Hypoxia in Plaque Macrophages
A New Danger Signal for Interleukin-1β Activation?

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The recruitment of inflammatory cells to the arterial wall and their critical role in increasing plaque size and complexity is now dogma in the field of atherosclerosis. Macrophages compose the majority of the inflammatory burden in plaques and incite many of the deleterious responses that exacerbate disease. Thus, the mechanisms by which macrophages are activated to secrete cytokines and other inflammatory mediators are of intense interest. The atherosclerotic milieu is replete with cellular stressors such as modified apolipoprotein B–containing lipoproteins (eg, oxidized low-density lipoprotein) and reactive oxygen species, which can act as triggers of the inflammatory response. These danger signals compose a repertoire of triggers of so-called sterile inflammation that characterizes atherosclerosis.

The prototypical mediator of sterile inflammation is the proinflammatory cytokine interleukin (IL)-1β. Because of its potent downstream effects, the production of mature, biologically active IL-1β is tightly regulated at 2 distinct steps. In the priming phase, activation of signaling pathways such as nuclear factor-κB by Toll-like receptors, other cytokines, or IL-1β itself, leads to the transcriptional induction of precursor IL-1β (pro–IL-1β). Generation of the mature form is then governed by activation of a complex of proteins known as the inflammasome. Although several distinct complexes with unique triggers have been described, the NLRP3 inflammasome composed of nucleotide-binding oligomerization domain-like receptor family member (NLRP3), apoptosis-associated speck-like protein (ASC), and pro–caspase-1 is the most relevant to metabolic diseases such as atherosclerosis. A variety of pathogen- or endogenously derived danger signals (known as pathogen- or damage-associated molecular patterns) can activate the NLRP3 inflammasome, leading to proteolytic activation of caspase-1 and consequent cleavage of pro–IL-1β to the mature form. The process of inflammasome activation and its contribution to atherogenesis has been the focus of significant recent investigation in the field. Cholesterol crystals, which were previously thought to be inert byproducts of aberrant lipid metabolism in the atherosclerotic plaque, have now been shown to be potent inducers of the NLRP3 inflammasome and IL-1β secretion in macrophages, akin to other crystalline damage-associated molecular patterns. Even the proinflammatory action of oxidized low-density lipoprotein seems to occur in part through CD36-mediated uptake into lysosomes and conversion to cholesterol crystals.

Given the ability of IL-1β to further enhance the proinflammatory response and recruitment of immune cells, its critical role in exacerbating atherosclerotic progression has long been appreciated. Thus, the discovery of endogenous stressors found in the atherosclerotic milieu with profound effects on the macrophage IL-1β response is of significant interest. In this regard, the role of one such cellular stressor, hypoxia, on inflammatory signaling and atherosclerosis is intriguing. The presence of hypoxia, which is observed in both human atheroma and animal models of atherosclerosis, is consequential particularly in advanced plaques with increased hypercellularity and lesion complexity. Local hypoxia is thought to arise from a combination of increased metabolic demand in macrophages and reduced oxygen supply stemming from increased diffusion distances across the complex lesion. The role of hypoxia as a trigger for numerous atherogenic cellular responses is supported by a large body of literature. A universal feature of these responses seems to involve the stabilization and transcriptional activation of hypoxia-inducible factor-1α (HIF-1α). At lower oxygen levels, cells are known to produce mitochondrial reactive oxygen species, leading to the stabilization of HIF-1α. HIF-1α induces proteolytic, proangiogenic, proapoptotic, and other destabilizing factors that increase plaque complexity. Hypoxia also distinctly alters cellular metabolism, where a shift to anaerobic glycolysis occurs. This affects cellular energy balance and the availability of metabolic intermediates required for optimal cellular function. An important outcome is the alteration of macrophage lipid homeostasis toward a foam cell formation phenotype. Not surprisingly, hypoxia-induced cellular derangements in the plaque result in accelerated atherosclerosis in several proatherogenic animal models.

Despite the many parallels between hypoxic and proinflammatory effects on plaque progression, few direct links between hypoxia and proinflammatory signaling pathways (including IL-1β signaling) have been described. Hypoxic conditions can activate the nuclear factor-κB pathway through as yet undefined mechanisms. Several reports have shown that hypoxia can induce the transcription of proinflammatory cytokines, especially IL-1β. Most recently, work by Tannahill et al detailed a novel mechanism by which lipopolysaccharide stimulates IL-1β transcription through HIF-1α, a process that depends on succinate and metabolic switching of
macrophages to glycolysis. Although the synergistic secretion of IL-1β on exposure to both lipopolysaccharide and hypoxia was noted, the specific role of hypoxia in IL-1β transcription was not evaluated. An overview of the current understanding of the regulation of IL-1β production with links to hypoxia in atherosclerotic macrophages is shown in Figure 1A.

