Review

Endothelial cell phenotypes in the rheumatoid synovium: activated, angiogenic, apoptotic and leaky

Jim Middleton[1], Laure Americh[1], Regis Gayon[1], Denis Julien[1], Luc Aguilar[1], Francois Amalric[1,2] and Jean-Philippe Girard[1,2]

[1]Endocube S.A.S., Prologue Biotech, Labege cedex, France
[2]Laboratoire de Biologie Vasculaire, Equipe Labellisée 'LA LIGUE 2003', IPBS-CNRS UMR 5089, Toulouse cedex, France

Corresponding author: Jim Middleton (e-mail: jim.middleton@rjah.nhs.uk)

Received: 3 Nov 2003 Revisions requested: 3 Dec 2003 Revisions received: 28 Jan 2004 Accepted: 4 Feb 2004 Published: 8 Mar 2004

Arthritis Res Ther 2004, **6**:60-72 (DOI 10.1186/ar1156)

Abstract

Endothelial cells are active participants in chronic inflammatory diseases. These cells undergo phenotypic changes that can be characterised as activated, angiogenic, apoptotic and leaky. In the present review, these phenotypes are described in the context of human rheumatoid arthritis as the disease example. Endothelial cells become activated in rheumatoid arthritis pathophysiology, expressing adhesion molecules and presenting chemokines, leading to leukocyte migration from the blood into the tissue. Endothelial cell permeability increases, leading to oedema formation and swelling of the joints. These cells proliferate as part of the angiogenic response and there is also a net increase in the turnover of endothelial cells since the number of apoptotic endothelial cells increases. The endothelium expresses various cytokines, cytokine receptors and proteases that are involved in angiogenesis, proliferation and tissue degradation. Associated with these mechanisms is a change in the spectrum of genes expressed, some of which are relatively endothelial specific and others are widely expressed by other cells in the synovium. Better knowledge of molecular and functional changes occurring in endothelial cells during chronic inflammation may lead to the development of endothelium-targeted therapies for rheumatoid arthritis and other chronic inflammatory diseases.

Keywords: endothelial cells, phenotypes, rheumatoid, synovium

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease affecting the joints, and is associated with increased morbidity and mortality [1–3]. The synovium or synovial membrane, which surrounds the joint cavity, becomes massively hypertrophied in RA. This tissue, known as pannus, can become invasive, penetrating and degrading the cartilage and bone, resulting in joint deformities, in functional deterioration and in profound disability.

The lining layer, or intima, of the synovium is normally one to three cells thick and it comprises macrophage-like cells and fibroblast-like cells [4]. This layer undergoes thickening and hypertrophy in RA, largely due to the increased recruitment of monocytes from the blood supply in the deeper layer, or subintima, of the tissue [5,6]. Other inflammatory cells such as T cells (mainly CD45RO) and B lymphocytes migrate from the blood into the synovium and can form ectopic lymphoid follicles around blood vessels. These structures resemble the lymphoid follicles of lymph nodes. In addition, neutrophils migrate into the synovium and end up in large numbers in the synovial joint fluid.

The role of endothelial cells in RA

Endothelial cells are active participants in the inflammatory process. They are involved in diverse activities including

COX = cyclooxygenase; CRH = corticotropin-releasing hormone; FGF = fibroblast growth factor; HEV = high endothelial venules; HGF = hepatocyte growth factor; ICAM = intercellular adhesion molecule; IL = interleukin; IFN = interferon; MCP-1 = monocyte chemoattractant protein-1; MHC = major histocompatibility complex; MMP = matrix metalloproteinase; OA = osteoarthritic; RA = rheumatoid arthritis; TIMP = tissue inhibitor of metalloproteinase; TNF = tumour necrosis factor; VCAM = vascular cell adhesion molecule; VEGF = vascular endothelial growth factor.

the regulation of leukocyte extravasation, angiogenesis, cytokine production, protease and extracellular matrix synthesis, vasodilation and blood vessel permeability, and antigen presentation [7].

In RA, endothelial cells in the synovium are generally held to play a central role in the pathophysiology. The cells achieve this in several ways. First, as a component of blood vessels in the subintima, endothelial cells allow the migration of leukocytes such as T cells, B cells, monocytes, neutrophils and dendritic cells into the joint tissues and fluid. Endothelial cells undergo activation, expressing adhesion molecules and presenting chemokines, leading to leukocyte migration from the blood into the tissue. Second, the permeability of endothelial cells increases, leading to plasma extravasation, to oedema formation and to swelling of the joint [8]. Third, endothelial cells proliferate as part of the angiogenic process, which allows a supply of oxygen and nutrients to the growing pannus. There is also a net increase in the turnover of endothelial cells since the number of apoptotic endothelial cells increases as well as the number of proliferative cells [9]. Finally, endothelial cells express various cytokines, cytokine receptors and proteases that are involved in angiogenesis, in proliferation and in tissue degradation.

