REVIEW

Hypoxia and inflammatory synovitis: observations and speculation

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In recent years considerable research interest has been directed at studying the biological consequences of tissue hypoxia. As this work progresses it becomes increasingly apparent that tissue hypoxia has complex biological consequences. Much is now known about the natural defences of the body to hypoxia, including heat shock protein synthesis and angiogenesis. These systems are normally under rigid control, but this would seem not so in the rheumatoid joint. This accumulation of knowledge has prompted the belated reawakening of interest in joint hypoxia; it is now clear that an understanding of the physiological and pathological effects of joint hypoxia is of great importance to both clinical and research rheumatology.

This review outlines joint hypoxia from a historical perspective and offers explanations for the phenomenon. Some of the more pertinent implications of hypoxia in the context of inflammatory synovitis are discussed.

The evidence

SYNOVIAL FLUID

A major advantage in the assessment of joint physiology both in health and disease is the accessibility of synovial fluid from the larger joints. Accordingly, many comparative studies have been performed. In 1970 Lund-Olesen reported the results of a major survey comprising synovial fluid samples from 85 rheumatoid knees.¹ The mean rheumatoid synovial fluid oxygen tension (P_{O_2}) was found to be 27 mmHg, significantly lower than in acute traumatic effusions (mean 63 mmHg), although osteoarthritic knees were often hypoxic (mean 43 mmHg). Treuhaft and McCarty,² in a similar study of 55 rheumatoid patients, agreed with Lund-Olesen in finding that 27% of rheumatoid knees show profound hypoxia with a P_{O_2} less than 15.2 mmHg. Other workers have independently verified this phenomenon. In addition to a low P_{O_2} , many studies have shown alterations in rheumatoid synovial fluid physiology which would be expected in a hypoxic state—namely, a raised carbon dioxide tension (P_{CO_2} —up to 150 mmHg), raised lactate (up to 10 mmol/l), lowered glucose, and acidosis (pH as low as 6.6).³⁻¹¹ The most notable study summarising these changes is by Falchuk,³ who showed that joints with the lowest P_{O_2} (as low as 9 mmHg) also exhibited large increases in P_{CO_2} and lactate; the same joints showed severe microvascular obliteration in the synovial membrane.

It is important to note that physiological measurements in synovial fluid can vary with the patient's activity. This is evident from a recent study, which showed that movement of an inflamed joint can induce significant further decrease in P_{O_2} from a resting baseline.¹² This observation has particular relevance to the concept of joint 'ischaemia-reperfusion' injury.

SYNOVIAL MORPHOLOGY

The sequence of pathological changes in rheumatoid arthritis is closely associated with alterations in the synovial microvasculature. From the mid-eighteenth century to date there have been many detailed subjective descriptions of synovial morphology in health and disease. Much of the described microvascular derangement in synovitis would seem to be both a consequence and cause of further hypoxia. The hypoxia of multilamination of vascular basement membrane, characteristic of rheumatoid arthritis,¹³ is seen in other vascular beds in response to hypoxia.^{14 15} It also imposes a barrier to oxygen transport. Arteriovenous shunting has been implicated as a cause of focal pockets of ischaemic synovium. This effect could be achieved in several ways, which have been reported independently. The obliteration of terminal vascular plexi^{2 16 17} provides one possible cause of arteriovenous shunting. Microvascular damage such as this also leads to increased permeability and local oedema through impaired fluid resorption. Another ischaemia promoting rheumatoid characteristic is that of irregularly distributed synovial vasculitis.¹⁸ Microvessel plugging by inflammatory cells also serves to reroute blood from the peripheral capillary beds. This is associated to a varying degree with microthrombi¹⁹ and extravascular aggregates of platelets.²⁰

Interestingly, the digital arteries in rheumatoid arthritis have also been found to show occlusive obliteration in the proximity of joint effusions.^{21 22} It is thought, however, that the capillary and venular changes leading to stagnation of synovial blood flow²³ (at least focally) are the most important factors that convincingly implicate disseminated microangiopathy in rheumatoid pathogenesis.²⁴

These observations, which are for the most part subjective, go some way towards explaining synovial fluid hypoxia. Until recently, however, there has been a paucity of quantitative (morphometric) information on the functional

