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NATURE|VOL 422 | 17 APRIL 2003 |www.nature.com/nature

Immunology

Oxygen and the inflammatory cell

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The discovery that a single protein allows certain immune cells both to respond to low oxygen levels and to induce inflammation may provide a new target for drugs to treat diseases characterized by excessive inflammation.

Infected tissues, wounds, rheumatic joints, and parts of tumours that have outgrown their blood supply would seem to have little in common. Yet such sites share two features: they have lower concentrations of oxygen than healthy tissues (they are 'hypoxic'), and they are infiltrated by leukocytes, major cell types of the innate immune system. Writing in *Cell*, Cramer and colleagues1 propose that a single protein mediates both the response of these cells to hypoxia and their ability to participate in inflammation — a coordinated immune response to tissue injuries such as those mentioned above2. The protein, hypoxia inducible factor-1a (HIF-1a), regulates the expression of at least 30 genes when oxygen levels are low3. Cramer *et al.* show that HIF-1a also controls several key aspects of inflammation: the redness and swelling of injured tissues, and the ability of leukocytes to enter these sites. It is striking that a single molecule should emerge as a master regulator in two such diverse and significant settings as hypoxia and inflammation. Leukocytes come in various guises, two major subtypes being neutrophils and macrophages. In one of the earliest responses to injury, neutrophils in an affected tissue's venules — small blood vessels that receive input from capillaries — stop flowing with the blood. Instead, they stick to the inside of the venules and form clumps that can grow to occlude the vessel, reducing blood flow. In addition, some neutrophils cross the vessel wall, migrating into the tissue. The reduced blood flow and increased cell numbers lead to local depletion of oxygen. Then, macrophage precursor cells arrive.

Hypoxia causes macrophages, neutrophils and other cells to activate a variety of their genes, including that encoding vascular endothelial growth factor (VEGF), which makes blood vessels leaky4. This gene activation is achieved by means of HIF-1a, which binds to the promoters (regulatory elements) of target genes. To do so, HIF-1a must first bind its partner, HIF-1b, and this interaction is controlled by oxygen levels. When oxygen is abundant, there is little HIF-1a — it is destroyed under the direction of the von Hippel–Lindau (VHL) protein — and what there is can't bind HIF-1b. At low oxygen levels these twin restraints are lifted5 (Fig. 1, overleaf).

Given that HIF-1a is active in hypoxic leukocytes, Cramer *et al.*1 wanted to find out whether it is needed in inflammation. To study the function of a protein, researchers often inactivate its gene in experimental organisms such as mice, to see what happens. But disrupting the HIF-1a gene kills mice when they are embryos, precluding any subsequent study of inflammation. So Cramer *et al.* used a special technique in mice to delete the protein only in leukocytes. The approach involved using the promoter of the lysozyme gene, which is largely active only in leukocytes, to switch on an enzyme that inactivates the HIF-1agene.

The researchers found that isolated macrophages and neutrophils from the mutant mice had reduced levels of VEGF and phosphoglycerate kinase compared with wild-type cells. The latter enzyme is needed to generate ATP, the main cellular energy store, from glucose by glycolysis; this oxygen-independent process is the predominant means by which leukocytes generate energy. As might be expected, the leukocytes' ATP levels were also low.

In vitro, isolated macrophages showed defective migration and a reduced ability to kill bacteria. Moreover, mice with HIF-1a deficient leukocytes developed almost no redness or swelling in response to chemical irritants or arthritis-causing protein complexes, and fewer leukocytes infiltrated inflammatory sites (Fig. 1). By contrast, when the authors generated mice whose leukocytes lacked VHL, the inflammatory response was greatly increased, presumably because, without VHL, HIF-1a was no longer destroyed. Cramer *et al.* conclude that HIF-1a is needed for leukocytes to generate ATP in the low-oxygen conditions of injured tissues, and hence for these cells to function.

These remarkable findings suggest that HIF- 1a might make a good target for drugs aimed at reducing the excessive inflammation associated with many diseases. Does the decrease in VEGF expression in HIF-1a-deficient leukocytes contribute to the reduced inflammatory response? Cramer *et al.*'s findings suggest that it might be partly responsible: they generated mice lacking VEGF, and found that the animals developed less swelling in injured sites (although they still showed extensive leukocyte infiltration). But the reduced swelling and redness associated with HIF-1a mutant leukocytes might instead, or additionally, indicate a deficiency in the activity of mast cells. Mast cells are components of the innate immune system that act as sentinels stationed around blood vessels and beneath mucosal surfaces.

They orchestrate the early phases of inflammation, including redness, swelling and leukocyte recruitment 2, as the arthritis model used by Cramer and colleagues shows 6. Like leukocytes, mast cells can express lysozyme, and so may also have been lacking HIF-1a in the mutant mice. If so, these cells might also have had decreased ATP levels and been sluggish in triggering inflammation. This again hints at the potential benefits of manipulating HIF- 1ato treat inflammation.

That idea might seem to have a drawback: HIF-1a-deficient macrophages are less efficient at killing bacteria *in vitro*1, suggesting that dampening inflammation might come with the price tag of a diminished ability to fight infection. But the situation could be different *in vivo*, and here it is interesting to look at nitric oxide (NO). Inflammation activates a gene that encodes the high-output form of NO synthase, an enzyme that makes NO; hypoxia, via HIF-1a, contributes to this activation7. NO then promotes swelling and other aspects of inflammation, and helps macrophages kill microbes. It also mediates the avoidance and dormancy responses of fruit-fly larvae to hypoxia8, and controls the number of mitochondria — the cell's energy producing organelles — in mouse tissues9.

