Forum Minireview

Pathophysiological Response to Hypoxia — From the Molecular Mechanisms of Malady to Drug Discovery:

Inflammatory Responses of Hypoxia-Inducible Factor 1 α (HIF-1 α) in T Cells Observed in Development of Vascular Remodeling

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Abstract. Recent studies have shown that the cellular immune response to the hypoxic micro-environment constructed by vascular remodeling development modulates the resulting pathologic alterations. A major mechanism mediating adaptive responses to reduced oxygen availability is the regulation of transcription by hypoxia-inducible factor 1 (HIF-1). Impairment of HIF-1-dependent inflammatory responses in T cells causes an augmented vascular remodeling induced by arterial injury, which is shown as prominent neointimal hyperplasia and increase in infiltration of inflammatory cells at the adventitia in mice lacking Hif-1 α specifically in T cells. Studies to clarify the mechanism of augmented vascular remodeling in the mutant mice have shown enhanced production of cytokines in activated T cells and augmented antibody production in response to a T-dependent antigen in the mutant mice. This minireview shows that HIF-1 α in T cells plays a crucial role in vascular inflammation and remodeling in response to cuff injury as a negative regulator of the T cell-mediated immune response and suggests potential new therapeutic strategies that target HIF-1 α .

Keywords: arteriosclerosis, T cell, hypoxia-inducible factor 1 α (HIF-1 α), hypoxia, vascular remodeling

1. Introduction

Multi-cellular organisms use molecular oxygen for efficient ATP production, and oxygen consumption increases with increase in the mass and metabolic activity of the organism. However, exposure to oxygen must be limited in order to avoid damaging effects of reactive oxygen species (ROS) on cellular responses. Thus, all of the major physiological systems of vertebrates participate in complex homeostatic mechanisms that are designed to maintain the oxygen concentration to which each cell is exposed within a critical range for survival (1).

Cells closest to a perfused blood vessel are exposed to relatively high oxygen concentrations, which decline as

distance from the vessel increases. Thus, cells that are exposed to dynamic changes in oxygen concentrations in the body are adapted to function as cellular responses. Physiological and pathophysiological functions of cells are generally modulated according to the cellular oxygen concentration. Hypoxia is an elemental physiological stimulus that occurs in response to tissue growth during normal development (2 – 6) and in disease states, such as anemia, hemorrhage, inflammation, and pneumonia, and is a selective pressure for the evolution of adaptive responses. A major mechanism mediating adaptive responses to reduced oxygen availability is the regulation of transcription by hypoxia-inducible factor 1 (HIF-1) (7).

This minireview will focus on the role of HIF-1 in T cells in inflammatory responses to a hypoxic microenvironment constructed by pathophysiological events during development of vascular remodeling and on the mecha-

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nisms by which adaptive T cell inflammatory responses are impaired by genetic disruption of HIF-1 α in T cells.

2. Hypoxia-inducible factor

HIF-1 is a transcription factor that regulates gene expression in response to hypoxia, and it is composed of heterodimers of an oxygen-sensitive α -subunit and constitutively expressed β -subunit (also known as arylhydrocarbon receptor nuclear translocator, ARNT). HIF-1 α regulates the expressions of genes in response to hypoxia to maintain physiological oxygen homeostasis (8, 9). HIF transcription factor regulates numerous biological events that accelerate both oxygen delivery and adaptation to oxygen deprivation by regulating genes that are involved in glucose uptake, energy metabolism, angiogenesis, erythropoiesis, cell proliferation and apoptosis, cell–cell and cell–matrix interactions, and barrier function (10, 11).