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adequacy of the synovial vasculature. In the normal joint the synovial membrane is only a few cells thick, but incorporated into this is a dense vascular plexus. The important vascular parameters which govern the efficacy of the oxygen delivery system are capillary number density, capillary spatial distribution, and blood flow. Morphometry of tissue sections of normal human knee synovium has supplied data on the first two of these features.^{25 26} From these data the functionally optimal range of vascular parameters for the synovium can be extrapolated. The normal data from human knees correlate well with the results of similar morphometric analysis of rabbit knee synovium.²⁷ When the same criteria were used to evaluate chronically inflamed rheumatoid synovia a significant *reduction* in vascularity was shown in the functionally important superficial region of the synovium.^{25 28} Capillary number density in rheumatoid synovium is significantly reduced to about one third of normal, reflecting not only an increased spatial distribution of the microvasculature but also a grossly thickened synovial lining. The average capillary distance from the joint cavity is significantly increased from 32.5 μm to 93.3 μm . This apparent 'burial' of the vasculature is due to the thickening of synovial lining, which apparently proceeds at a greater pace than angiogenesis.

Why does the inflamed rheumatoid joint become hypoxic?

There are two explanations which individually could provide the answer to why inflamed joints are hypoxic—namely, the increased metabolic demand of the inflamed synovium, and inadequate oxygen delivery through poor perfusion of the inflamed joint. In fact, both are characteristic of the inflamed joint.

SYNOVIAL METABOLISM

The oxygen consumption of the rheumatoid synovial membrane per gram of excised tissue is about 20 times that of normal. This work by Dingle and Page-Thomas^{29 30} and by Roberts *et al*³¹ also showed that the activity of the glycolytic (Embden-Meyerhof) pathway for ATP production was markedly greater in rheumatoid synovium than in normal synovium. The raised metabolic rate of the rheumatoid synovium concomitantly raises the demand for ATP. The intracellular production of this molecule can be achieved by either the aerobic or anaerobic oxidation of glucose via the tricarboxylic acid cycle or the glycolytic pathway respectively. The oxygen dependent tricarboxylic acid cycle is a much more efficient producer of ATP than the anaerobic system and so is generally favoured in normoxic tissues. The rheumatoid synovium favours the anaerobic glycolytic pathway, which indicates its hypoxic nature. In support of this, Henderson *et al* have shown that synovial lining cells in rheumatoid arthritis contain significantly more glyceraldehyde-3-phosphate and lactate dehydrogenase activity than those of normal tissue.³² These are the major enzymes of the glycolytic pathway. Their

increase is more reasonably explained by a response to tissue hypoxia than by raised metabolic activity as mitochondrial oxidation in the synovium is not similarly enhanced.³³

The terminal end product of the anaerobic oxidation of glucose is lactate. The ratio of lactate to glucose,¹⁰ therefore, should give an indication of oxygen status in the synovium. This is indeed the case, as Goetzl *et al* have shown that lowered glucose and raised lactate concentrations correlate well with falls in PO_2 and pH.⁶

SYNOVIAL PERFUSION

There have been numerous attempts to measure blood flow directly by a variety of methods. Unfortunately, none has succeeded in measuring the absolute flow per unit volume of synovium.

To meet the metabolic demands of the inflamed synovium a marked increase in perfusion would be required. Although some studies have reported some increase in perfusion,^{3 35} the extent is probably less than adequate. In any event, the validity of these studies is in question because of methodological problems.^{28 36} Any such study must take into account the possibility of arteriovenous shunting giving the impression of perfusion but resulting in ischaemic, non-perfused synovial pockets. Falchuk *et al* concluded that synovial perfusion was increased in rheumatoid arthritis but overlooked the possibility of arteriovenous shunting in assuming that the measured synovial fluid PO_2 equalled that of the venous blood draining the joint.³ Other studies⁷ using clearance of ^{125}I and tritiated water³⁷ indicate that joint perfusion in chronic synovitis is markedly reduced. Whereas intra-articular temperature in acutely inflamed joints is higher than that found in normal joints, joint temperature in severe proliferative chronic arthritis may be lower than normal,⁷ essentially implying that blood flow in these most affected joints is insufficient to provide adequate perfusion.