As part of this spectrum of biological activities, synovial endothelial cells in RA express a variety of phenotypes that can be characterised as being activated, angiogenic, apoptotic and leaky. The intent of the present review is to examine the pattern of human endothelial cell gene expression associated with these phenotypic alterations and to examine whether certain genes are selectively regulated in endothelial cells and not in other cell types. (See Table 1 for a summary of genes.)

Morphological and ultrastructural activation

Changes to the endothelium are among the first pathophysiological events that occur in the human RA synovium, and these changes occur in venules and capillaries rather than in arterioles [8,10]. During the first month of synovitis, these changes include hypertrophy with the cells becoming cuboidal in morphology, the development of gaps between endothelial cells and the presence of multiple concentric layers in the basement membrane. Associated with these changes is the transendothelial migration of numerous mononuclear and polymorphonuclear inflammatory cells.

In another study, synovial biopsies from one patient with monoarthritis who was subsequently found to have RA showed endothelial cell proliferation with no detectable inflammatory infiltrate [8,10]. Endothelial cell proliferation may thus be the initial event in RA. However, another study suggested that the recruitment of mononuclear cells from the blood into the perivascular areas and the lining layer occurs before endothelial morphological alteration and proliferation [11].

The cuboidal morphology of the endothelial cells of synovial venules resembles that of high endothelial venules (HEV), which are the postcapillary venules of lymphoid tissues specialised in lymphocyte migration [12]. This change presumably represents a response to cytokines and other factors that occur in the synovial milieu, and relates to the increased leukocyte trafficking into the tissue [13]. The postcapillary venules of the rheumatoid synovium in patients with active, untreated disease exhibit HEV-like morphology, especially in regions near lymphocyte aggregates, whereas tissue samples from patients whose disease has been modified by treatment exhibit a flatter endothelium [13,14].

In the skin of primates, HEV-like blood vessels are induced by stimulation with tumour necrosis factor (TNF)-α and IFN-γ, which also elicit the adhesion and extravasation of inflammatory cells [15]. Other studies have shown that HEV are not absolutely necessary for transendothelial migration of T cells as migration also occurs through flat endothelium, although the transit time is considerably longer [16]. The HEV-like morphology may thus be associated with the increased leukocyte recruitment that occurs in synovial inflammation, due to the enhanced or selective presentation of chemokines and adhesion molecules, whereas the flatter endothelium may be more associated with basal leukocyte trafficking.

Many of these changes are not specific to RA, since cuboidal endothelial cells have been demonstrated in a variety of inflammatory diseases [12]. In addition, multiple concentric layers in the basement membrane of capillaries have been observed in muscle capillaries in other rheumatic diseases such as systemic lupus erythematosis and systemic sclerosis [17,18], and in nonrheumatic diseases such as Duchenne muscular dystrophy [19].

Gene expression associated with leukocyte migration

Adhesion molecules

As part of leukocyte migration occurring at sites of inflammation, circulating leukocytes adhere to the luminal surface of the endothelium. This interaction, according to the current paradigm, involves the sequential engagement of leukocyte and endothelial adhesion molecules. First, selectins and their carbohydrate counterligands mediate leukocyte tethering and rolling. Leukocyte integrins and their ligands on endothelial cells, including intercellular adhesion molecules (ICAMs), then mediate firm leukocyte adhesion [20].

Endothelial cells have been shown to express a variety of adhesion molecules in the RA synovium. As a part of endothelial activation, several of these molecules are

