Hypoxia Regulates Macrophage Functions in Inflammation

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Hypoxia Regulates Macrophage Functions in Inflammation

Craig Murdoch, Munitta Muthana, and Claire E. Lewis

The presence of areas of hypoxia is a prominent feature of various inflamed, diseased tissues, including malignant tumors, atherosclerotic plaques, myocardial infarcts, the synovia of joints with rheumatoid arthritis, healing wounds, and sites of bacterial infection. These areas form when the blood supply is occluded and/or unable to keep pace with the growth and/or infiltration of inflammatory cells in a given area. Macrophages are present in all tissues of the body where they normally assist in guarding against invading pathogens and regulate normal cell turnover and tissue remodeling. However, they are also known to accumulate in large numbers in such ischemic/hypoxic sites. Recent studies show that macrophages then respond rapidly to the hypoxia present by altering their expression of a wide array of genes. In the present study, we outline and compare the phenotypic responses of macrophages to hypoxia in different diseased states and the implications of these for their progression and treatment. The Journal of Immunology, 2005, 175: 6257–6263.

Macrophages are versatile myeloid cells, which circulate in the bloodstream for 1–2 days as monocytes before migrating into tissues to terminally differentiate into “resident” tissue macrophages. They represent the front line of defense against infection by bacteria, viruses, and other pathogens. Extensive monocyte extravasation is also an early event in the onset of inflammation, wound healing, and various diseases, where they exhibit a tissue-specific array of functions. Areas of ischemia and hypoxia (extremely low oxygen tensions) are present in various inflamed, diseased tissues due to the occlusion of the local blood supply or the inability of local vessel growth to keep pace with the growth and/or infiltration of cells in that area. Macrophages accumulate in large numbers in such sites and then respond rapidly to the hypoxia present with altered gene expression, thought to be mediated, in part, via their up-regulation of the transcription factors, hypoxia-inducible factors (HIFs)1 1 and 2 (1, 2). These consist of a distinct hypoxia-inducible α subunit and a common, constitutively expressed β subunit. In the presence of oxygen, both α subunits are rapidly degraded in the cytoplasm, but in hypoxia, they fail to degrade and translocate to the nucleus where they bind first to the β subunit and then to the hypoxic response elements of hypoxia-induced genes (3).

A number of studies have documented the distinct changes in gene expression that occur in macrophages when they experience hypoxia in vitro (see summary in Table I). These include up-regulation of molecules required for macrophage survival, tissue revascularization (and thus reoxygenation of the ischemic site), and recruitment and activation of more macrophages and/or other inflammatory cells (4). In this review, we compare the responses of macrophages to hypoxia in vitro with those described, to date, for these cells in a number of inflamed and/or diseased tissues. We then discuss the possible implications of such responses for the progression and treatment of disease.

Malignant tumors

There is now compelling evidence for inflammatory cells playing a crucial role in tumor progression fostering the survival, proliferation, invasion and metastasis of tumor cells, and promoting tumor angiogenesis (4, 5). Tumor-associated macrophages are a prominent inflammatory cell type in many forms of human tumor, (6) and a number of tumor-derived chemoattractants are thought to recruit monocytes across the tumor vasculature, including M-CSF, CCL2 (formally MCP-1), and vascular endothelial growth factor (VEGF) (7).

Pollard’s group used a macrophage-depleted transgenic mouse model to demonstrate a central role for tumor-associated macrophage in the progression of spontaneous mammary tumors (8). Tumors containing fewer tumor-associated macrophage exhibited slower progression from preinvasive to malignant lesions and reduced lung metastases compared with tumors grown in normal littermates. This finding helps to explain why the presence of large numbers of tumor-associated...
Use of hypoxic cell markers like pimonidazole (PIMO) has demonstrated the presence of both transient (avascular, non necrotic) and chronic (perinecrotic) areas of hypoxia in both human and experimental tumors (9, 10). Tumor-associated macrophages accumulate in these areas due to a number of mechanisms, including the hypoxic release of such macrophage chemoattractants as endothelial monocyte-activating polypeptide II, endothelin 2, and VEGF by tumor and/or stromal cells (7). Moreover, hypoxia appears to immobilize tumor-associated macrophage by directly decreasing their mobility (11). The insidious functions of tumor-associated macrophage in these areas has been inferred by studies showing that their number in such hypoxic sites correlates with increased levels of tumor angiogenesis, lymph node involvement, and/or poor prognosis in breast cancer (7).

