Viewpoint
Hypoxia: not merely a regulator of angiogenesis?
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Introduction
Maintaining oxygen homeostasis is of vital importance for the survival and development of mammalian cells. Hypoxia (below-normal levels of oxygen in air, blood and tissue) can potentially lead to cellular dysfunction and ultimately cell death and is a feature of many pathological conditions. Understanding how reduced oxygen levels may contribute to or even promote disease is a significant facet of developing new therapeutic options. A recent study by Cramer *et al.* gives a fresh insight into these mechanisms and raises questions over whether hypoxia is more than just a regulator of angiogenesis [1].

How do cells respond to changes in oxygen concentration?
The way in which cells 'sense' and respond to changes in oxygen concentration in their environment has attracted considerable interest. An important and well-characterised master regulator of the adaptive response to alterations in oxygen tension is hypoxia-inducible factor (HIF), a transcriptional complex containing two (α and β) members of the basic-helix-loop-helix PAS (period-aryl hydrocarbon nuclear receptor translocator-single minded) family. Several HIF-α isoforms exist, including HIF-1α and HIF-2α. HIF molecules bind specifically to hypoxia-responsive elements in the promoter or enhancer regions of various genes, which include erythropoietin, vascular endothelial growth factor (VEGF), glycolytic enzymes and genes involved in iron metabolism [2].

Oxygen levels regulate HIF primarily through a mechanism involving oxygen-dependent proteolysis of HIF-α [3]. Under normoxic conditions, HIF-α subunits have a very short half-life. This is because prolyl 4-hydroxylase domain enzymes, that require oxygen as an obligatory cosubstrate, hydroxylate conserved proline residues in the HIF-α subunit, which allows the von Hippel-Lindau E3 ubiquitin ligase complex to bind to HIF-α and target it for proteasomal destruction [2,4]. In addition, the recruitment of transcriptional coactivators by HIF-α is regulated by oxygen-dependent hydroxylation of asparaginyl residues within the subunit [5]. The critical dependence of prolyl and asparaginyl hydroxylation on oxygen means that, under conditions of hypoxia, HIF-α accumulates in the nucleus where, upon binding to constitutively expressed HIF-1β and recruitment of coactivators, it recognizes hypoxia-responsive elements within promoters of target genes, leading to their transcriptional activation.

Hypoxia in RA
Hypoxia has been postulated to contribute to a number of pathologies, including tumour growth and metastasis, and, of relevance to this article, rheumatoid arthritis (RA). The main features of RA are an inflamed, heavily infiltrated and thickened synovium, with pannus formation and subsequent invasion and destruction of cartilage and bone. One consequence of synovial hyperplasia in RA is an increase in the distance between the proliferating cells and the nearest blood vessels. It has been postulated that this increase causes local hypoxia, and, indeed, a paper published more than 30 years ago showed that oxygen tension is low in human RA synovial fluids [6]. More recently, a study using sensitive microelectrodes demonstrated that synovial membrane oxygen tension is significantly lower in patients with RA [7,8]. These data are supported by findings showing hypoxia in the joints of arthritic mice using different models of disease [9,10]. The augmented proliferation of synovial cells also imposes an additional demand on the vasculature, further promoting the hypoxic state, and several studies have demonstrated that the oxygen consumption of the RA synovium is elevated [11]. Moreover, the increase in synovial volume is likely to promote hypoxia, by reducing synovial capillary flow. Resting intra-articular pressure in chronically inflamed RA joints has been found to be elevated, and this effect would be compounded during the movement of joints inducing acute ischemia [12].

HIF = hypoxia-inducible factor; RA = rheumatoid arthritis; VEGF = vascular endothelial growth factor.

As expected, given that the RA joint is profoundly hypoxic, HIF-1α was shown to be expressed by macrophages in the RA synovium, but was absent from healthy synovial tissue [13]. Studies using an animal model of arthritis demonstrated that HIF-1α and another transcription factor, Ets-1, colocalised with areas of hypoxia in inflamed joints [9]. More recently, HIF-2α was also described in RA [14].

It is generally thought that the primary consequence of the hypoxic RA synovial environment is enhanced angiogenesis, since VEGF is an important HIF-inducible molecule. Within a few hours of exposing different cell cultures to hypoxia, VEGF mRNA levels are dramatically increased [15]. For example, my colleagues and I have reported that RA synovial cells respond to hypoxia by upregulating VEGF [16]. Certainly, VEGF is expressed in RA and the synovial vascular density is altered [16]. However, the recent publication from Napoleone Ferrara's and Randall Johnson's groups has raised the highly intriguing possibility that hypoxia and HIFs may have other roles in RA besides the regulation of angiogenesis [1].