Table 1

Genes expressed by, or on[a], endothelial cells in the rheumatoid synovium

Gene	Distribution pattern[b]	References
Adhesion molecules		
E-selectin	E, RA > C, P	[141]
P-selectin	E, RA = C, P	[27]
Counterligands for L-selectin (MECA-79 epitope)	E, P	[25]
HECA 452 epitope	EO, RA > C, P	[142]
ICAM-1	EO, RA > C, P	[33,141]
ICAM-2	EO, RA = C, P	[28]
VCAM-1	EO, M, P	[29,30]
Fibronectin	EO, RA > C, M, P	[31,32]
CD31 (PECAM-1)	EO, RA = C, P	[27]
CD146	E, RA = C, P	[34]
Vascular adhesion protein-1	E, P	[35]
CD44	EO, P	[27]
VLA1–VLA6 integrins (α1–6β1)	EO, P	[23]
αVβ3 integrin	EO, RA > C, P	[27,69]
Chemokines		
IL-8 (CXCL8)	EO, RA > C, M, P	[50]
MCP-1 (CCL2)	EO, RA > C, M, P	[46,50]
SLC (CCL21)	EO, M, P	[52,57]
ENA-78 (CXCL5)	EO, RA > C, P	[48]
SDF-1 (CXCL12)	EO, M, P	[49,58,60]
MIP-1α (CCL3)	EO, RA > C, P	[47]
ELC (CCL19)	EO, P	[52]
Chemokine receptors/binding molecules		
CXCR3	EO, P	[59]
CXCR4	EO, P	[58,60]
Duffy antigen	E, M, P	[56]
Heparan sulphate proteoglycan	EO, P	[55]
Angiogenesis regulators and their receptors		
FGF-1 and FGF-2	EO, M, P	[74,75]
Platelet-derived growth factor receptor	EO, M, P	[77]
Hepatocyte growth factor activator and receptor	EO, RA = C, M, P	[78]
Vascular endothelial growth factor	EO, P	[79,80]
Vascular endothelial growth factor receptors (VEGFR1–VEGFR3)	E, RA > C, M, P	[81,82]
Transforming growth factor beta 1	EO, RA > C, P	[83]
Endoglin (transforming growth factor beta receptor)	EO, RA = C, P	[83]
Angiopoietin-1	EO, RA > C, P	[87]
Tie-1 receptor	EO, RA > C, P	[88,89]
Angiogenin	EO, P	[75]
Thrombospondin	EO, RA = C, P	[69,90]
Pleiotrophin	EO, P, M	[143]
Angiotensin converting enzyme	EO, P	[91]
Ets 1 transcription factor	EO, RA > C, M, P	[92]
Axl tyrosine kinase	EO, P	[93]
Proinflammatory cytokines, other mediators and their receptors		
IL-1	EO, P	[97]
Type 1 IL-1 receptor	EO, P	[97]
IL-1 receptor antagonist	EO, P	[97]
Tumour necrosis factor alpha	EO, RA > C, P	[100]
Tumour necrosis factor receptors	EO, P	[99,103]
IL-15	EO, P	[110]
IL-17 receptor	E, RA > C, P	[112]
Substance P receptor	E, P	[67]
Parathyroid hormone-related protein	E, M, P	[144]

Table 1 (continued)

Genes expressed by, or on[a], endothelial cells in the rheumatoid synovium

Gene	Distribution pattern[b]	References
Kinin B2 receptor	EO, RA = C, P	[116]
Corticotropin-releasing hormone receptor type 1	EO, RA > C, M, P	[113]
Urocortin	EO, P	[114]
Endothelin-1	E, P	[145]
Stem cell factor	EO, RA = C, P	[118]
Somatostatin receptor (sst2a)	EO, P	[119]
Midkine	EO, RA > C, P	[117]
Osteoprotegerin	EO, P	[146]
Proteases		
MMP-1 (collagenase)	EO, RA > C, M	[120]
MMP-3 (stromelysin)	EO, M	[122]
MMP-9 (gelatinase B)	EO, RA > C, P	[123]
MMP-13 (collagenase 3)	EO, RA > C, P	[124]
Membrane type 1 matrix metalloproteinase	EO, RA > C, M	[125]
Cathepsin B and cathepsin L	EO, RA > C, M	[120]
Urokinase plasminogen activator receptor	EO, RA > C, P	[127]
Tissue-type plasminogen activator	E, RA = C, P	[128]
TIMP-1 and TIMP-2	EO, RA > C, P	[130]
Other genes		
MHC class II	EO, P	[147]
c-*fos*	EO, RA > C, P	[138]
Ras	EO, P	[126]
Pentraxin (PTX3)	EO, P	[139]
Thy-1 glycoprotein	E, P	[148]
Prostaglandin E	EO, RA > C, P	[133]
Cyclooxygenase-2	EO, RA > C, P	[132]
Phospholipase A2 activating protein	EO, P	[134]
Inducible nitric oxide synthase	EO, RA > C, M, P	[136]
Annexin IV and annexin VI	EO, P	[140]

[a]In the case of soluble mediators such as cytokines, the presence of a protein within a cell does not necessarily mean that it is produced there. It may be produced elsewhere and bound and internalised by the cell of interest. [b]E, endothelial selective distribution, a potential marker for these cells; EO, gene expressed by endothelial and other cells in the synovium; RA < C, gene expression lesser in RA synovium than in control synovium; RA > C, gene expression greater in RA synovium than in control synovium; RA = C, gene expressed equally in RA synovium and control synovium; P, protein studied; M, mRNA studied.

induced or upregulated by cytokines such as TNF-α, IL-1 and IFN-γ [21]. There is good evidence for the presence of selectins and their counterligands that could promote leukocyte tethering and rolling [13,22]. E-selectin shows an endothelial-selective distribution in synovia where its expression is upregulated in RA compared with osteoarthritic (OA) tissue [23]. This adhesion molecule is a marker of endothelial activation in lymphocyte-rich regions of the RA synovium [24]. In addition, synovial endothelial cells situated in inflammatory infiltrates stain positively with the monoclonal antibody MECA-79 [25]. This antibody recognises sulphated carbohydrate structures on counterligands for L-selectin [26], which are expressed on HEV from lymphoid and chronically inflamed tissues [12]. P-selectin has been detected on RA endothelial cells where its distribution is endothelial selective, and there is no difference in the level of expression between RA and control samples [27].

