

EDITORIAL

What is hypoxia-inducible factor-1 doing in the allergic lung?

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Emerging evidence points to a role for hypoxia-inducible transcription factors in allergic airway inflammation and asthma. The hypoxia-inducible factor (HIF) family of heterodimeric transcription factors is now known to regulate the activation of different innate immune cell types, and asthma bears the 'footprints of HIF activation'. In this issue of the *Journal*, Huerta-Yepez and colleagues used genetic and pharmacologic approaches to explore the role of HIF-1 in mouse models of allergic airway inflammation. These studies support the idea that activation of HIF-1 is required for allergen sensitization and open the door to future therapeutic studies targeting the HIF pathway in allergic diseases.

The ability to sense and respond to changes in oxygen (O_2) concentration is a fundamental property of all nucleated cells. Exposure to hypoxia results in alterations in gene expression in multiple tissues mediated by the transcription factors of the hypoxia-inducible factor (HIF) family. HIFs are heterodimers comprised of different α subunits and the common HIF-1 β subunit (1). HIF-1 β (also known as aryl hydrocarbon receptor nuclear translocator or ARNT) is a constitutively nuclear factor that dimerizes with its binding partners to form an active transcriptional complex. In contrast to HIF-1 β , the expression and activity of HIF-1 α is regulated by oxygen (O_2)-dependent hydroxylation. Under normoxic conditions, degradation of HIF-1 α is controlled by binding of the von Hippel-Lindau (VHL) protein, which is the recognition component of an E3 ubiquitin-protein ligase that targets HIF-1 α for proteasomal degradation (2). VHL binds to HIF-1 α that is hydroxylated on specific proline residues (Fig. 1) (3). The prolyl hydroxylases that are responsible for this modification use O_2 as a substrate under normoxic conditions. Under hypoxic conditions, however, HIF-1 α prolyl hydroxylation and degradation are attenuated, allowing HIF-1 α to translocate to the nucleus, interact with HIF-1 β , and bind to specific DNA recognition sequences. This oxygen-sensing mechanism provides a means by which changes in O_2 concentration can be rapidly transduced into changes in gene expression.

HIF-1 α was originally identified in a search for DNA-binding factors that induced erythropoietin gene expression in response to hypoxia (4) and is now known to regulate the expression of an array of genes involved in metabolism, cell survival, and angiogenesis (5). Activation of HIF-1 is strongly associated with cancer growth and metastasis, and HIF-1 pathway antagonists are under active development with some candidates already in clinical trials. More recent studies have suggested that HIFs play a wider role in immunity and inflammation, and distinct roles of HIF-1 α

and HIF-2 α are starting to emerge (6, 7) (discussed further below). Standard cell culture conditions (95% air and 5% CO_2) expose cells to 20% O_2 , which is markedly higher than the O_2 concentrations to which most immune cells are exposed *in vivo*. Tissue oxygen concentrations are even lower during inflammation as edema interferes with the diffusion of oxygen from the microvasculature and the infiltration of inflammatory cells results in increased O_2 consumption. The concentration of oxygen in secondary lymphoid organs is very low (8), suggesting that HIFs may be activated in lymphocytes during their normal circulation in the body. Although oxygen concentration can affect the acute activation of CD4⁺ lymphocytes (9), more research is needed to understand potential effects of varying oxygen concentrations on the differentiation and survival of immune cells.

In the paper published in this issue of the journal, Huerta-Yepez and colleagues (10) used several approaches to explore the role of HIF-1 in allergic airway inflammation. First, the authors used a conditional deletion strategy and found that HIF-1 β -deficient mice were protected from allergen-driven airway inflammation using a well-established model of sensitization to ovalbumin plus alum. Second, the authors complemented this genetic approach with pharmacologic pathway agonists and antagonists (EDHB and 2ME, respectively). Third, the authors showed that HIF-1 α and HIF-2 α expression are upregulated in cells and endobronchial biopsies from human subjects following allergen challenge. Taken together, these studies suggest that HIFs play a role in allergic airway inflammation.

