylation of specific proline residues in HIF-α, enabling capture by an E3 ubiquitin ligase complex, leading to proteasomal destruction of HIF-α. FIH-1 and PHD1-3 belong to a superfamily of 2-oxoglutarate and iron dependent dioxygenases, which require molecular oxygen as a co-substrate [58]. Thus, under conditions where O₂ supply limited, as is the case in RA synovium, HIF-α subunits accumulate and activate gene transcription. In RA synovial tissue, HIF-1α-positive cells correlate with the number of blood vessels and with inflammatory endothelial cell infiltration, proliferation and synovitis [59].

Hypoxia alters the expression of a number of endothelial genes, including those involved in the inflammatory response. For example, increased expression of chemokines such as CCL15 and IL-8/CXCL8 has been described in endothelial cells exposed to hypoxia, suggesting that altered oxygen tension may influence leukocyte activation [60,61]. Increased leukocyte adhesion to endothelial cells exposed to low oxygen tension has also been described [62,63], and hypoxia may synergise with inflammatory cytokines such as TNFα to upregulate E-selectin and ICAM-1 [64]. Transcriptomic and proteomic analyses have shown that hypoxia activates endothelial cells to express cytokines, growth factors, extracellular matrix protein genes, collagens and members of the PHD family in a HIF-1-dependent manner, and that hypoxia increased basement membrane invasion and tube formation by endothelial cells [65-67]. Of potential relevance to RA, hypoxia increases endothelial permeability, affecting adhesion molecules such as VE-cadherin and Rho GTPases regulating the actin cytoskeleton, such as RhoA and Rac1 [68].

Hypoxia may also affect endothelial activation indirectly, by activating synovial cells to express factors which stimulate endothelial cell responses. Hypoxia increases expression by synovial cells of pro-angiogenic factors such as VEGF [32], as well as chemokines IL-8/CXCL8 [69], CCL20 [70] and SDF-1/CXCL12 [71,72]. Increased levels of pro-inflammatory cytokines such as IL-6, and of matrix-metalloprotease (MMP) enzymes MMP-1 and MMP-3 [69] together with enhanced synovial cell invasiveness, in response to hypoxia have also been reported. In a recent study, interaction be-

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Taken together, these observations suggest that at least part of the clinical effectiveness of TNFα blockade in RA is due to deactivation of vascular endothelium, leading to reduced inflammation, cell trafficking (and, as a consequence, diminished synovial cellularity) and angiogenesis, and normalisation of coagulation and fibrinolytic systems (summarised in Tab. 1). This is supported by a recent finding regarding Certolizumab pegol, a humanised anti-TNFα antibody approved for clinical use for RA. Certolizumab significantly blocked TNFα-induced E-selectin, VCAM-1 and ICAM-1 expression on microvascular endothelial cells, as well as chemokine expression and endothelial tube formation, and adhesion of HL60 leukaemia cells to endothelial cells [54]. These data demonstrate that blockade of the inflammatory cascade in RA, using approaches such as TNFα inhibitors, diminishes endothelial activation and is associated with clinical benefit.

ENDOTHELIUM AND INFLAMMATION – INTERACTION WITH HYPOXIA

Mammalian cells and tissues are exposed to various oxygen tensions, depending on their location, frequently as low as 5% in the case of venular endothelial cells [55]. A complex interplay between altered oxygen levels and inflammation is involved in the pathogenesis of inflammatory diseases such as RA. The micro-environment in the inflamed joint is characterised by a low partial pressure of oxygen, first demonstrated more than 40 years ago. Mean synovial fluid O₂ in knee joints was reported to be lower in RA patients [56] than in osteoarthritis patients or in healthy controls. Despite these intriguing observations, it was only recently that we were able to directly measure synovial oxygen tension in RA patients using a highly sensitive gold microelectrode. We observed that synovial tissue in RA patients was indeed hypoxic, with O₂ lower than in non-inflamed synovium in patients without RA [56]. This hypoxic milieu leads to a cascade of enhanced expression of hypoxia-regulated transcription factors and hypoxia-responsive genes, and increased levels of pro-inflammatory cytokines and angiogenic factors, establishing a link between synovial hypoxia and inflammation in RA [25,57].