ICAM-1 is a ligand for the β_2 integrins of leukocytes and is present on most RA synovial endothelial cells, as well as on macrophages and fibroblasts [21,23,28]. ICAM-1 expression is upregulated on these cell types in RA compared with normal synovium. There is also increased ICAM-1 expression on cuboidal HEV-like endothelia compared with 'flat' endothelia. ICAM-1, like E-selectin, is hence considered a marker of endothelial activation. ICAM-2, another β_2 integrin ligand, is mainly expressed on synovial endothelial cells and not by other cell types [23,28]. However, this adhesion molecule is expressed by most RA endothelia and normal endothelia, suggesting that it is not an activation antigen on these cells. ICAM-3 has been detected on cells of myeloid origin in the synovium, but there is little or no expression of this molecule on the endothelium [28]. All three ICAMs are expressed by RA synovial dendritic cells that also harbour MHC class II antigens.

Vascular cell adhesion molecule (VCAM)-1 mRNA and protein is present in the RA endothelium, albeit weakly, and is mainly expressed in lining cells and macrophages [29,30]. The CS-1 isoform of fibronectin is expressed on the luminal surface of the endothelium and by lining layer cells in RA synovia, with less expression occurring in control synovia [22,31,32]. Both VCAM-1 and CS-1 fibronectin are ligands for α_4 integrins expressed by lymphocytes and monocytes, and may be functional in lymphocyte adhesion to synovial endothelial cells [33].

CD31 (PECAM-1) is present on most synovial endothelial cells, as well as on macrophages and lining cells, and its expression on endothelia is comparable in RA and normal synovia [23,27]. CD146 is a cell adhesion molecule that belongs to the immunoglobulin supergene family and is potentially involved in leukocyte–endothelial interactions [34]. CD146 is expressed almost exclusively by the vascular endothelium in RA synovia and in normal synovia. The high levels of soluble CD146 found in RA synovial fluid, particularly in early disease, may reflect increased endothelial activity and angiogenesis. Vascular adhesion protein-1 was originally isolated from synovial endothelial cells, and it localises selectively to endothelial cells and not to other cell types in human RA synovia [35]. Vascular adhesion protein-1 expression is increased in joint inflammation and is a marker of activated endothelium in the pig and the dog [36]. Other adhesion molecules expressed by RA synovial endothelial cells include CD44 and cadherins [13,23,27].

The functional importance of adhesion molecules in human RA has been shown by the administration of antibodies *in vivo*. For example, anti-ICAM-1 treatment in RA patients with active disease causes a reduction in disease activity [37] and blocks trafficking of T cells [38]. In another study, anti-TNF treatment with the antibody infliximab decreases serum E-selectin and ICAM-1 levels, suggesting that this may reflect diminished activation of endothelial cells in the synovium, leading to reduced migration of leukocytes into human RA joints [39]. Furthermore, administration of anti-E-selectin in animal models of RA results in a marked decrease of polymorph and monocyte migration into arthritic rat joints, and results in inhibition of T-cell recruitment, causing reduced T-cell-mediated inflammation [40]. VCAM-1 blockade also reduces the clinical severity in collagen-induced arthritis in mice, most probably by altering B-cell trafficking [41].

Chemokines

Chemokines play several roles at inflammatory sites. They initiate firm adhesion of leukocytes to the endothelium by activating integrins on the leukocyte cell surface [42,43]. Chemokines then direct leukocyte migration across the endothelium and through the extracellular matrix into the tissue [44].

There have been numerous reports showing the expression of chemokines in the RA synovium and the joint fluid [45], and some of these studies have detected the presence of chemokine proteins in the endothelium. The chemokines with an endothelial distribution include ENA-78 (recently designated CXCL5), IL-8 (CXCL8), SDF-1 (CXCL12), MCP-1 (CCL2), MIP-1 (CCL3), SLC (CCL21) and ELC (CCL19) [46–52]. These studies suggest that RA endothelial cells can produce numerous chemokines. However, this cannot be stated unequivocally since nearly all the studies excluded mRNA data. It is therefore not possible to determine whether the endothelial cells themselves produce the chemokines or whether they are produced elsewhere in the synovium, such as by macrophages or fibroblasts, and the endothelium is binding and internalising these mediators as part of the mechanisms of chemokine transcytosis and presentation to blood leukocytes [20,53,54]. In this respect, chemokine binding sites for IL-8, MCP-1, MIP-1 and RANTES, which may transcytose and present chemokines, have been shown to be expressed by the synovial endothelium [55,56].

A few studies, however, have examined mRNA and protein expression. In the cases of IL-8, MCP-1 and SLC, their mRNAs and proteins are present in the RA synovial endothelium [50,57] indicating that such cells can both synthesise and present these chemokines. In contrast, SDF mRNA occurs in RA synoviocytes and the protein is present in endothelial cells, where it is presented attached to heparan sulphate [58]. There may therefore be selectivity in the types of chemokines produced by synovial endothelial cells.

