One Special Question to Start with: Can HIF/NFkB be a Target in Inflammation?

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One Special Question to Start with: Can HIF/NFkB be a Target in Inflammation?

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Abstract: Hypoxia and Inflammation are strictly interconnected with important consequences at clinical and therapeutic level. While cell and tissue damage due to acute hypoxia mostly leads to cell necrosis, cells that are located closer to vessels are able to survive adapting their phenotype through the expression of a number of genes, including proinflammatory receptors for alarmins. These receptors are activated by alarmins released by necrotic cells and generate signals for master transcription factors such as NFkB, AP1, etc. which control hundreds of genes for innate immunity and damage repair. Clinical consequences of chronic inflammatory reparative response activation include cell and tissue remodeling, damage in the primary site and, the systemic involvement of distant organs and tissues.

Thus every time a tissue environment becomes stably hypoxic, inflammation can be activated followed by chronic damage and cell death or repair with vessel proliferation and fibrosis. This pathway can occur in cancer, myocardial infarction and stroke, diabetes, obesity, neurodegenerative diseases, chronic and autoimmune diseases and age-related diseases. Interestingly, proinflammatory gene expression can be observed earlier in hypoxic tissue cells and, in addition, in activated resident or recruited leukocytes.

Herewith, the reciprocal relationships between hypoxia and inflammation will be shortly reviewed to underline the possible therapeutic targets to control hypoxia-related inflammation in a number of epidemiologically important human diseases and conditions.

Keywords: Hypoxia, Inflammation, Tissue and cell repair, Molecular rehabilitation, Stress response

HYPOXIA AND ADAPTATION TO HYPOXIA

Hypoxia is a reduction of normal level of tissue oxygen tension, occurring for several reasons in some physiological processes such as early embryonic growth or stem cell niche maintenance [1] and in a number of pathological conditions such as infarction, tumors, diabetes, obesity, pathological hypertrophy, neurodegenerative diseases and many others [1].

Hypoxia has two effects: 1-necrosis of cells in the region more distant from tissue vessels. 2-adaptation of cells in the region along the hypoxic gradient close to the vessels. Necrosis: the O2 shortage causes inhibition of ATP production, rapid fall of the energy charge, and loss of ionic gradients with the alteration of cytosolic calcium homeostasis [2, 3]. The increase of cytosolic Ca++ concentration above 10-6 M, dramatically activates the peroxidative metabolism [2], an irreversible contraction and degradation of the cytoskeleton [3, 4] and the activation of Ca”+-dependent cytosolic proteases (calpains) and DNA-ases [5, 6], all leading to a rapid and irreversible cell degradation. Plasmamembrane rupture due to lipoperoxidation is responsible for the definitive loss of the ionic gradients and for the release of intracellularly segregated molecules, many of which are called alarmins for their ability to signal the cell damage to specific receptors on adjacent cells. Adaptation of cells surviving to the hypoxia (when they are closer to the vessels) occurs through the activation of HIF1α and the expression of a number of genes, such as VEGF, Glut1 and HKII, TERT [7], stemness genes and, very importantly in our case, endogenous alarmin receptors, such as RAGE, P2X7 [8], Toll-like receptors [9], NOD-like receptors [10], etc. (Fig. 1). The beneficial effects of these genes can be observed in multiple cell or tissue functions and include: vasodilatation and neoangiogenesis to increase the local blood supply, metabolic shift from oxidative mitochondrial metabolism to anaerobic glycolysis, activation of telomerase in stem cells, reprogramming somatic cells to stem cells [11].