Under normoxic conditions, HIF-1 α is synthesized and subjected to hydroxylation on proline residues by prolyl hydroxylase domain (PHD) proteins (principally PHD2) that use molecular oxygen and α -ketoglutarate as substrates. Prolyl hydroxylation of HIF-1 α is required for the binding of von Hippel-Lindau protein (VHL), which recruits a ubiquitin ligase complex (12, 13), leading to the degradation of HIF-1 α by the proteasome (14). Factor inhibiting of HIF-1 (FIH-1) binds to HIF-1 α and negatively regulates the transactivation function (15) by hydroxylating an asparagine residue, which blocks interaction of the HIF-1 α transactivation domain with the co-activator p300/CBP (16). In addition to the negative regulation of HIF-1 α activities by oxygen-dependent hydroxylation pathways, oxygen-independent pathways regulating HIF-1 α activity have been reported. In one of these pathways, resulting in up-regulation of HIF-1 α functions, several factors relevant to inflammation and oxidative stress, including nitric oxide, tumor necrosis factor (TNF)- α (17), angiotensin II (18), interleukin (IL)-1, insulin, and insulin-like growth factors (19, 20), have been shown to induce nonhypoxic HIF-1 α stabilization. The mechanism by which these extracellular signals enter cells is mostly by binding of growth factors, cytokines, and other ligands to the receptor tyrosine kinases and G-protein–coupled receptors on the cell surface, leading to the activation of phosphatidylinositol-3-kinase (PI3K) and MAP kinase pathways.

Genetic ablation of HIF-1 α in the mouse results in embryonic lethality at mid-gestation, and the embryo shows defects of vascularization, somites and neural crest, decreased size, and defects in ventricle development (21–23). Although regulated HIF-1 α function is clearly required for embryonic development, the use of

conditional knockout technology has allowed functional studies of hypoxia-response genes in adult mice (24, 25).

3. Vascular responses to hypoxia in development of vascular remodeling

Hypoxia occurs when oxygen supply is decreased and/or demand is increased (8, 26–28). Previous reports have shown that arterial wall oxygen levels were measured in animal models, using oxygen microelectrodes or a fluorescently labeled hypoxia marker in hypercholesterolaemic or balloon-injured rabbit models (29–31), and that they showed hypoxia. On the other hand, in an atherosclerotic lesion, oxygen diffusion could be limited due to intimal thickening or obstruction by calcification, while hypoxia could also originate from the high oxygen demand of metabolically active inflammatory cells (32). Hypoxia of the vessel wall is expected when the intimal thickness exceeds the maximum oxygen diffusion distance of 100–250 μ m (33). Nevertheless, hypoxia is typically present in macrophage foam cells in human and rabbit arterial plaques, suggesting that the inflammatory content also affects the presence of plaque hypoxia. Indeed, human subluminal (20–30 μ m) macrophages were already hypoxic despite their location well within the oxygen diffusion distance (34). Additionally, arterial tissue with inflammation is virtually always chronically hypoxic because of its high metabolic demand (35). Therefore, the hypoxia threshold seems to be dependent not only on the oxygen diffusion distance but also on the inflammatory microenvironment.

Hypoxia may also have a functional role in atherogenesis beyond angiogenesis due to hypoxia-mediated pro-inflammatory effects. Indeed, hypoxia in macrophages increases cytokine production (32, 35) and matrix metallo-proteinase secretion (36). Hypoxia also decreases migration of macrophages in vitro (37), potentially resulting in accumulation of macrophages in the inflammatory region. These findings have suggested that hypoxia also affects the formation of ROS (38) and the oxidation and loading of low-density lipid (LDL) (39–41). Generation of ROS in the absence of oxygen is generally accepted, although some contradictory findings have been reported, depending on cell type and quantification method (42, 43). ROS generation during hypoxia is reported to be produced from mitochondrial complex II, NADPH oxidase, and xanthine oxidoreductase (42–45). Therefore, the effects of hypoxia-mediated generation of ROS on atherosclerosis suggest aggravated intimal thickening compared to normoxic exposure of a balloon-injured rabbit (46) and apolipoprotein E-deficient mice (47). Although these studies have shown an effect of low

oxygen levels on the initiation and aggravation of vascular remodeling, it remains unclear how decreased oxygen in the pathophysiological microenvironment causes vascular remodeling.