Chemokine receptors and other binding molecules are expressed by the synovial endothelium. CXCR3 is expressed by endothelial cells and mononuclear cells in the lymphoid aggregates of RA synovium [59]. CXCR4 is present in endothelial cells, synoviocytes and inflammatory infiltrates in RA and OA synovial tissue [58,60]. The Duffy antigen/receptor for chemokines, a promiscuous chemokine binding protein, is present on RA and control synovial endothelial cells, where it shows a selective distribution on venules [56]. In addition, heparan sulphate proteoglycans, which bind and present a wide range of chemokines and other cytokines, are expressed by the synovial endothelial cells of the RA synovium [55].

Several studies have shown the functional importance of chemokines in animal models and human RA, and some of these have been on chemokines produced by synovial endothelial cells. For example, administration of an anti-IL-8 antibody in rabbit experimental arthritis almost completely blocks the infiltration of neutrophils into the joints and provides protection from tissue damage in the early phase of inflammation [61]. In addition, treatment with an MCP-1

antibody in rat collagen-induced arthritis decreases the number of macrophages recruited to the joints and therefore reduces ankle swelling [62]. Some of the effects of infliximab, a blocking antibody to TNF, in human RA are mediated by chemokines since patients show reduced synovial expression of IL-8 and MCP-1 and decreased leukocyte migration into joints [63].

Gene expression associated with angiogenesis

The formation of new blood vessels, termed angiogenesis, occurs in the rheumatoid synovium. It is generally accepted that angiogenesis is central to maintaining and promoting RA [7,64–66]. The increased endothelial surface area provides the potential for enhanced leukocyte recruitment. In addition, angiogenesis is required for the formation and maintenance of the pannus since the increased volume of this invasive tissue needs a supply of oxygen and nutrients. It is proposed that angiogenesis occurs in several stages. First, at the new branch point the endothelium becomes activated by cytokines, the blood vessel permeability increases and the basement membrane is disrupted by the release of proteolytic enzymes from the endothelium. The endothelial cells then proliferate and migrate towards a chemotactic stimulus to form a new tube, and a new basement membrane is deposited. The increased permeability of the blood vessels may be mediated by proinflammatory agents, such as substance P, and results in tissue oedema. In this context, substance P receptors have been identified on endothelial cells in the RA synovium [67].

Evidence for endothelial cell proliferation in the RA synovium is the expression of cell-cycle-associated antigens (PCNA and Ki67), the $\alpha v\beta 3$ integrin, which is associated with vascular proliferation, and vascular endothelial growth factor (VEGF) [68,69]. Interestingly there is an increased turnover of blood vessels in the RA synovium with focal regions of angiogenesis and vascular regression. Increased cell death in the endothelium was shown by elevated labelling of DNA fragments by terminal uridyldeoxynucleotide nick-end labelling. This occurred in the same synovial tissue samples where there was a concurrent increase in the expression of proliferative markers. These changes are related to the remodelling of the synovial vasculature, leading to reduced vascular densities adjacent to the synovial lining region and to increased vascular densities in the deeper synovium.

It should be mentioned that the vascularity of the RA synovium is the subject of ongoing discussions. One study has shown the mean number of vessels per unit volume of synovium was higher in RA than in control tissue [70], whereas another reported that the vessel density was reduced in the RA synovium [71]. These differences may, in part, be explained by variation in sampling between these studies [72,73].

Regulators of angiogenesis

Angiogenesis is regulated by the balance of angiogenic activators and inhibitors, many of which have been found in the rheumatoid joint. Several of these substances are thought to act indirectly by upregulating the expression of more potent and specific stimuli. Several growth factors that are capable of promoting angiogenesis are mitogens that act on a broad range of cell types. For example fibroblast growth factor (FGF)-1 and FGF-2 stimulate proliferation, migration and differentiation. FGF-2 mRNA and protein are expressed by RA synovial endothelial cells, as well as by fibroblasts and lining cells, and there is no expression detected in normal synovia [74,75]. Platelet-derived growth factor (PDGF) is present in the RA synovium and is angiogenic [76]. It is also a potent mitogen for fibroblasts and is chemotactic for fibroblasts and smooth muscle cells. PDGF receptor mRNA and protein have been demonstrated in vascular endothelial and smooth muscle cells and in some stromal cells in the RA synovium [77]. The actions of the angiogenic factor hepatocyte growth factor (HGF) are dependent on its activation by HGF activator and binding to a specific HGF receptor. Both the activator and receptor mRNAs and proteins are expressed by endothelial cells, fibroblasts and macrophages in RA and OA synovia [78].