Acute exacerbation of chronic hypoxia

A distinguishing property of the diarthrodial joint is its ability to move. The inflamed joint of a mobile patient is unavoidably subjected to movement. This situation we believe has profound significance in joint pathogenesis through the effects of ischaemia-reperfusion injury.³⁸

The environment for ischaemia and reperfusion in rheumatoid synovitis is created by the unique topography of the component parts of a joint, which predisposes the synovium to pressure induced fluctuations in blood supply. The synovium is the innermost confine of the joint space and its fluid. This movable and compressible stroma is backed by the more rigid confines of the musculature and ligaments. It might be expected, therefore, that increasing the volume of fluid effusion concomitantly with synovial centripetal thickening (cf rheumatoid arthritis) generates a pressurised system. This is indeed the case. We have confirmed that the resting intra-articular pressure in chronically

inflamed joints is slightly above atmospheric pressure (+5–10 mmHg) compared with that of normal joints which is subatmospheric (–5 mmHg).³⁹ When normal joints (knee) are exercised the pressure falls further, whereas in chronically inflamed joints intra-articular pressure rises, sometimes as high as 300 mmHg and always above 40 mmHg. The capillary perfusion pressure in inflammation is of the order of 20–30 mmHg. It is therefore suggested that in chronically inflamed joints the intra-articular pressure rises during exercise above a critical level sufficient to occlude parts of the capillary bed, thus inducing acute ischaemia in an already hypoxic environment. Recent independent observations have supported the hypothesis of ischaemia induced by intra-articular pressure. James *et al* have shown in rheumatoid patients with knee effusions that increases in intra-articular pressure in the range encountered during daily activity can compromise blood flow.⁵ This effect is associated with increased synovial fluid lactate, raised PCO₂, and decreased pH. McDonald and Levick have shown in rabbit knees that intra-articular pressures as low as 19 mmHg cause synovial capillaries to assume a more flattened, elliptical profile.⁴⁰

In mobile patients, who may be subjecting their joints to pressure induced ischaemia, subsequent rest allows reperfusion of blood and reoxygenation of the synovium, albeit to an inadequate degree. This eventuality, we believe, has pathological repercussions consequent on the generation of reactive oxygen metabolites which accompanies postischaemic reperfusion. The evidence for this hypothesis has recently been summarised.³⁹

BIOCHEMICAL CONSEQUENCES OF SYNOVIAL HYPOXIA

In normoxic synovium energy is derived from oxidative metabolism of fatty acids and glucose. The contribution of anaerobic glycolysis is small, and glycogen stores are maintained. In the hypoxic synovium the tricarboxylic acid cycle is substituted by anaerobic glycolysis³² using glycogen. This metabolic shift induces synovial lactic acidosis. When the pH becomes sufficiently low anaerobic glycolysis is also inhibited, resulting in chronic ATP depletion.^{41–42} In this environment several important biochemical events take place which have major significance in the pathology of synovitis. Calcium imbalance is arguably the most influential of these events. Intracellular homeostasis, with respect to Ca²⁺, is dependent upon energy in the form of ATP. When ATP availability is compromised, as in the hypoxic synovium, cytosolic Ca²⁺ levels rise. Mitochondria are avid accumulators of Ca²⁺, and when overloaded with Ca²⁺ their function is impaired,⁴³ resulting in the further depletion of ATP levels and the maintenance of a calcium influx.⁴⁴ Many important cellular functions and control mechanisms are dependent on Ca²⁺. The consequences of hypoxia derived Ca²⁺ imbalance must be considered as possible contributing factors in the pathogenesis of rheumatoid

arthritis. The failure of cellular phosphorylation mechanisms also leads to accumulation of adenosine (a putative angiogenic factor) and of its breakdown products, including hypoxanthine and xanthine, which are the substrates for the xanthine oxidase enzyme system (see below). The outcome of these events in the hypoxic synovium confers the facility for the inappropriate generation of reactive oxygen metabolites upon reperfusion of blood. There is much evidence to show that postischaemic reperfusion of the synovium evokes the generation of reactive oxygen metabolites despite the underlying hypoxic environment which is not conducive to most conventional radical generating mechanisms.⁴⁵ One proposed mechanism which can achieve this is dependent on the enzyme xanthine oxidase^{46–47} which is present in the synovium.⁴⁸ The non-pathological dehydrogenase form of this enzyme oxidises hypoxanthine and xanthine to uric acid using NAD⁺ as an electron acceptor. Under ischaemic conditions, however, this enzyme can be converted to an NAD⁺ independent form which catalyses the same reaction using molecular oxygen as an electron acceptor, resulting in superoxide anion generation.⁴⁶ The inappropriate production of superoxide in a biological system can have catastrophic repercussions, particularly when the system facilitates the redox environment required for consequent hydroxyl radical (•OH) formation. The synovium is such a system enabling the spontaneous or enzymatic conversion of superoxide to hydrogen peroxide, which can then fuel a Fenton-type reaction catalysed by compartmentalised iron,⁴⁹ forming the cytotoxic •OH species thus:

BIOLOGICAL CONSEQUENCES OF REACTIVE OXYGEN METABOLITES

Biochemical analysis of synovial fluid shows the effects of radical damage to endogenous biomolecules in rheumatoid arthritis. For example, the end products of lipid peroxidation,⁵⁰ fluorescent IgG,^{51–53} and inactivated α₁ proteinase inhibitor⁵⁴ are indicative of radical attack and are characteristic findings in rheumatoid synovial fluid. Interestingly, Garret *et al* have recently shown that superoxide anion stimulates osteoclast bone resorption in vitro and in vivo.⁵⁵ Many other examples of oxidative damage to the joint have been reported and are reviewed elsewhere.⁵⁶ Apart from these damaging or cytotoxic properties of free radicals, they also have the ability to interfere subtly with finely controlled cellular mechanisms. One example of this occurs in the control of vascular tone by vasoactive substances.

OTHER BIOLOGICAL CONSEQUENCES OF HYPOXIA TRANSFORMED BIOCHEMISTRY

An extensive range of publications shows that hypoxia has very profound effects in biological systems. In this review we have selected for discussion some of the more pertinent effects which we believe have implications in rheuma-

toid arthritis. Our examples are confined for the most part to the endothelial cell. All other cell types, however, show profound changes when subjected to hypoxia.

Vasoactivity

An obvious vascular response to tissue ischaemia would be to lower its resistance to blood flow by vascular relaxation. This has been shown to be the case in some systems where hypoxia induced release of prostacyclin (prostaglandin I_2) is sufficient to elicit endothelium dependent dilatation.⁵⁷ Pohl and Busse have also shown that hypoxia stimulates the release of an endothelium derived relaxing factor from rabbit femoral artery and aorta.⁵⁸ In the pulmonary circulation, however, it has been known for many years that hypoxia causes vascular constriction.⁵⁹ The benefit of this is in rerouting most of the blood flow to the best oxygenated parts of the lung. Other pulmonary studies have shown that hypoxia blocks endothelium derived relaxing factor production,^{60 61} and induces the release of a constricting factor⁶² from the endothelium. These conflicting reports highlight the heterogeneity of endothelial cell responses to the same stimulus in different situations and environments, allowing the possibility of an apparently appropriate response eventually to become pathological.

Hypoxia has other quite pronounced effects on endothelial cells, for example in von Willebrand factor release (discussed later), reducing plasminogen activator activity,⁶³ and increasing superoxide dismutase activity.⁶⁴ The endothelium also produces other very powerful vasoactive substances, which may be mediated by hypoxia. These include platelet activating factor⁶⁵ and the highly potent vasoconstrictor endothelin.⁶⁶ In addition, endothelial cells have a pivotal role in the initiation, regulation, and maintenance of the inflammatory process⁶⁷ through the induction of changes in their interactions with blood constituents. These factors, when considered in parallel with the susceptibility to perturbation by hypoxia of the endothelial cell, confer its prominent role in the pathogenesis of ischaemic states.

The activity of endothelium derived relaxing factor has been ascribed to the nitrogen centred radical nitric oxide (NO),⁶⁸ which is synthesised in endothelial cells by a Ca^{2+} dependent enzymatic conversion of L-arginine. Nitric oxide can interact with superoxide to produce the highly cytotoxic hydroxyl radical through peroxynitrite.⁶⁹ In vitro this interaction abrogates the endothelium derived relaxing factor activity of NO as shown by the ability of superoxide dismutase to enhance and prolong the relaxant effect of NO.^{70 71} Vascular tone is controlled by the opposing actions of relaxing and contracting factors released from the endothelium in response to neurogenic stimuli or platelet products. Superoxide has itself been implicated as an endothelium derived contracting factor^{72 73} along with endothelin. It is feasible, therefore, that an effect of hypoxia in the synovium is paradoxical vasoconstriction,⁷⁴ effectively worsening the situation.