4. Immune response in the development of injured arterial vascular remodeling

Neointimal hyperplasia in immune-deficient animals (48 – 52) shows a clear link between injury and the immune response to it. Although the specific antigens involved remain unknown, various reports on the roles of specific cell types and immune factors provide some insights. For example, cell injury alone can result in the release of intracellular materials, including uric acid (53) and heat-shock proteins, that can act as adjuvants, resulting in immune activation (54). In addition, damage-associated molecular pattern (DAMP), the endogenous molecules from the damaged and/or stressed cells, signal through pathogen-associated molecular pattern receptors (55), resulting in the activation of inflammatory responses. Therefore, it is easy to speculate that injury will result in both antigen-specific and nonspecific immune responses (56).

The mechanisms of arterial vascular remodeling induced by injury are thought to include at least i) intimal hyperplasia, through the recruitment of inflammatory cells and smooth muscle-like cells and elaboration of the extracellular matrix (ECM); ii) adventitial fibrosis, which will limit the ability of the perivascular ECM to positively remodel; and iii) increased medial tone (secondary to reduced vasodilator production or response or increased vasoconstrictor activity), which can contribute to intimal responses by increasing wall shear stress (57).

Recent data suggest that immune cells play critical roles in the development of angiotensin II-induced, deoxycorticosterone acetate salt-sensitive, and Dahl-salt-sensitive hypertension and in the progression of vascular remodeling (58). The potent pleiotropic cytokine interferon- γ (IFN- γ), a product of T-helper (Th)1 T cells and natural killer (NK) cells, affects the activation state of multiple cell types and also regulates major histocompatibility complex and costimulatory molecule expression and the production of cytokines, chemokines, adhesion molecules, and ECM. IFN- γ can also be produced by activated macrophages (59) and by smooth muscle cells (SMCs) stimulated by IL-12 and IL-18 (60). These facts suggest that injured arterial vascular remodeling could be caused by inflammatory responses mediated by activation of macrophages and SMCs and modulated by a hypoxic condition in inflamed vessels and surrounding tissues.

5. Inflammatory responses to arteriosclerosis and vascular remodeling under a hypoxic condition

Arteries and veins consist of multiple layers: the intima composed of endothelial cells, pericytes, and a basement membrane; the media composed mostly of SMCs and their ECM; and in the largest vessels, the adventitia composed mostly of fibroblasts and their extracellular matrix. Vascular response to arterial injury in arteriosclerosis or in-stent restenosis leads to neointimal formation and inward remodeling. Recent studies have shown that the immune system plays an important role in the development of vascular remodeling in response to the injury (57). Studies using mice have shown that arterial injury is associated with local accumulation of antibodies and that mice lacking functional T and B cells exhibit increased neointima formation, indicating that adaptive immune responses to neoantigens in the damaged tissue modulate the vascular remodeling (49 – 51).

During the development of vascular remodeling, a hypoxic microenvironment accompanying arteriosclerosis or stent-mediated over-distention in the injured vascular region is thought to be one of the factors modulating proliferation of myofibroblasts and increased matrix synthesis in the adventitial region (61). It has also been reported that hypoxia accelerates the progression of atherosclerosis (47, 62) and modulates vascular remodeling after arterial injury (46). Several studies have provided evidence of hypoxia within the arterial wall in atherosclerosis in an animal model (29) and human disease (63, 64). It has also been reported that adaptation to low oxygen tension in local tissues is important for activities of immune cells because they are often exposed to different oxygen tensions that markedly affect cellular metabolism as they survey different tissue microenvironments (65). These findings indicate that adaptation of immune cells to a hypoxic condition in vascular remodeling modulates the pathogenesis. However, there are many unanswered questions as to how the immune response is regulated in vascular remodeling under a hypoxic condition and how it may be targeted for therapeutic intervention (66). Little is known about the functions of T cells in development of vascular remodeling in a hypoxic microenvironment.