Macrophages up-regulate both HIFs 1 and 2 and various HIF target genes in hypoxic/necrotic areas of human tumors (1, 2). For example, they up-regulate the potent proangiogenic factor, VEGF, in hypoxic, avascular areas of human breast carcinomas (12), suggesting that they cooperate with tumor cells (which also up-regulate VEGF in response to hypoxia) to ensure the eventual revascularization of these areas. They also express the enzyme, matrix metalloproteinase (MMP)-7 in avascular areas of various forms of human tumors (1). This accords well with the finding that macrophages express increased levels of MMP-7 mRNA when exposed to hypoxia in vitro. (1) MMP-7 is an important enzyme in the context of the tumor microenvironment because it stimulates tumor cell invasion through the basement membrane into normal surrounding tissues, as well as endothelial cell migration during tumor angiogenesis (13).

Many of the other hypoxia-regulated cytokines and enzymes listed in Table I have also been shown to be expressed by tumor-associated macrophage in murine or human tumors (4). These include cytokines or enzymes with known proangiogenic and/or proinvasive effects such as fibroblast growth factor 2.

Table I. Gene expression by macrophages under hypoxic condition*

<table>
<thead>
<tr>
<th>Factor</th>
<th>Function(s)</th>
<th>Up-Down-regulation</th>
<th>mRNA</th>
<th>Protein</th>
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<td>VEGF</td>
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<td>PIGF</td>
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<td></td>
<td>78</td>
</tr>
<tr>
<td>COX-2</td>
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<td>Up</td>
<td>Yes</td>
<td></td>
<td>78</td>
</tr>
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<td></td>
<td>proangiogenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leptin</td>
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<tr>
<td>Tissue factor</td>
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<td>Up</td>
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<td></td>
<td>and prothrombotic</td>
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<tr>
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<td>CXCL12 receptor</td>
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<td>CD80</td>
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<td>Robo4</td>
<td>Slit receptor and</td>
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<td></td>
<td>antiangiogenic</td>
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<td></td>
<td></td>
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<td>MIF</td>
<td>Antimigratory and</td>
<td>Up</td>
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<td>Proinflammatory</td>
<td>Up</td>
<td>Yes</td>
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<td>60</td>
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</table>

*Abbreviations used (not defined in text): GLUT-1, glucose transporter 1; a glucose receptor; HGF, hepatocyte growth factor; NMB-R, neuromedin B receptor; PIGF, placental like growth factor. * Indicates where cDNA array data have been confirmed by real-time PCR.
(FGF2), TNF-α, macrophage inhibitory factor (MIF), cyclooxygenase-2, tissue factor, inducible NO synthase (iNOS), urokinase plasminogen activator (and its receptor), and the immunosuppressive cytokines, IL-10 and PGE₂. However, the role of hypoxia in regulating tumor-associated macrophage expression of these within tumors has yet to be demonstrated.

It has been suggested that given their propensity for hypoxic areas, macrophages could be used as delivery vehicles to target hypoxia-regulated gene therapy to such sites (which are largely inaccessible to other blood borne therapeutic agents). In a recent study, primary human macrophages were engineered to carry the gene for the prodrug activating enzyme, cytochrome p4502B6, under the control of a hypoxic response element. When they migrated into the hypoxic centers of tumor spheroids (small, spherical tumor masses grown in vitro), they expressed the p450 enzyme and this produced marked tumor cell killing when spheroids were administered the prodrug, cyclophosphamide, in vitro (as this was converted to its cytotoxic metabolite by macrophages expressing p450 in hypoxic areas of spheroids and then diffused out of these cells to kill neighboring tumor cells) (14).

Atherosclerosis

Atherosclerosis is initiated by dysfunction of endothelial cells at lesion-prone sites in the walls of arteries, which promotes a procoagulant and proinflammatory vasculature and results in monocyte infiltration into the arterial intima (15). These cells differentiate into macrophages, which then internalize large amounts of oxidized low-density lipoprotein forming cholesterol-laden macrophages called “foam” cells, which in turn give rise to fatty streaks in the arterial wall (16). As the atherosclerotic lesion develops, the arterial wall thickness increases and oxygen diffusion into the intima is markedly reduced (17). Bjornheden et al. (18) showed the presence of hypoxic regions at distances of 200 μM away from the endothelial surface in rabbit atherosclerotic plaques. These hypoxic regions contain large numbers of foam cells revealing that these cells experience hypoxia during the development of atherosclerotic lesions.