Leucocyte chemotaxis and adhesion

The initiating factors of inflammatory cell infiltration are adherence to endothelium and chemotaxis. The activation pathways and mechanisms of initiation for adhesion are reviewed elsewhere.⁷⁵ Synovial inflammatory cell infiltration is a major characteristic of rheumatoid arthritis, as is the expression of membrane associated adhesion molecules. The contribution of hypoxia to this situation may have considerable significance, particularly where oxygen free radicals are a consequence. Several reports show that superoxide, hydrogen peroxide, or both, can generate a chemoattractant for neutrophils from extracellular fluid.^{76 77} The identity of the neutrophil chemotactic factor(s) generated in ischaemia is unknown. Possible candidates are cyclooxygenase and lipoxygenase metabolites of arachidonic acid, such as 12-hydroxyeicosatetraenoic acid, which is found to be raised in hypoxic states⁷⁸ and is a neutrophil chemoattractant.⁷⁹ In support of this, Farber *et al* have shown that cultured endothelial cells can respond to hypoxia by producing a neutrophil chemoattractant, which is suppressible by lipoxygenase inhibitors.⁸⁰

The adhesion component of leucocyte infiltration can be facilitated by oxygen radicals⁸¹ and by ischaemia alone.^{82 83} Interestingly, the adhesion of neutrophils to ischaemic arterial vasculature is associated with a loss of endothelium dependent vasorelaxant activity.⁸³

Microvascular barrier function

In any inflammatory condition the manifestation of the inflammatory response is controlled by the permeability of the microvascular blood/tissue barrier. Increased transvascular exchange of macromolecules, emigration of leucocytes, and oedema formation are indicative of modified barrier function and are characteristic of synovial inflammation. The dynamics of barrier function, particularly solute exchange, are modulated physiologically by a complex assortment of mediators. During hypoxia, however, homeostasis is disrupted by, as yet, poorly understood mechanisms involving mediators of altered barrier function, such as platelet activating factor, complement, leukotrienes, and prostaglandins. The mechanism for increased permeability, when associated with interendothelial gap formation, is thought to involve changes in the actin cytoskeleton of the endothelial cell.⁸⁴ These changes can be elicited by calcium imbalance as actin binding proteins which regulate the endothelial cytoskeleton are calcium dependent.⁸⁵ Increased permeability during ischaemia or hypoxia has been reported from many vascular and culture systems—for example, heart,^{86–88} lung,^{89 90} brain,⁹¹ and cultured bovine endothelium.⁹²

In addition to the effects of hypoxia alone, rheumatoid synovial microvascular permeability can be altered by the membrane damaging effects of oxygen free radicals.^{93 94}

von Willebrand factor

von Willebrand factor, otherwise known as

factor VIII related antigen, is a large adhesive glycoprotein which is synthesised in endothelial cells and megakaryocytes and plays an important part in the adhesion of platelets to damaged vessel walls. Small subunits of von Willebrand factor are released constitutively by endothelial cells, accounting for normal circulating plasma concentrations. Larger multimers of von Willebrand factor are stored in specific organelles called Weibel-Palade bodies within endothelial cells. Interestingly, these bodies are common in the synovium and have been implicated as a marker of angiogenesis in rheumatoid arthritis.⁹⁵ Various physiological stimuli can induce acute release of these multimers from the Weibel-Palade bodies *in vitro*. Raised concentrations of plasma von Willebrand factor have been found in various disease states, including rheumatoid arthritis.⁹⁶⁻⁹⁷ This phenomenon has been taken to indicate vascular damage.⁹⁸ Significantly, there is evidence that Ca^{2+} influx mediates the release of von Willebrand factor from endothelial cells.⁹⁹⁻¹⁰⁰ This has obvious bearing on the recent finding that simulated ischaemia (an inducer of Ca^{2+} influx) in cultured endothelial cells causes membrane changes, Weibel-Palade body exocytosis, and an associated release of von Willebrand factor, which is detectable by enzyme linked immunosorbent assay (ELISA).¹⁰¹ The probability that this mechanism operates in the rheumatoid synovium is emphasised by the immunohistochemical demonstration of a patchy von Willebrand factor distribution in the most hypoxic regions, suggesting von Willebrand factor release.¹⁰¹ This may provide an explanation for the aggregates of platelets in the synovium²⁰ and for microthrombi which can be caused by von Willebrand factor release from Weibel-Palade bodies.¹⁰² Platelet aggregation elicits several notable endothelial cell responses, including endothelium derived relaxing factor release¹⁰³ and angiogenesis. In the context of the former response, Ashmore *et al* have shown that adenine nucleotides released from aggregating platelets stimulate a rise in intracellular Ca^{2+} .¹⁰⁴ The effects of platelet induced Ca^{2+} increase are not necessarily limited to endothelium derived relaxing factor release but may also represent a common mechanism for the release of other endothelium derived mediators, such as von Willebrand factor, plasminogen activator, prostacyclin, and endothelin.