6. Inflammatory responses of HIF-1 in T cells to the development of vascular remodeling and the role of HIF-1 as a negative regulator

T cells had been not implicated in vascular remodeling until much later, when they had been found in injured vascular tissue (67). Subsequently, it was reported that neointimal thickening after injury was increased in T cell-depleted or athymic nude rats (50). These early re-

ports confirmed the role of the immune system in the response to arterial injury. Recent studies have also demonstrated that a hypoxic microenvironment during the development of arterial injury-induced vascular remodeling possibly affects the inflammatory progression and subsequent progressive pathological alterations (29, 46, 47, 62–64). A vascular remodeling model, femoral arterial injury induced by an external vascular polyethylene cuff model (68, 69), showed that hypoxia was present at the vascular wall around the cuff-injured artery and that HIF-1 α -expressing T cells infiltrated around the hypoxic vascular region (70). These findings suggest that the function of infiltrating T cells around cuff-injured vessels in the development of vascular remodeling might be modulated by HIF-1 α in a hypoxic microenvironment, even though there are many other modulators of HIF-1 α besides hypoxia in the vascular wall, such as inflammation and oxidative stress induced by factors such as nitric oxide, tumor necrosis factor (TNF)- α (17), angiotensin II (18), IL-1, insulin, and insulin-like growth factors (19, 20). It has also been shown that hypoxia alone is not sufficient and that T cell receptor (TCR)-mediated signaling is required for accumulation of HIF-1 α in peripheral T cells (70, 71). TCR engagement does not influence hypoxia-dependent stabilization but stimulates synthesis of HIF-1 α , probably via a PI3K/mammalian target of the rapamycin pathway (71). The evidence described above also suggests that HIF-1 α in T cells is involved in T cell activation for immune responses in vivo.

A recent study demonstrated that cuff placement at the femoral artery caused prominent neointimal hyperplasia in Hif-1 α -T cell-deficient mice compared to that in control mice (70). This finding of augmentation of arterial hyperplasia in the mutant mice and the results of several experiments on T cell-mediated immune responses in vivo and in vitro suggested that HIF-1 α in T cells plays a crucial role in vascular inflammation and remodeling in response to cuff injury as a negative regulator of the T cell-mediated immune response, which includes regulating the production of cytokines and specific antibodies as described below. Another study has also shown that the T cell response in both in vitro and in vivo models of acute inflammation is negatively regulated by HIF-1 α (72), indicating that a deficiency of HIF-1 α in T cells improved septic pathogenesis via their functional inhibition in a genetic mouse model. Thus, HIF-1 α in T cells is required for regulation of adaptive immune responses, including both cellular and humoral immunity.

HIF-1 α has been reported to be involved in neointima formation after vascular injury and to mediate the upregulation of stroma cell-derived factor 1 (SDF-1), which is expressed in the vascular region (73). In vitro experi-

ments on the chemotactic activity of HIF-1 α -deficient T cells in response to SDF-1 showed no distinctive responses of HIF-1 α -deficient T cells or control cells to SDF-1 under a hypoxic condition, suggesting that the HIF-1 α deficiency in T cells does not alter the function of T cells in SDF-1-mediated migration under a hypoxic condition. These results are consistent with results of other studies showing that migration of even HIF-1 α -deficient T cells to areas around cuff-injured vessels was not impaired in mice. These results suggest that aggravation of vascular inflammation and remodeling in response to cuff injury may not result from dysfunction of chemotactic activity of T cells in Hif-1 α -deficient mice and that another T cell-mediated aberrant immunological response may be involved in its pathogenesis.