The findings of Huerta-Yepez and colleagues are in keeping with the growing appreciation of the 'footprints of HIF activation' in asthma. For example, the expression of several HIF-1 target genes is increased in the airway in asthma including vascular endothelial growth factor (VEGF) (11, 12). In addition to promoting vascular remodeling and lymphangiogenesis, VEGF contributes to T-cell activation in response to inhaled allergens through distinct effects on dendritic and T cells (13, 14). A nonbiased proteomic analysis revealed that several hypoxia-inducible, HIF-1-regulated proteins are upregulated in the lung in a mouse model of asthma, including several enzymes involved in glycolysis (15). Other molecules associated with both Th2-driven asthma and hypoxia/HIF include Muc5AC, CXCR4, arginase, and some members of chitinase/Fizz family members (16).

Because HIF-1 α , HIF-2 α , and HIF-1 β are required for mouse development (17), homozygous-null germline knockout approaches cannot be used to study the role of these factors in

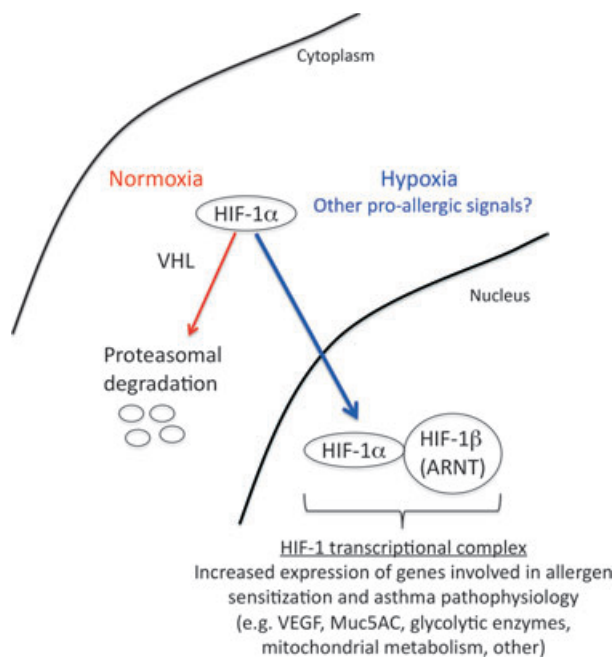


Figure 1. Under normoxic conditions (red arrow, left side), hypoxia-inducible factor (HIF)-1 α is hydroxylated by proline hydroxylases (not shown), binds von Hippel-Lindau (VHL), and is targeted for proteasomal degradation. Under hypoxic conditions (blue arrow, right side), HIF-1 α translocates to the nucleus and interacts with HIF-1 β (ARNT) to form a transcriptionally active HIF-1 complex. HIF-1 α nuclear translocation might also be promoted by other 'nonhypoxic' pro-allergic signals. HIF-1 induces the expression of genes that contribute to asthma pathophysiology and might also lead to allergic airway inflammation by regulating cellular and mitochondrial metabolism.

adult mice. In a preliminary study, we reported that heterozygous-null mice partially deficient in HIF-1 α were protected from lung eosinophilia in a mouse model of allergic airway inflammation (18). Huerta-Yepez (10) used a conditional strategy to circumvent the embryonic lethality of HIF-1 β . Conditional deletion refers to the technique whereby a gene of interest can be deleted in a cell-type specific and/or inducible manner. A widely used strategy involves insertion of recognition sites for the Cre recombinase flanking a crucial part of the targeted gene of interest. These recognition sites (referred to as lox P sites) are bound by Cre, which excises the intervening genetic segments and recombines the free ends of DNA. Two general approaches can then be used to conditionally delete genes of interest. First, by breeding mice with targeted lox P sites (referred to as 'floxed') to separate transgenic strains in which Cre expression is regulated by tissue-specific promoters, deletion of the targeted gene can be restricted to specific cell types. Second, transgenic mice in which Cre expression can be induced pharmacologically can be used to study acute effects of gene deletion. Both of these conditional deletion strategies can circumvent embryonic lethality of genes that are required during development and provide insights into their roles in adult mice.