Platelet activating factor

Platelet activating factor is the name given to a family of structurally related acetylated phosphoglycerides, or lipid autacoids. It is well known to be a potentially critical mediator in diverse pathological processes. In this respect many of the most important physiological actions of platelet activating factor are platelet independent. Platelet activating factor is synthesised by a variety of cell types after appropriate stimulation.¹⁰⁵ An increasing volume of evidence indicates that its synthesis or release can be stimulated in ischaemia.¹⁰⁶⁻¹⁰⁸ Furthermore, it has been suggested that platelet activating factor release accounts for some of the damage seen in

ischaemic conditions as treatment with platelet activating factor antagonists affords protection against such damage.¹⁰⁹⁻¹¹⁰ Platelet activating factor has been implicated in many disorders through various aspects of its multiple biological activities. In the ischaemic synovium of rheumatoid arthritis its most important effects are probably as an accessory factor to increased vascular permeability¹¹¹⁻¹¹² or in the amplification cycle of endothelial cell injury.¹⁰⁵ Hence, platelet activating factor is a potential participant in the still evolving cycle of events which generate persistent synovial inflammation.

Angiotensin converting enzyme

Endothelial cells contain angiotensin converting enzyme, which converts vasoinactive angiotensin I to the vasoconstrictor angiotensin II and inactivates bradykinin.¹¹³⁻¹¹⁴ It is of significance to this review that angiotensin II stimulates endothelial cells to synthesise platelet activating factor.¹¹⁵ In a variety of vascular systems both increased¹¹⁶⁻¹¹⁷ and decreased¹¹⁸ angiotensin converting enzyme activity have been reported after exposure to hypoxia. This may reflect once more the heterogeneity of endothelial cell responses to hypoxia in various tissues. The finding that angiotensin converting enzyme activities are markedly increased in rheumatoid synovial fluid¹¹⁹ may indicate a direct effect of hypoxia or acute release due to ischaemic damage to synovial endothelial cells. The part played by angiotensin converting enzyme in rheumatoid arthritis warrants further investigation, inasmuch as high activity in synovial fluid would exacerbate ischaemia through the down-regulation of a vasodilator (bradykinin) concomitantly with the generation of the vasoconstricting peptide angiotensin II.

Attempts of the joint to limit hypoxic damage

The immediate local response to tissue hypoxia is elicited by the vasculature, using vasoactive substances which serve to enhance perfusion. It is clear that the facility for this response is impaired in rheumatoid synovitis. A more long term strategy for the re-establishment of adequate perfusion is neovascularisation of hypoxic tissue. The facility for this, termed angiogenesis, most certainly exists in the rheumatoid synovium, to an extent which some believe to be pathological.

Under conditions of physiological stress, such as hypoxia, cell survival may become dependent on the expression of a specialised set of proteins, termed heat shock proteins. There is reason to believe that these proteins have an important protective role in the chronically hypoxic rheumatoid synovium.

ANGIOGENESIS

The formation and growth of new blood vessels is under rigid control, so much so that in health angiogenesis only occurs in wound healing and during endometrial regeneration. Functional aberrations of the physiological regulation of new capillary growth such as seen in ischaemia and reperfusion are implicated in the patho-

genesis (if not aetiology) of many neoplastic and non-neoplastic diseases.