Rydberg et al. (19) showed that macrophages subjected to hypoxia in vitro up-regulate the expression of lipoxygenase-2, an enzyme implicated in low-density lipoprotein oxidation. In addition, hypoxia also up-regulates very low-density lipoprotein (VLDL) receptors on macrophages, and high levels of VLDL receptors are expressed by plaque macrophages in vivo (20, 21). The VLDL receptor mediates the uptake of β-VLDL, chylomicron remnant, and lipoprotein LP(a) (21–23) suggesting that hypoxia contributes markedly to lipid metabolism in atherosclerotic lesions.

Recruitment of T lymphocytes and proliferation and migration of smooth muscle and endothelial cells are essential for atherosclerotic plaque formation and development. CXCL8 (formally IL-8) is a chemotactic and mitogenic for smooth muscle and endothelial cells (24). Foam cells isolated from human atherosclerotic tissue displayed elevated levels of CXCL8 release compared with macrophages in culture (25). Furthermore, low-density lipoprotein receptor knockout mice with CXCR2 (one of the receptors for CXCL8)-deficient bone marrow cells show reduced atherosclerosis compared with that seen in wild-type mice (26)), suggesting that CXCL8 contributes to atherosclerotic plaque development. Recently, CXCL8 has been shown to be up-regulated by foam cells found in hypoxic zones in rabbit and human atherosclerotic plaques (27). Thus, it appears that hypoxia-induced secretion of CXCL8 from foam cells may lead to the recruitment of smooth muscle cells and T cells into the atherosclerotic plaques and thus to plaque progression.

Several other genes have been shown to be up-regulated by foam cells in response to hypoxia. One such molecule is oxygen-regulated protein 150 (ORP150). Exposure of mononuclear phagocytes to hypoxia induces ORP150 expression and ORP150 expression is restricted to foam cells within atherosclerotic lesions (28). The precise biological function of ORP150 is currently unknown, but it may be linked to macrophage survival in hypoxia (29). This suggests that ORP150 may protect foam cells from environmental stress allowing them to carry out their role of cholesterol scavenging and tissue remodeling within lesions. MIF is also highly expressed by foam cells in atherosclerotic lesions (30). MIF expression within plaques is likely to promote neovascularization because MIF has been shown to mediate proliferation of cultured endothelial cells (31). Treatment of a strain of mice, which spontaneously develop atherosclerotic lesions (apolipoprotein E-deficient mice) with neutralizing anti-MIF Ab, led to a reduction of a variety of inflammatory mediators typically associated with atherosclerosis (32), implying that MIF may also play an important role in arterial intima inflammation. A number of cytokines and enzymes known to be induced by hypoxia are expressed by foam cells in human atherosclerotic plaques, including VEGF (33), platelet-derived growth factor (PDGF) (34), TNF-α, IL-1 (35), CCL2 (36), MMP-1 and -7 (37, 38). However, the expression of these factors by hypoxic foam cells in comparison to nonhypoxic foam cells in vivo has yet to be determined.

The secretion of proteolytic enzymes by foam cells eventually leads to weakening of the fibrous cap and ultimately rupture of the plaque; an event that results in thrombus formation or discharge of debris into the bloodstream, producing microemboli (39). Both these events can occlude arteries and prevent blood supply to tissues, resulting in localized hypoxia. If this occurs in coronary arteries, occlusion may result in persistent myocardial ischemia. In fact, regions of hypoxia have been detected in animal models of myocardial infarction (MI) (40). Moreover, Azzawi et al. (41) found significantly more macrophages in hearts with ischemic disease than in control hearts. Studies of MI induced in rats showed that HIF-1α and -2α were up-regulated in macrophages in infarcted tissue (42), suggesting that in MI macrophages are present in hypoxic myocardium. Although the factors secreted by this population of hypoxic macrophages have yet to be determined, macrophage expression of hypoxia-inducible growth factors in MI can be amplified by exposure to the macrophage-derived, antibiotic peptide PR39, which is expressed along the border of acute MI in rodent models (43). Li et al. (44) showed that PR39 induced angiogenesis in vitro and increased myocardial neovascularization in mice by inhibiting ubiquitin-proteasome degradation of HIF-1α, leading to increased cellular levels of HIF-1α and thus growth factor expression. Thus, it appears that infiltrating macrophages in infarcted regions can play an important role in cardiac tissue regeneration after MI. It remains to be seen whether PR39 has potential as a therapeutic agent in MI patients.
Rheumatoid arthritis (RA)

RA is characterized by persistent inflammation of the synovium in affected joints, with leukocyte infiltration, proliferation of synovial fibroblasts, and destruction of bone and cartilage (45). Migration of cells into joints needs abundant vasculature, which is generated by angiogenesis, and this is a prominent feature of RA. Although new blood vessels deliver oxygen to the inflammatory cell mass, the vascular network is dysfunctional so the synovium remains a markedly hypoxic environment (46). The presence of hypoxia in the human RA synovium was shown using microelectrodes and in the joints of a rat model of RA using PIMO (47, 48). Repetitive cycles of hypoxia and reoxygenation, together with oxidants produced by macrophages and neutrophils, lead to the generation of reactive oxygen species and tissue damage in RA joints (46).