Moreover, in hypoxic conditions, NO inhibits cytochrome oxidase10, an enzyme that is essential for oxygen-dependent ATP generation by mitochondria; other products of NO synthase impair ATP formation by glycolysis. So, in wild-type leukocytes under hypoxic conditions *in vivo*, ATP levels might fall farther than would be predicted from the decreased oxygen supply alone. Conversely, HIF-1a-deficient leukocytes might express less NO synthase than wild-type cells, so their ATP generation could be less impaired. That suggests that ATP levels in HIF-1a-deficient and normal leukocytes in hypoxic inflammatory sites might

be more similar than they are in isolated cells cultured in air1. If so, the cells might also be more similar in their ability to fight bacteria *in vivo* than *in vitro*.

Other interactions between NO and hypoxia deserve mention because they can affect the activation of HIF-1a (Fig. 1). In hypoxic macrophages, if mitochondrial oxygen consumption by NO is blunted, the amount of intracellular oxygen available for other reactions11, including further NO synthesis, will increase. When NO inhibits the mitochondrial transfer of electrons to oxygen, electrons leak out, eventually giving rise to superoxide. This species or its products can activate HIF-1a3. Furthermore, high output NO synthase can stabilize HIF-1a12.

NO also induces haem oxygenase-1 (whose product, carbon monoxide, might inhibit HIF-1a13). On balance, low oxygen alone may not fully explain the activation of HIF- 1a in inflammation. Instead, the combination of low oxygen and high NO may be important. Researchers who seek to develop anti-inflammatory treatments based on this new understanding of HIF-1a might be discouraged by the difficulty of inhibiting a protein whose function is not enzymatic, and whose known enzymatic regulators (the prolyl hydroxylases) inhibit rather than activate it. But let us hope that investigators will continue to probe the relationship between hypoxia and inflammation that has been highlighted in such a surprising way by Cramer and colleagues.

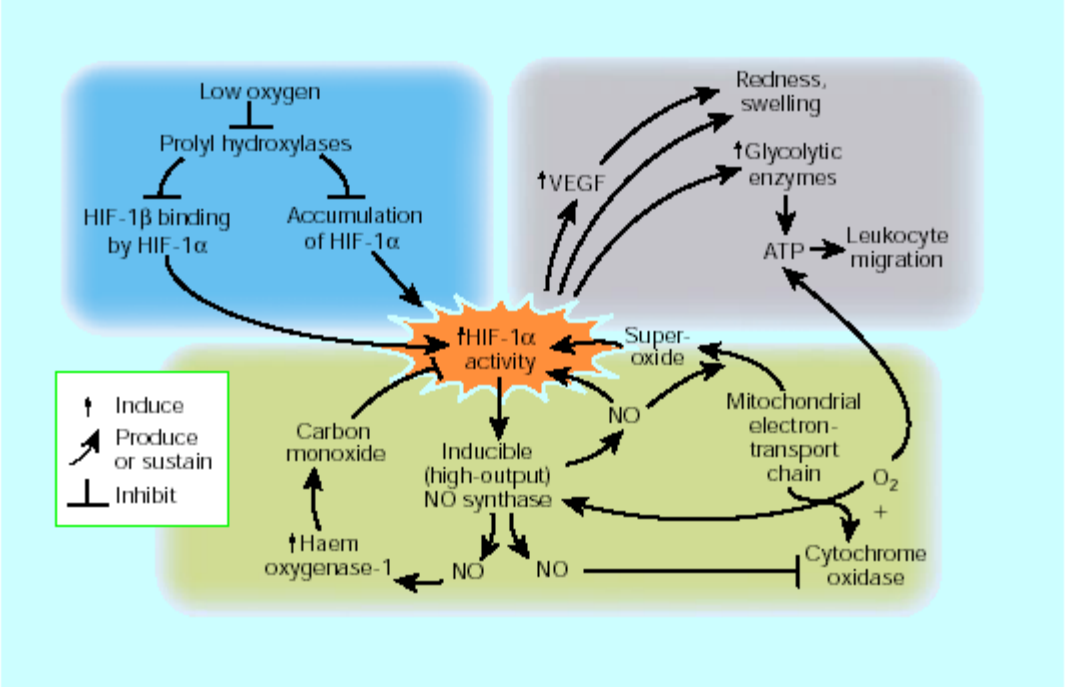

Figure 1 **Factors that may control, or be controlled by, hypoxia-inducible factor-1**a **(HIF-1**a**) in inflammatory sites with low oxygen levels. The blue shaded area shows that hypoxia inhibits prolyl hydroxylase enzymes, which would otherwise restrict HIF-1**a **activity. The grey area shows the findings of Cramer *et al.*1: HIF-1**a **is needed for several aspects of inflammation, namely the redness and swelling of injured tissues and, via glycolytic enzymes, leukocyte migration into injured areas. As shown previously, HIF-1**a **also induces the production of vascular endothelial growth factor (VEGF).**

The green area shows additional considerations, taking into account that HIF-1a **increases the production of nitric oxide (NO). Specifically, NO can induce haem oxygenase-1, which produces carbon monoxide; this in turn inhibits HIF-1**a**. But NO also increases HIF-1**a **activity, both directly, and indirectly via the production of superoxide (through its effect on mitochondrial electron transport). And NO inhibits cytochrome oxidase, leading to reduced oxygen-dependent ATP synthesis, which leaves some oxygen available for further production of NO.**

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