Investigating the functions of HIF-1α

In this seminal study by Cramer *et al.*, targeted cre-loxP-mediated deletion of HIF-1α in myeloid lineage cells achieved over 75% HIF-1α deletion efficiency in macrophages and granulocytes [1]. Unlike the embryonic lethality seen with HIF-1α knockout animals [17], mice lacking HIF-1α in only neutrophils and monocytes were without obvious phenotype under normal conditions. As expected, peritoneal macrophages failed to upregulate VEGF under hypoxic conditions. Macrophages have the capacity to switch from an aerobic to an anaerobic glycolytic pathway for ATP production, but deletion of HIF-1α resulted in an inability to upregulate molecules involved in glycolysis (phosphoglycerate kinase and the glucose transporter Glut-1). Furthermore, loss of HIF-1α was associated with impaired macrophage aggregation and migration. Although the RA synovium is hypoxic, it is unclear whether oxygen tension may differ spatially across the tissue. Conventionally, it has been thought that the fibroblasts at the leading edge of the invasive pannus are most hypoxic, since these cells are furthest from the synovial blood vessels. However, the RA synovium is infiltrated by many cells of lympho-haematopoietic origin including macrophages, which are therefore also likely to be exposed to low oxygen levels. This is supported by the observation that CD68-positive cells in RA express HIF-1α [13]. Indeed it is relevant that, in the RA synovium, glucose oxidation via an anaerobic, rather than aerobic, pathway has been reported, suggesting that under conditions of hypoxia in RA macrophages turn on a survival response by switching to anaerobic glycolysis [18]. Thus, in RA, increased levels of HIF-1α may not only induce VEGF expression but also promote macrophage survival and retention.

Subsequent experiments in the study by Cramer *et al.* addressed whether conditional loss of HIF-1α has functional consequences on inflammatory responses *in vivo* [1]. Using a model of phorbol ester-induced acute inflammation in the ear, dramatically reduced inflammation (shown as diminished CD45-positivity) and decreased oedema were shown in HIF-1α-deficient animals, paralleled by reduced myeloperoxidase activity in tissue homogenates. Crucially, mice with conditional deletion of VEGF were strikingly different to those lacking HIF-1α, exhibiting instead quite extensive infiltration, although with reduced oedema. This suggests that the reduced inflammation in HIF-1α conditional knockouts is not simply due to decreased VEGF expression. In a model of chronic cutaneous inflammation, leukocyte trafficking was also abrogated in animals lacking HIF-1α in myeloid cells.

The study most relevant to RA in the Cramer *et al.* publication involved the use of a murine model of arthritis [1]. KRN T cell receptor transgenic mice were crossed with NOD mice and arthritis was induced by serum transfer. This led to joint inflammation, oedema and destruction of bone and cartilage. Importantly, in HIF-1α conditional knockout animals, development of arthritis was significantly reduced, with diminished ankle swelling and decreased synovial infiltration and joint destruction. The mechanism involved in the reduced inflammation seen in these knockouts is not clear, but is likely to involve macrophages losing the ability to maintain energy homeostasis, thus impairing subsequent responses such as adhesion and migration.

Advancing our understanding of RA

It is well known that hypoxia and synovial infiltration are seen in RA. The observations described in the study by Cramer *et al.* extend our awareness by suggesting that HIFs regulate not only angiogenesis, but also inflammation (both acute and chronic) and, in particular, the inflammatory cascade in RA [1]. It is of significance that in parallel to the oxygen-dependent pathway, HIFs may also be regulated by receptor-mediated signals, although this pathway is less well understood [19]. These more subtle changes in HIF-α levels and/or transcriptional activation are stimulated by growth factors and cytokines such as TNFα and IL-1, both of which play vital roles in RA pathogenesis [20–22]. Interestingly, in synovial fibroblasts, IL-1 appears to increase mRNA for HIF-1α [23]. HIF may thus represent an important convergence point, integrating cellular responses to low oxygen tension and to inflammatory cytokines, and regulating both angiogenesis and inflammation. In RA, based on the study by Cramer *et al.*, upregulation of HIFs, as a result of both local hypoxia and increased proinflammatory cytokines, may promote macrophage infiltration and survival and hence the inflammatory cascade. More broadly, this study indicates that hypoxia may be intimately involved in the

upregulation of inflammation in a host of diseases in which oxygen tension is reduced, including psoriasis, atherosclerosis, RA and malignancies. Consequently, interrupting the HIF pathway could not only interfere with angiogenesis, but also directly reduce inflammation and cell trafficking, making it a potentially significant target in the development of new therapies for these diseases.

Competing interests
None declared.

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