For example, Johnson and colleagues used lysozyme promoter-driven Cre to conditionally delete HIF-1 α in neutrophils and monocytes and observed profoundly reduced tissue injury in mouse models of cutaneous inflammation and arthritis (19). Interestingly, there was defective expression of HIF-1 target genes encoding VEGF and glycolytic enzymes in HIF-1 α -null macrophages studied ex vivo even under nonhypoxic conditions. Whereas deletion of HIF-1 α abrogated leukocyte influx, VEGF deletion impaired tissue edema without affecting inflammation. This paper established that HIF-1 α was essential for myeloid-driven inflammation in a VEGF-independent manner. A more recent study reported that HIF-2 α is preferentially induced by Th2 cytokines in myeloid cells and contributes to alternative macrophage activation (7).

Huerta-Yepez et al. used an inducible deletion strategy to study the role of HIF-1 β in allergic airway inflammation. This involved breeding floxed HIF-1 β mice (referred to as *Arnt*^{F/F} mice) with mice expressing Cre under the control of the Mx-1 promoter. Mx1 is involved in innate anti-viral defenses and is highly inducible by type I interferons. By injecting mice with the double-stranded RNA polyI:C, which leads to robust type I IFN production, Huerta-Yepez et al. were able to induce widespread deletion of HIF-1 β in multiple cell types. Although immunohistochemical staining indicated prominent baseline expression of HIF-1 β in bronchiolar epithelial cells and blood vessels that was significantly reduced following polyI:C injection, it seems likely that HIF-1 β was deleted in other cell types as well. It is not possible to distinguish between the roles of HIF-1 β during allergen sensitization, allergen challenge, or both from the present study. The observation that HIF-1 β deletion-attenuated Ova-specific IgE production suggests that this factor is involved in alum-driven Th2 sensitization, which involves inflammasome-dependent dendritic cell activation. It will be interesting in future studies to dissect the role of HIF family members in pro-allergic innate immune responses in more detail.

The authors also showed that in both humans and mice, allergen challenge leads to marked upregulation of HIF-1 α and HIF-2 α expression in airway epithelial cells and cells retrieved from nasal and bronchoalveolar lavage. At least some of the HIF-1 α expression was localized to the nucleus, indicative of a transcriptionally active HIF-1 complex. As airway epithelial cells are continually exposed to ambient oxygen concentrations, how could the oxygen-sensing mechanism described above become activated in these cells? One possibility is that alternative 'nonhypoxic' modes of HIF-1 α activation operate in these cells, which is a ripe area for future research. It is worth noting that mouse models of chemical colitis have uncovered a protective role for HIF-1 in promoting intestinal epithelial barrier integrity (20), whereas HIF-1 plays a pathogenic role in promoting lung inflammation in a mouse model of trauma/hemorrhagic shock (21). It will be important in future studies to define the precise role of different HIF family members in the airway epithelium (e.g. with epithelial-specific conditional deletion), as well as in different inflammatory cell types (e.g. dendritic cells and lymphocyte subsets). It is quite possible that HIF-1 α may have protective effects in some contexts. For example,

chimeric mice with complete loss of HIF-1 α in lymphocytes manifest autoimmunity, suggesting a role for HIF-1 in down-regulating immune responses (22). Future studies investigating the potential association of HIFs with redox homeostasis and mitochondrial metabolism in the allergic airway may also prove worthwhile (23).

In summary, the identification of HIF-1 as a transcription factor involved in allergic airway inflammation is an important advance. Future studies of this oxygen-sensing pathway in different cells types and human subjects with asthma should enhance our understanding of asthma pathophysiology. The clinical development of HIF antagonists may also open new doors for therapeutic intervention.

Author contribution

TR and GLS edited and revised the manuscript, SNG wrote the first and final drafts.

Conflict of interest

None of the authors has a conflict of interest with the topic of the editorial.

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