VEGF, in contrast, is a relatively endothelial-specific angiogenic factor. It has dual activities by eliciting endothelial cell division and increasing vascular permeability, which may increase oedema, and hence joint swelling, in RA. VEGF levels in the sera and synovial fluids of patients with RA are markedly higher than in OA patients or normal patients [66]. In the RA synovium, VEGF mRNA is expressed by lining cells and VEGF protein is present on lining cells, on sublining stromal cells and on the endothelium of small vessels of the pannus and other locations [79,80]. The mRNAs and proteins for the VEGF receptors VEGFR-1, VEGFR-2 and VEGFR-3 are expressed by RA synovial endothelial cells, where they are more abundant than in controls [81,82]. These receptors show an endothelial-selective distribution and localise in the vicinity of VEGF-producing cells. Interestingly, VEGF production is upregulated by hypoxia, and the RA joint is more hypoxic than normal. This has lead to the suggestion that the formation of new blood vessels in the pannus may be mediated by hypoxia-driven expression of VEGF [66].

Endoglin is an angiogenesis inducer. This glycoprotein is a receptor for transforming growth factor beta and also acts as an adhesion molecule, containing an arginine–glycine–aspartic acid (RGD) motif. Endoglin is expressed by RA synovial, OA synovial and normal synovial endothelial cells, with little difference in the level of expression between the three groups [83]. The endoglin ligand (transforming growth factor beta 1), however, is upregulated on RA synovial

endothelial cells compared with OA and normal endothelial cells [83].

Some chemokines are angiogenic, such as IL-8, and others are angiostatic, such as IP-10 and MIG. Activation of their respective chemokine receptors results in the stimulation or inhibition of endothelial cell proliferation and chemotaxis [84]. Chemokines are mainly expressed by macrophages and fibroblasts in the RA synovium but also by endothelial cells, as shown for IL-8 [50,51,85]. Little is known about the expression of chemokine receptors on RA synovial endothelial cells. Some recent studies, however, have shown that CXCR3 (the receptor for IP-10 and MIG) is expressed by the RA synovial endothelium, suggesting that this receptor mediates the angiostatic response to these chemokines [59]. CXCR4, which mediates the proangiogenic activity of SDF, is present in the synovial endothelium [58,60]. The Duffy antigen may also have an angiogenic function, and this receptor is expressed by synovial endothelial cells [56,86].

The angiopoietins and their receptors Tie-1 and Tie-2 play a key role in the development of the vasculature. Angiopoietin-1 in adults localises to the endothelium, the lining cells and the macrophages in the RA synovium, where levels are higher than in OA or normal synovium [87]. The expression of Tie-1 and Tie-2 has been reported in the RA synovium, and there is significant upregulation of Tie-1 on endothelial cells, on lining cells and on macrophages in RA compared with normal [87–89]. Angiogenin is a 14 kDa plasma protein that has angiogenic effects, stimulating endothelial cell proliferation. It codistributes with basic FGF in the rheumatoid joint, localising to lining cells, to macrophages and to endothelial cells [75].

Thrombospondin is an inhibitor of angiogenesis and localises primarily to blood vessels, including endothelial cells, and to macrophages in the RA synovium [69,90] Another angiogenesis regulator is angiotensin II, and angiotensin converting enzyme localises to endothelial cells and to macrophages in RA synovia [91].

The Ets 1 transcription factor has been intimately linked to the regulation of angiogenesis and it is induced in endothelial cells by VEGF. There is upregulation of Ets 1 expression in the RA synovium compared with OA synovium, with Ets 1 mRNA and Ets 1 protein localising to endothelial cells of newly formed vessels [92]. Many growth and survival factors use receptors belonging to the tyrosine kinase family. One of these tyrosine kinases, Axl, has been detected in RA synovia, localising to the capillary endothelium, to vascular smooth muscle cells of blood vessels and to other cells [93].

Cell adhesion molecules play a role in the regulation of angiogenesis. In addition to mediating leukocyte adhesion to the endothelium, VCAM and E-selectin potentiate angiogenesis, with the latter contributing to the morphogenesis of the capillary tube [94]. The invasion, migration and proliferation of endothelial cells during angiogenesis are also regulated by integrins [7]. These molecules are expressed at the cell surface of activated endothelial cells and interact with a large array of extracellular matrix proteins. Several integrins have been shown to be expressed by the RA endothelium [13]. These include $\beta1$ integrins, such as $\alpha4\beta1$ and $\alpha5\beta1$ that bind to fibronectin, and $\alpha6\beta1$ that interacts with laminin. In addition, collagen-binding integrins are expressed by the vascular endothelium, including $\alpha2\beta1$. αV can associate with several β chains and can have multiple specificities, including interactions with vitronectin and fibronectin. $\alpha V\beta3$ is expressed by several cell types including activated leukocytes and endothelial cells. It is minimally, if at all, expressed on resting or normal blood vessels but is highly expressed in RA synovial blood vessels, where it is viewed as a marker for endothelial activation [27,69].

The relevance of angiogenesis in the pathophysiology in RA has been shown in animal models. For example, TNP-470 (a compound that exerts antiangiogenic, as well as other, effects) was found to suppress established disease associated with a marked inhibition of pannus formation and neovascularisation in type II collagen-induced arthritis in rats [66,95]. In addition, a soluble form of the Flt-1 VEGF receptor significantly reduces disease severity and joint destruction in murine collagen-induced arthritis [96]. In human RA, the anti-TNF antibody infliximab reduces synovial vascularity as assessed by immunostaining for CD31, von Willebrand factor and $\alpha V\beta3$ integrin [66]. Observations suggest that part of the beneficial effects of anti-TNF treatment in RA may be related to decreased production of VEGF and reduced angiogenesis.