Recent studies have shown that T and B cells contribute to the modulation of vascular remodeling by arterial injury (49–51). Results of these studies have suggested that the adaptive immune system evoked by arterial tissue injury serves to limit the extent of the subsequent vascular repair process and that the mechanisms involved in restriction of the vascular repair process might include inhibition of smooth muscle cell growth by T cell-derived IFN- γ and antibody-mediated removal of proinflammatory debris. It has also been reported that aberrant production of IFN- γ and antibodies in mice lacking the *Hif-1 α* gene in T cells might result in enhancement of arterial injury-mediated vascular remodeling, including neointima formation, suggesting that the immune response by HIF-1 α in T cells is strictly regulated during vascular remodeling (70, 72).

Previous studies indicated the possibility that Th1 cytokines, especially IFN- γ , are key factors for inducing the development of vascular intimal hyperplastic lesions as well as more typical vascular remodeling (74). This is supported by the results of our study showing enhanced IFN- γ production in activated T cells lacking expression of the *Hif-1 α* gene (70). On the other hand, production of transcripts encoding IL-4 and IL-13, Th2-related cytokines, is also not impaired, suggesting possible roles for HIF-1 as a negative regulator in T cell activation. Furthermore, enhanced IL-2 production in Hif-1 α -deficient T cells may result in increased T cell numbers in peripheral lymphoid organs in mutant mice. Taken together, these results suggest that mechanisms involved in excess vascular remodeling resulting from HIF-1 α deficiency in T cells include aberrant production of cytokines in injured arterial tissues.

However, it remains to be elucidated how HIF-1 α regulates TCR-mediated T cell activation as a suppressive modulator in Th1 and Th2 cytokine production. The mechanisms involved in aberrant cytokine production in Hif-1 α -deficient T cells remain to be elucidated, but they

may include additional transactivation of the IL-2 gene by cross-talk between the arylhydrocarbon receptor (AhR) and HIF-1 α / β signaling pathways. Indeed, previous studies revealed that the IL-2 promoter region contains novel distal regulatory elements for the AhR, another competitive heterodimeric partner of HIF-1 β , which can induce IL-2 and can cooperate in modulation of the proximal promoter (75). This finding suggests that deficiency in HIF-1 α allows the AhR to recruit more HIF-1 β , resulting in activation of AhR/HIF-1 β signaling and resultant IL-2 production in T cells.

Regarding autoreactive inflammation in the development of vascular remodeling by arterial injury, a recent study showed increased production of antibodies against specific antigens in Hif-1 α -deficient mice, indicating possible roles of HIF-1 α in enhancement of autoreactivity. It has also been reported that autoimmunity and inflammatory tissue damage were found in chimeric mice with complete Hif-1 α deletion in T cells and B cells (76). However, it remains to be elucidated how HIF-1 α in T cells modulates autoreactivity against injured artery tissue. To examine autoimmunity in Hif-1 α -deficient T cells in vivo, morphological phenotypes in the kidney and liver were characterized and the number of regulatory suppressor T cells in the thymus was determined. However, no significant difference was found in morphology of these organs, even though the amounts of antibodies in serum were increased, or in the number of Foxp3-positive cells, a marker of regulatory suppressor T cells, which are localized in thymic medullar regions (70). Thus, the autoreactive phenotype observed in Kojima's study (76) may result from a lack of normal B cell functions caused by deficiency of HIF-1 α in B cells in T cell.

7. Conclusions

HIF-1 has been thought to be the therapeutic target for several diseases (77–80), while the detailed molecular mechanisms by which HIF-1 could control vascular remodeling in pathophysiological events are still unclear. In this review, we summarized the effects of a hypoxic microenvironment generated by vascular remodeling induced by artery injury, especially the effect on HIF-1 α -mediated T cell inflammatory responses to the progression of vascular remodeling. Overall, the modulation of initiation and progression of vascular remodeling in response to arterial injury by HIF-1 α in T cells may suppress immune responses in hypoxic regions in the body and represent a protective mechanism against unnecessary and excess immune responses, such as those in autoimmune and allergy diseases. This implies that control of the hypoxic microenvironment by modulation of HIF-

1 α activity in arteriosclerosis or in-stent restenosis remains a possible therapeutic target for local intervention.

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