Table 1. Summary of the effects of anti-TNFα on the vasculature in RA.

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tween hypoxia, HIF and the Notch signalling pathway was shown to play an important role in hypoxia-induced angiogenesis. Notch-1 was highly expressed in inflamed synovial tissue and was located predominantly to perivascular/vascular regions, and inhibition of Notch-1 by RNA interference significantly attenuated hypoxia-induced angiogenesis and endothelial cell function [73].

Synovial hypoxia is therefore likely to contribute to RA by promoting inflammation, angiogenesis, cellular infiltration and cartilage degradation. However, recent emerging evidence suggests the opposite, adding some controversy to the previous well established dogma, and increasing the need of further studies for a better understanding of the role of hypoxia/HIF in RA. A very good example is the role of HIF in anaemia, which as in many chronic inflammatory diseases, is one of the most common extra-articular manifestations of RA, estimated to occur in 30-60% of RA patients [74]. Anaemic patients have more severe RA and also have more affected joints and higher levels of functional disability and pain. Studies have shown that treating anaemia in RA patients leads to reduced joint swelling and had a positive effect on patients’ quality of life. A key mediator of anaemia in RA is hepcidin, a regulatory hormone that limits iron availability and suppresses erythropoiesis under conditions of inflammation. Expression of hepcidin is induced by IL-6, a major player in the pathogenesis of RA and increased levels of serum hepcidin were directly linked to the occurrence of coronary artery atherosclerosis in RA patients [75]. Under conditions of hypoxia however, the expression of hepcidin is repressed to permit physiological adaptation to tissue oxygen tension. It has been reported that inhibition of the PHD enzymes by deferoxamine or dimethyloxaloylglycine was also able to down-regulate hepcidin expression, independently of HIF [76]. However, recently Liu and colleagues has shown that suppression of hepcidin was mediated by HIF, indirectly through erythropoietin-induced erythropoiesis [77].

CONCLUSIONS

The response of vascular endothelium to cytokines, chemokines and growth factors governs subsequent resolution or perpetuation of the inflammatory cascade in vivo. Inappropriate or excessive responses result in consequences such as leukocyte extravasation, immune activation and angiogenesis, thus contributing to certain diseases such as RA.

Some questions regarding the function of endothelium in chronic inflammatory disorders such as RA still remain unanswered, for example regarding the relative roles of the different adhesion molecules and chemokines. However, it is not unreasonable to suggest that targeting the vasculature in RA, for example using angiogenesis inhibitors, in combination with other therapies such as anti-TNFα, may lead to a more persistent reduction in synovial volume and hence modify disease progression, but confirmation of this hypothesis requires appropriate clinical trials. It is likely that our understanding of RA is a model of chronic inflammatory disease will allow elucidation of the potential of developing new therapeutic approaches for treatment of other disorders in which inflammation, hypoxia and the vasculature are involved, such as atherosclerosis, psoriasis, diabetes and cancer.

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Śródbłonek wyścielający naczynia krwionośne — rola w przewlekłych chorobach zapalnych

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STRESZCZENIE

Śródbłonek wyścielający naczynia krwionośne odgrywa kluczową rolę w regulacji odpowiedzi organizmu na cytokiny prozapalne, chemokin i czynniki wzrostu (pochodzące zarówno z samych komórek śródbłonkowych jak i innych komórek — np. leukocytów czy fibroblastów), wpływając na aktywację leukocytów, ich adhezję oraz migrację z miejsca zapalenia do przylegających tkank. Zablokowanie odpowiedzi zapalnej w RA znacząco wpływa na unaczynienie, potwierdzając współzależność między zahamowaniem aktywacji śródbłonka i leczeniem chronicznych stanów zapalnych.