Proinflammatory cytokines, other mediators and their receptors

In the RA synovium IL-1α and IL-1β are mainly secreted by macrophages, but also localise to endothelial cells and lymphocytes [97–99]. The type 1 IL-1 receptor is expressed by the endothelium, by the lining cells and by other sublining cells in the RA synovium and the cartilage–pannus junction [97]. A similar pattern of IL-1 receptor antagonist is found, yet there is less expression. In addition, antigenic TNF-α mainly localises to macrophages in the RA lining layer and in the sublining, with some endothelial and lymphocyte staining [76,100–102]. The TNF receptors p55 and p75 are expressed by endothelial cells and a variety of other cell types in the lining and sublining layers [99,103]. IL-1 and TNF stimulate the production of degradative proteases and cytokines such as IL-1, IL-6, IL-8 and MCP-1 by joint tissue cells, contributing to joint destruction. Many of these cytokines are also involved in angiogenesis. In addition, IL-1 and

TNF upregulate the expression of ICAM-1, VCAM-1 and E-selectin on endothelial cells, stimulating leukocyte migration into the joint [76,104].

There have been numerous functional studies showing that blockade of cytokines have anti-inflammatory effects on RA in animal models and humans. Perhaps the most striking have been studies on TNF [105]. In humans, the use of an antibody to TNF (infliximab) or to the soluble TNF receptor (etanercept) has significantly contributed to therapy in RA. Use of infliximab results in a significant reduction in pain, stiffness, number of swollen joints, serum C-reactive protein and Paulus criteria [106,107]. Interestingly, longer trials reveal that the antibody, in conjunction with methotrexate, reduces radiographic joint damage in 50% of patients [108].

There is some evidence that anti-inflammatory cytokines, such as IL-10 and IL-13, may inhibit leukocyte–endothelial adhesion and endothelial expression of adhesion molecules [109]. In the presence of activated leukocytes, however, IL-10 may also enhance adhesion molecule expression. IL-13 may have proangiogenic effects as it stimulates endothelial chemotaxis, inferring the expression of IL-13 receptors by these cells [76]. IL-15 protein localises to the endothelium and other cell types in the RA synovium [110]. This cytokine stimulates T-cell migration into RA synovia engrafted into the SCID mouse. IL-17 is produced by the RA synovium, induces production of metalloproteinases and could activate the endothelium enhancing leukocyte extravasation [111]. In this respect, the IL-17 receptor is expressed in RA synovia, mainly on endothelial cells, where its expression is higher than in OA synovia [112].

Elevated levels of corticotropin-releasing hormone (CRH) are produced locally in the inflamed synovium, and a role for CRH is indicated in the pathogenesis of inflammatory joint disease [113]. The CRH receptor type 1 mRNA and protein are abundantly expressed by RA synovial endothelial cells, as well as by mast cells, where this receptor may be involved in vascular permeability changes and in angiogenesis. The receptor is not expressed in normal synovia. In another study, staining for CRH receptor and the CRH ligand urocortin was demonstrated in RA endothelial cells and in a variety of other cell types [114]. Kinin levels are raised in RA and have been implicated in the pathogenesis of RA by causing release of cytokine and noncytokine mediators such as IL-1, TNF, PAF, histamine and prostaglandin E2, which contribute to joint destruction, leukocyte influx, pain, oedema and angiogenesis [115]. Kinin B2 receptors have been detected on synovial endothelial cells, on lining cells and on fibroblasts in RA and in OA samples [116].

Midkine is a retinoic acid-inducible heparin-binding cytokine that stimulates neutrophil chemotaxis. The protein is detected on the endothelial cells and synoviocytes in inflamed synovia, and less in noninflamed tissue [117]. The protein for stem cell factor, but not its receptor (c-kit), localises to synovial endothelial cells, macrophages and fibroblasts in RA and OA synovia [118]. In addition, the somatostatin receptor (sst2a) protein localises to the RA synovial endothelium and to macrophages [119].

Proteases

Proteases function in the degradation of the basement membrane and other regions of the extracellular matrix, playing a role in angiogenesis and enhancing leukocyte migration into the tissue [120]. Microvascular endothelial cells are also present in the invasive synovial pannus, such as at the cartilage–pannus junction [97,99,103,121]. Proteases released by endothelial cells could therefore contribute to cartilage and bone destruction caused by the pannus. In this respect, however, it is not known how much of a contribution endothelial cells make compared with other cell types such as fibroblasts and macrophages.