The neovascularisation of a wound progresses, logically, towards the centre where hypoxic macrophages are stimulated to produce angiogenic factors.¹²⁰ This is fundamental evidence which supports the hypothesis that exposure to hypoxia causes the blood vascular system to adapt its structure to allow greater amounts of blood to flow to the tissues at any given perfusion pressure gradient. Much more evidence in support of this has been reported pertaining to pathological hypoxia. Angiogenesis is a well documented physiological response to myocardial infarction.¹²¹ In response to hypoxia the infarcted tissue is repaired by neovascularisation emanating from the surrounding myocardium. It has been suggested that this is achieved by a low molecular weight angiogenesis factor that can be extracted from human myocardial infarcts.¹²² Hypoxia driven neovascularisation in the eye is said to be one of the most common causes of blindness, for example as in diabetic retinopathy,¹²³ neovascular glaucoma,¹²⁴ and the retinopathy of prematurity.¹²⁵ Pertinent to the latter disorder, a 15-lipoxygenase metabolite of arachidonic acid 15-hydroxyeicosatetraenoic acid, which is pro-angiogenic, has been shown to be markedly raised in hypoxic neonatal vasculature.¹²⁵

The persistent synovitis characteristic of rheumatoid arthritis equates with persistent, though perhaps not fully effective, angiogenesis. The resulting fibroproliferative, inflammatory pannus invades and elicits the destruction of articular cartilage, leading to joint deformation and loss of function. Clearly the rheumatoid joint is a victim of angiogenesis, although morphometry suggests that this does not keep pace with synovial proliferation.²⁵ This situation, as has been discussed earlier, promotes hypoxia in the peripheral region of the synovium, which is rich in macrophages. The evidence that rheumatoid synovial tissue macrophages¹²⁶ and hypoxic macrophages¹²⁰ release a substance capable of inducing angiogenesis invites the hypothesis that hypoxia drives synovial angiogenesis. The macrophage derived angiogenic factor is thought to be tumour necrosis factor α . Tumour necrosis factor α has been localised histochemically to the synovial lining cells in rheumatoid arthritis, but no prominent staining is seen in osteoarthritis non-inflammatory samples.¹²⁷ The accumulation of adenosine is a feature of hypoxic cells. Adenosine¹²⁸ and other hypoxia induced nucleotide degradation products¹²⁹ have shown proangiogenic activities. Hyaluronan, a constituent of synovial fluid which can be oxidatively degraded, has both angiogenic and antiangiogenic capabilities. Its degradation products are pro-angiogenic,¹³⁰ whereas high molecular mass hyaluronan has inhibitory effects on endothelial cell proliferation.¹³¹ In addition to the list of possible angiogenic factors which coexist in rheumatoid arthritis, the synovium has been shown to contain a low molecular weight angiogenesis factor,^{132 133} similar if not identical to that extractable from tumours¹³⁴ and myocardial infarcts.¹²²

HEAT SHOCK PROTEINS

A more suitable term to describe these ubiquitous intracellular proteins is 'stress proteins'. This is because the expression of these proteins can be effected by a heterogeneous assortment of stressors other than heat.¹³⁵ Stress protein mRNAs are actively transcribed on the activation of a number of specific genes which are normally dormant or only partially active. Their function is still being researched, but it is thought that they confer protection to stressed cells in a variety of ways. The 32 kDa stress protein, for example, is induced by hydrogen peroxide and results in the efficient scavenging of reactive oxygen metabolites.¹³⁶ Repeated or continuous stressing of cells producing specific stress proteins results in tolerance to the initial stressor and to certain others simultaneously. Of relevance to this discussion, is the recognition that both hypoxia^{137 138} and reactive oxygen metabolites^{138 139} evoke stress protein responses. The heat shock protein response in arthritis has been reviewed recently by Winrow *et al*,¹⁴⁰ who describe the histochemical localisation of certain heat shock proteins to the inflamed rheumatoid synovium and speculate on the function of these in limiting hypoxic-reperfusion injury.

The heterogeneity of cellular responses to hypoxia has already been alluded to. Similarly, the viability and proliferation parameters of different cell types vary significantly in reduced Po_2 . It is not unreasonable to suppose that heat shock proteins play a part in this heterogeneity owing to their differential cellular expression in response to a particular stressor. It is an intriguing possibility that the specific cell populations comprising the cellular milieu of rheumatoid synovium are regulated by the combination of hypoxic stress and heat shock protein response.

Comment

Variable tissue hypoxia is a feature of chronic synovitis. The biological consequences of this are diverse, complex, and profound. We believe that researchers within rheumatology take little note of this when conducting *ex vivo* experiments. Is it reasonable to study a cell's behaviour or cell to cell interactions in a culturing environment ($\text{Po}_2 \sim 152$ mmHg, $\text{Pco}_2 \sim 38$ mmHg, and $\text{pH} \sim 7.4$) and draw conclusions thought relevant to chronic synovitis when those cells exist *in vivo* in an environment that is *profoundly* different? The answer is clearly *no*.

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