

to 400 km, based on the depths at which seismic shear waves travel at elevated speeds — shear waves have their particle motion perpendicular to the direction of propagation. Such higher seismic wave speeds indicate the presence of a region possessing distinct properties, undoubtedly cooler than its surroundings, but also likely to be distinct in composition.

Gung and colleagues¹ help to reconcile these results. Their work is based on images of Earth structure derived from shear waves with different polarizations. The approach — seismic tomography — is akin to the medical tomography used to study the interior of the human body. In the seismic version, however, illumination of Earth's interior depends on the distribution of earthquakes generating seismic waves and of seismic stations producing high-quality data. Many different classes of wave-propagation phenomena are exploited to improve sampling deep into the Earth. The dominant energy from earthquakes is radiated as shear waves in which the deformation is transverse to their propagation path. Purely horizontally polarized shear waves have somewhat simpler characteristics than those with propagation in a vertical plane and so have been used in many studies. Gung *et al.* point out that the studies indicating a thick root to the continental shields make extensive use of data from the horizontally polarized waves.

When the authors segregate the information from vertically and horizontally polarized shear waves, they find that the resulting images show significant differences. The structure with fast wave speeds beneath most continental shields extends to at most 250 km for the vertically polarized waves, whereas the equivalent structures for horizontally polarized waves extend deeper, to nearly 400 km. Gung *et al.* suggest that this form of seismic anisotropy, with horizontally polarized shear waves travelling faster than those with vertical polarization, is due to the influence of mantle flow in the region between 250 and 400 km. Similar anisotropy has been found under the ocean basins^{3,4} in the depth range from 80 km to 250 km. The transition to anisotropy caused by mantle flow would then mark the seismic definition of the base of the 'tectosphere', with a depth of no more than 250 km beneath the ancient continental shields.

In most parts of the world it is difficult to provide any direct test of the results of Gung *et al.*¹ But the distribution of earthquakes around the shield of northern Australia, a region that I have studied, is such that higher-frequency seismic waves can be used to probe structure beneath the shield. Here, similar anisotropy is found, with faster travel of horizontally polarized waves⁵ through a zone below 220 km that produces significant dissipation of wave energy and is hence likely to have lowered viscosity, promoting flow. A

complicated structural transition to lowered seismic wave speeds beneath the ancient shield occurs at about 210 km and has itself been attributed to a different kind of anisotropy⁶. The patterns of seismic anisotropy are subtle and the strong effects associated with polarization are accompanied by variations with the direction of propagation⁷.

Overall, Gung *et al.* have provided a satisfying way forward in tackling a long-standing puzzle in Earth science. But on the evidence from northern Australia — and as might be expected — the picture will not be straightforward. The seismically defined base of the tectosphere is likely to be quite

complex, depending on variations in the local tectonic environment. ■

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Immunology

Oxygen and the inflammatory cell

Carl Nathan

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-1 α (HIF-1 α), regulates the expression of at least 30 genes when oxygen levels are low³. Cramer *et al.* show that HIF-1 α 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, neutro-

phils 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-1 α , which binds to the promoters (regulatory elements) of target genes. To do so, HIF-1 α must first bind its partner, HIF-1 β , and this interaction is controlled by oxygen levels. When oxygen is abundant, there is little HIF-1 α — it is destroyed under the direction of the von Hippel-Lindau (VHL) protein — and what there is can't bind HIF-1 β . At low oxygen levels these twin restraints are lifted⁵ (Fig. 1, overleaf).

Given that HIF-1 α 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-1 α 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-1 α gene.

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-1 α -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-1 α was no longer destroyed. Cramer *et al.* conclude that HIF-1 α 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-1 α 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-1 α -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-1 α 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 recruitment², as the arthritis model used by Cramer and colleagues shows⁵. Like leukocytes, mast cells can express lysozyme, and so may also have been lacking HIF-1 α 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-1 α to treat inflammation.

That idea might seem to have a drawback: HIF-1 α -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-1 α , 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 fruitfly 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

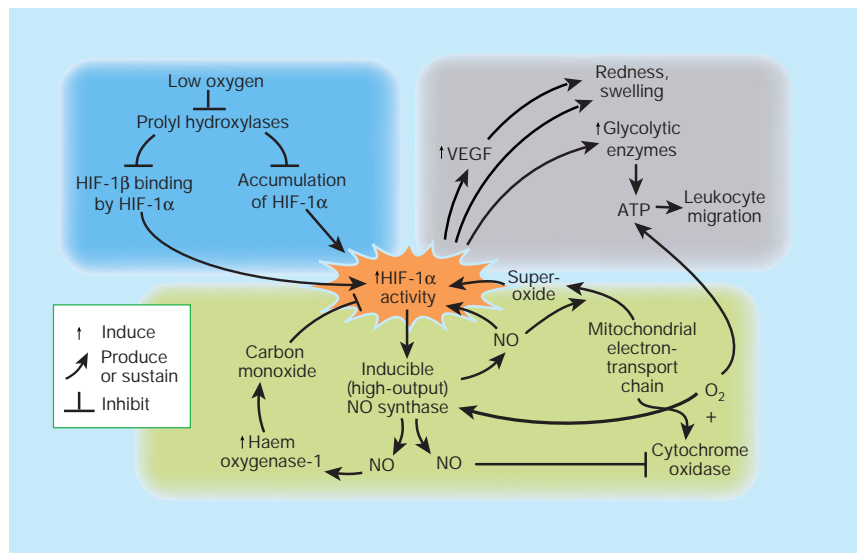


Figure 1 Factors that may control, or be controlled by, hypoxia-inducible factor-1 α (HIF-1 α) in inflammatory sites with low oxygen levels. The blue shaded area shows that hypoxia inhibits prolyl hydroxylase enzymes, which would otherwise restrict HIF-1 α activity. The grey area shows the findings of Cramer *et al.*: HIF-1 α 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 α also induces the production of vascular endothelial growth factor (VEGF). The green area shows additional considerations, taking into account that HIF-1 α increases the production of nitric oxide (NO). Specifically, NO can induce haem oxygenase-1, which produces carbon monoxide; this in turn inhibits HIF-1 α . But NO also increases HIF-1 α 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.

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-1 α -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-1 α -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-1 α (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-1 α ³. Furthermore, high-output NO synthase can stabilize HIF-1 α ¹². NO also induces haem oxygenase-1 (whose product, carbon monoxide, might inhibit HIF-1 α ¹³). On balance, low oxygen alone may not fully explain the activation of HIF-

1 α 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-1 α 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. ■

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