Several proteases are produced by the RA synovial endothelium. In early RA the mRNAs for matrix metalloproteinase (MMP)-1 (collagenase) and the cysteine proteases cathepsin B and cathepsin L are expressed by synovial endothelial cells, as shown by *in situ* hybridisation [120]. There is only scant expression of these enzymes in normal synovia. MMP-3 (stromelysin) mRNA mainly occurs in the lining layer in RA synovia but is also expressed by endothelial cells [122]. The MMP-9 (gelatinase B) and MMP-13 (collagenase 3) proteins occur in endothelial cells, fibroblasts and leukocytes within the RA synovium, where they occur in elevated levels compared with the OA synovium [123,124]. Membrane type 1-MMP mRNA is detected in endothelial cells and fibroblasts of RA synovia, and there is less expression of the protease in control synovia [125]. Regarding the regulation of MMP gene expression, the oncogene Ras (which upregulates MMP-1 and cathepsins) has been shown to colocalise with cathepsin L in the vessels of the RA synovium [126].

Proteolytic joint destruction in RA is believed to be mediated, at least in part, by the plasminogen activation system. In this context the urokinase plasminogen activator receptor is expressed by RA synovial endothelial cells, occurring in higher levels compared with normal synovial endothelial cells. The receptor is also expressed by lining cells and sublining macrophages [127]. Tissue-type plasminogen activator also localises to the synovial endothelium in RA and in OA patients but not to other cell types [128].

Other enzymes such as heparanase and plasmin are produced and secreted from endothelial cells. These enzymes may play a role in angiogenesis by releasing growth factors such as FGF, VEGF and HGF that are bound to heparan sulphate in the extracellular matrix [84,129].

Tissue inhibitor of metalloproteinases (TIMPs) antagonise the effects of destructive MMPs. The expression of TIMP-1 and TIMP-2 has been shown in endothelial cells and lining cells in RA synovia but not in normal synovia [130]. It has been reported that the RA synovial endothelium produces decreased amounts of TIMP [131]. This may in part contribute to an imbalance between MMPs and TIMPs in the RA joint, favouring tissue destruction.

Other genes

Prostaglandins are important mediators of acute and chronic inflammation [132]. Prostaglandin E has been localised to the synovial endothelium, to lining cells and to inflammatory cells, where expression is higher in RA than in OA tissue [133]. The production of these mediators is catalysed by an enzyme cascade that includes cyclooxygenases (COXs). There are two isoforms of COX expressed in the synovium. COX-2 localises to endothelial cells, to mononuclear leukocytes and to fibroblasts, and COX-2 expression is increased in inflammatory arthritis compared with in noninflammatory arthritis [132]. COX-1, in contrast, is constitutively expressed, particularly by lining cells, and there is no difference in immunostaining between inflammatory and noninflammatory arthritis.

Another enzyme involved in prostaglandin synthesis is phospholipase A2. In this context, phospholipase A2 activating protein has been shown to be present in a variety of cells in the RA synovium, including endothelial cells, vascular smooth muscle and monocytes/macrophages [134].

Nitric oxide is synthesised by the action of a family of nitric oxide synthases, which are either constitutive or inducible. The production of nitric oxide plays a role in inflammation and physiological processes [135]. The expression of inducible nitric oxide synthase protein and mRNA has been shown in RA synovia, localising to the endothelium, to macrophage-like lining cells and, to a lesser extent, to fibroblasts [136]. There is only minimal labelling of these cells in normal synovium. Recent data suggest that nitric oxide production by inducible nitric oxide synthase has anti-inflammatory effects in experimental arthritis by mediating a reduction in leukocyte adhesion and infiltration [137].

Enhanced expression of activation markers, such as the protooncogene c-*fos*, has been reported in RA synovial endothelium compared with OA synovial endothelium [138]. The pentaxin PTX3 has a structure related to C-reactive protein and may play a role in inflammatory circuits in RA. PTX3 is expressed by the endothelium and synoviocytes in RA synovia [139]. Annexins are calcium-binding proteins with diverse functions including regulating inflammation and may have an intracellular role as cytoskeletal elements in exocytosis and cell differentiation. Endothelial cells in the RA synovium stain strongly for annexin IV and annexin VI, and weakly for annexin I and annexin II [140]. Lymphoid cells are also strongly positive for annexin VI in the RA synovium.

Conclusions

This review has reported 76 genes expressed by or on the synovial endothelium in the RA synovium (Table 1). Of these, 13 genes showed a preferential endothelial distribution and could be considered potential markers of these cells. In addition, 29 out of the reported 76 genes showed increased expression in the RA endothelium in comparison with non-RA control endothelial cells, and such genes may be implicated in the pathophysiology of RA. Future studies of these genes and other pathways specifically induced in RA endothelial cells may lead to the development of novel endothelium-targeted therapies for RA and to other forms of inflammatory arthritis.

Competing interests

None declared.

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Correspondence

Jim Middleton, Arthritis Research Centre, Medical School, Keele University at the Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry SY10 7AG, UK. Tel: +44 (0)1691 404149; fax: +44 (0)1691 404170; e-mail: jim.middleton@rjah.nhs.uk