Recent studies have shown HIF-1α expression by macrophages in the synovium of RA joints but not normal or osteoarthritic (nonhypoxic) joints (49). Furthermore, Cramer et al. (50) showed that when arthritis was induced in the joints of mice bearing a targeted deletion of the HIF-1α gene in myeloid cells, there was a marked reduction in joint swelling and reduced synovial infiltration by macrophages, implicating the hypoxic responses of these cells in RA pathology (51). A number of known hypoxia-regulated genes/proteins cited in Table I are also known to be expressed by macrophages in RA joints, including CXCL12 (51), CXCL8 (52), VEGF (53), IL-1β, and TNF-α (54). However, the exact role of hypoxia in regulating these in the RA joint has yet to be determined. In view of the abundance of macrophages in the synovia of RA joints, their expression of HIF-1 and various proinflammatory HIF-1 target genes, it would be interesting to see whether inflammation would be dampened by suppressing the expression/function of HIF-1 by macrophages. This could be achieved by injecting the synovial fluid of RA joints with a vector bearing small interfering RNA for HIF-1α.

Wounds

Macrophages are rapidly recruited to wounds to remove cellular debris and initiate revascularization and repair of the area. In a murine wound model, the initial inflammatory phase involves leukocyte recruitment and the formation of a fibrin matrix (55). Initially, neutrophils infiltrate the wound; their numbers peaking 1–2 days postwounding. This is accompanied by monocyte recruitment, but the latter peaks at days 2–5. Together, these phagocytes engulf debris and secrete factors that attract fibroblasts, lymphocytes, and endothelial cells into the wound that participate in neovascularization that re-establishes tissue architecture and restores perfusion (55).

Wounds exhibit areas of marked hypoxia due to lack of perfusion caused by vascular damage and the intense metabolic activity of cells infiltrating the wound (56). Oxygen tension measurements in a rodent model showed that immediately postinjury wound oxygen tension is reduced from 150 to 20–30 mm Hg, and this decreases to 5–7 mm Hg after 3–5 days (57). This led some to suggest that hypoxia (and other stress factors such as pH and glucose availability) might be essential for promoting angiogenesis and other repair mechanisms in wound healing (58). However, Haroon et al. (59) used PIMO labeling to show that hypoxia was largely absent on day 1 after punch biopsy wounds had been created in rats. Macrophages expressing various proangiogenic cytokines were found in these early, nonhypoxic lesions. This appeared to be coincidental with the onset of wound angiogenesis, suggesting that stimuli other than wound hypoxia are activated. However, by day 4, a fibrin clot had formed, and the disrupted vasculature and cellular demand for oxygen had resulted in widespread hypoxia. Significant macrophage accumulation was seen at this later stage, but a detailed analysis of their pattern of gene expression has yet to be performed. It is possible that their role in hypoxic lesions was to support and stabilize the newly formed vessels rather than promote the formation of new ones.

Studies in our laboratory have demonstrated expression of the transcription factor HIF-1 by macrophages in human dermal scar tissue (M. Crowther, N. J. Brown, and C. E. Lewis, unpublished observations), suggesting that macrophages are likely to respond to the hypoxia present in late wounds by up-regulating HIF-1 driven genes. Albina et al. (60) subjected macrophages isolated from rat wounds to hypoxia in vitro to determine their specific responses to hypoxia following conditioning by the wound microenvironment. iNOS gene transcription was increased along with the release of arginase, TNF-α, and IL-6. Macrophages isolated from nonwound sites and exposed to hypoxia only responded with increased TNF-α and IL-6 release (60). Catabolism of L-arginine produces reactive metabolites, which are found in rodent wounds and which may mediate some of the events in early inflammation (61). In rodents, iNOS is expressed in early wounds while arginase is expressed in late wounds (61). In early normoxic wounds, macrophages may use iNOS to metabolize L-arginine, but as the wound becomes more hypoxic, iNOS is switched off and L-arginine metabolism is shunted toward arginase (60). Other factors listed in Table I have also been detected in macrophages in experimental wounds of various animals. These include VEGF (62), platelet-derived growth factor (63), FGF-2 (64), TNF-α, IL-1β, PGE2 (66), ORP150 (67), and MMP-1 (68). However, it remains to be seen whether hypoxia regulates the expression of these proteins by macrophages in wounds in vivo.