In the current issue of Circulation Research, Folco et al suggest a novel link between hypoxia, inflammasome activation, and induction of the macrophage IL-1β response. To gain a holistic understanding of IL-1β production under hypoxic conditions, Folco et al interrogated the effect of moderate hypoxia in human macrophages and plaques at several levels of regulation, including transcription, pro–IL-1β processing, and inflammasome activation. First, they make the interesting observation that hypoxia synergistically elevates lipopolysaccharide-induced pro–IL-1β levels in a manner independent of transcription. Follow-up pulse-chase experiments confirmed slower pro–IL-1β degradation under hypoxia, an observation that implicates either proteasomal or autophagic dysfunction. Several prior reports have suggested a complex role for autophagy in IL-1β production. Autophagy can both facilitate the degradation of pro–IL-1β and dampen the ability of the NLRP3 inflammasome to convert pro–IL-1β to its active form. Using the potent lysosomal (and by extension, autophagy) inhibitor bafilomycin, Folco et al note that the pro–IL-1β accumulation under hypoxic conditions is abrogated. Buttressing their data with 2 markers of autophagy,
p62 and LC3, they conclude that disruption of autophagic degradation is a prominent mechanism by which hypoxia increases pro–IL-1β levels.

At first pass, a hypoxia-mediated disruption of autophagic degradation is at odds with several previous reports demonstrating reduced oxygen levels in potent induction of autophagy, a process that is HIF-1α dependent.26 In agreement with this literature, when Folco et al compare the rate of autophagic flux in their experimental model, the protein levels of two well-known targets of autophagic degradation (p62 and LC3) are indeed reduced under hypoxic conditions, suggesting increased autophagy. How could it be possible that autophagic degradation of pro-IL-1β can have slower kinetics with hypoxia while autophagy pathways are induced? Folco and colleagues suggest that selective autophagy might be the answer. The discriminatory capacity of cells to target specific proteins or organelles for autophagic degradation is known as selective autophagy and a repertoire of proteins have been described to date that mediate this process.28,30 A well-characterized mechanism for the selective targeting of proteins for autophagic degradation involves protein polyubiquitination, binding to the chaperone p62, and cargo delivery to autophagosomes via p62’s LC3-binding domain.29,30 As evidence for such a process occurring in macrophages, Folco et al use immunofluorescence microscopy to show a loss of p62 colocalization with pro-IL-1β in hypoxic conditions. This observation would imply that with hypoxia, p62’s interaction with competing proteins/organelles is favored, thus limiting p62 availability for selective pro–IL-1β degradation. At present, it is unknown whether pro–IL-1β undergoes polyubiquitination and the degree to which it interacts with and is cleared by a p62-dependent process, but this interesting possibility can be further evaluated in vitro (eg, reconstitution experiments where the ubiquitin-binding domain of p62 is manipulated). It is noteworthy that polyubiquitination of the inflammasome complex and p62-dependent autophagic degradation has also been proposed as an alternative mechanism of limiting IL-1β production.27

Folco et al go on to implicate hypoxia at another level of IL-1β production, the NLRP3 inflammasome. They show that induction of IL-1β secretion by lipopolysaccharide is synergistically activated only on incubation of macrophages with the known inflammasome trigger, cholesterol crystals, under hypoxic conditions. They support this finding by demonstrating selectivity for IL-1β (ie, no changes were noted in tumor necrosis factor-α and IL-6), concomitant elevation of cleaved (ie, activated) caspase-1, an abrogation of this activation by caspase-1 inhibition, and a similar synergistic relationship between hypoxia and another potent inflammasome activator nigericin. They suggest that at least part of the hypoxia-induced inflammasome hyperactivation is related to transcriptional increases in NLRP3, an essential component of the inflammasome complex. Finally, as a link to the in vivo setting, they demonstrate that cholesterol crystals, IL-1β, activated caspase-1, and several markers of hypoxia such as HIF-1α are highly colocalized to the same macrophage-rich areas of human atherosclerotic plaques. Surprisingly, the IL-1β response of human macrophages to lipopolysaccharide and cholesterol crystals showed mild nonsignificant elevations, akin to lipopolysaccharide and hypoxia. Although this is distinctly different that the robust elevations of IL-1β when similar assays are conducted in murine macrophages, differences in experimental design as the authors suggest or inherent differences between human and murine macrophages might be an explanation. Also, the precise mechanism by which hypoxia synergistically activates the inflammasome complex warrants further investigation. Is hypoxia-induced transcriptional upregulation of NLRP3 the predominant reason for enhanced inflammasome activity? Is hypoxia-induced NLRP3 transcription dependent on HIF-1α activation? An enticing alternative mechanism is hypoxia’s role in more direct cytoplasmic activation of the inflammasome complex. Lower oxygen levels are a potent trigger for mitochondrial reactive oxygen species,15 which in turn is one of the most potent triggers of the inflammasome complex.26 Furthermore, cholesterol crystals among other crystalline damage-associated molecular patterns are thought to activate the inflammasome by disrupting the membrane integrity of lysosomes.4 Thus, it would be interesting to evaluate whether hypoxia exacerbates crystalline-mediated lysosomal membrane integrity.

An overview of the role of hypoxia in atherosclerotic macrophage IL-1β production as proposed by Folco et al is shown in Figure 1B. Taken together, these data support the notion that hypoxia imparts similar effects as other damage-associated molecular patterns and relevant inflammasome activators such as cholesterol crystals and implicates hypoxia as a previously unrecognized danger signal in atherosclerosis. To control the indiscriminate activation of IL-1β, nature has placed multiple regulatory steps in the processing of this potent proinflammatory cytokine. Hypoxia now adds a fascinating twist to the ever-increasing complexity of IL-1β regulation, the mastery of which will expand our understanding of clinically important chronic inflammatory conditions such as atherosclerosis.

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References

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