Bacterial infection

Hypoxia is also a feature of tissues experiencing bacterial infection (69). This hypoxic state is due to increased oxygen consumption by the proliferation of bacteria, the accumulation of phagocytes at the infected site, and/or the vasoconstriction of vessels in the area that could impede delivery of oxygen to the site of inflammation. So macrophages are likely to experience hypoxia at sites of bacterial infection. However, there is currently no in vivo evidence that this directly alters/ regulates their antibacterial functions. However, it should be noted that HIF-1 levels can be up-regulated by rat and mouse macrophage cell lines in the absence of hypoxia by LPS (which is present in bacterial cell walls) (70). Furthermore, mouse macrophages rapidly up-regulate their expression of HIF-1α when exposed to Gram-positive or Gram-negative bacteria (71). Furthermore, Johnson’s group used transgenic mice with a targeted HIF-1α knockout in myeloid cells to demonstrate the importance of HIF-1α in the antibacterial functions of macrophages in vitro and in vivo under both normoxic and hypoxic conditions (50). Together, these findings indicate that HIF-1-mediated mechanisms play an important part in macrophage responses to bacterial infections and that these do not necessarily require activation by hypoxia.
It is clear from the evidence cited above that macrophages accumulate in areas of severe hypoxia in various inflamed sites, where they both respond to this microenvironmental cue and contribute to it by consuming oxygen themselves. Fig. 1 illustrates the marked overlap in pattern of gene expression shown by hypoxic macrophages in different diseased tissues. In most cases, this hypoxic phenotype appears to include the induction of a number of proangiogenic and proinflammatory genes, but the net effect of these on the surrounding tissue is likely to vary with context. For example, hypoxic macrophages may secrete proangiogenic cytokines and enzymes to drive angiogenesis in both tumors, arthritic wounds and healing wounds, but only in the latter case is this beneficial to the host. In the former, angiogenesis is known to accelerate disease progression. The fact that gene expression by these cells is not identical in different tissues may be due to the effect of local, tissue-specific cues on that gene expression by these cells is not identical in different tissues.

The prominent hypoxia-inducible transcription factor, HIF-1, is known to regulate many of the genes illustrated in Fig. 1 and to be up-regulated by macrophages in many of the ischemic tissues discussed in this article. However, care should be taken in assuming that HIF-1 up-regulation reflects exposure to hypoxia per se. As mentioned briefly in an earlier section, HIF-1 accumulates in macrophages in response to factors other than hypoxia. For example, oxidized low-density lipoprotein induces HIF-1α accumulation and HIF-1-dependent transcriptional activation in human monomac-6 cells (a macrophage cell line) under normoxic conditions (72). HIF-1 levels can also be up-regulated in macrophages by LPS (70), Gram-positive, and Gram-negative bacteria (71). Furthermore, HIF-1 has been shown to be up-regulated by proinflammatory cytokines and reactive oxygen species in several cell lines derived from different tissues but, as yet, not in macrophages (reviewed in Ref. 73).

It should also be noted that hypoxia can activate gene transcription in macrophages by mechanisms that are independent of HIFs. For example, hypoxia increased macrophage intracellular levels of hydrogen peroxide, which coincided with increased binding of the transcription factor AP-1 to the CXCL8 promoter and concurrent increase in CXCL8 mRNA expression (27), suggesting that AP-1 may be the main hypoxia regulated transcription factor regulating CXCL8 expression in hypoxic macrophages (although hypoxic activation of CXCL8 by C/EBPβ as well as AP-1 has also been demonstrated) (74). Furthermore, using C/EBPβ-deficient macrophage cell lines and peritoneal macrophages from C/EBPβ knockout mice, Albina et al. (75) showed that arginase-1 up-regulation by hypoxia in macrophages is modulated entirely by C/EBPβ. NF-κB, activating transcription factor-4, and Egr-1 have also been found to be up-regulated in human macrophages by hypoxia in vitro (L. El-barghati, C. Murdoch, and C. E. Lewis, unpublished observations) and in murine macrophages (76, 77). However, the role of these latter proteins in regulating gene expression by macrophages in inflamed, hypoxic tissues has yet to be determined.

Concluding remarks

References