Importantly, alarmins (Table 1) released by necrotic cells (HMGB1, ATP/ADP, membrane debris, nucleic acids, etc.) bind their receptors and activate NFkB with the expression of hundreds of genes of the inflammatory reparative response (IRR) [for ref. see Table 1]. Therefore, non leukocytic cells in hypoxic tissue microenvironment can present a NFkB-dependent proinflammatory gene response similar to that seen in activated leukocytes (Fig. 1).
INFLAMMATION OR INNATE DEFENSIVE AND REPARATIVE RESPONSE (IRR)

Inflammation is a tissue/cell response to a noxious agent and/or to a cell damage to protect, defend, repair and limit the harm of the organism. Acute inflammation was already well known to Hyppocrates and thereafter precisely described by Galeno as acute phenomenon with prevalent vascular involvement. The modern picture is more complex and detailed due to the knowledge of pathways and molecular players for its local activation, specific tissue reactions, production of effectors, recruitment of distant cells, systemic involvement, production of damage, fine tuning regulation, and inhibition of progression for resolution and repair.

IRR may result inadequate (that is ineffective in protecting and repairing), chronic (that is prolonged), inopportune (when activated untimely and awkwardly), excessive (when is not rightly controlled) during one or all its phases [12]. In these situations IRR is clinically chronic and contributes substantially to host damages, causing multiorgan dysfunctions. In the progression of IRR it is possible to recognize typical signs and symptoms such as the accumulation of exudate, fever, leukocyte infiltration, excess of ROS production, damage to surrounding tissue, and a continuous fibrotic repair which leads to fibrosis and to organ insufficiency [12].

Inflammation is triggered by exogenous damaging agents such as viruses, bacteria, fungi, other pathogens and extraneous agents, presenting on their surface characteristic chemical pattern (Pathogen-Associated Molecular Patterns or PAMPs), or by endogenous signals (Damage-Associated Molecular Patterns or DAMPs) [13], which are usually released by damaged cells (necrosis, membrane shedding, exosomes, apoptosis) (Table 1). Collectively PAMPs and DAMPs are also known as alarmins [14-22]. These
pleomorphic molecules activate, with different specificity, a
number of receptors belonging to highly conserved families
with diverse signaling pathways, converging in the activation
of NFkB, AP-1 and other TFs which are recognized as the
major players in the inflammation, controlling all the genes
participating to this response [13, 23].

**Alarmin receptors (ARs)** were firstly localized in tissue
resident leukocytes [13, 14], but at present have been
described in many other cells and tissues [13]. In fact, there
are evidences that alarmin receptors can be induced and
expressed *de novo* in resident or recruited stem cells and
pluripotent undifferentiated progenitors from many tissues,
der under stressing conditions such as acute or chronic hypoxia
[24] and cellular ROS increase [25, 26]. ARs after sensing
alarmins generate a signal cascade that activates NFkB (or
other TFs), leading to the expression of genes belonging to
the large families of the IRR, all involved in defence,
protection and repair of cell and tissues (Table 1).

Another molecular component of the innate response is
**inflammasome** constituted by large protein complexes
assembled by cytosolic alarmin receptors (NOD-like
receptors or NLRs) activated into the cytosol [27, 28]. While
surface membrane alarmin receptors interact with signals
coming from extracellular space (of exogenous origin or
released by damaged cells), inflammasomes are activated by
intracellular molecules already present in damaged, stressed
or aged cells. Examples of these molecules include
monosodium urate crystals (gout), cholesterol crystals
(hypercholesterolemia and atherosclerosis), islet amyloid
polypeptide (stored in the pancreatic beta cells in type 2
diabetes) [28], viral and bacterial DNA and simplier
molecules such as ATP, HMGB1 and membrane
phosphatidylserine which become available in damaged or
dying cells as membrane debris. Activated NLRs respond
rapidly to different cytosolic PAMPs and DAMPs, recruiting
and activating pro-caspase-1 and other pro-inflammatory
caspases [27]. Caspase-1 activates the major pro-
inflammatory cytokines (IL-1 and IL-18) and is also
responsible for degradation of many target proteins,
contributing to cell degradation [19, 27]. Defects in the
structure and activity of inflammasomes can be responsible
for a large number of chronic human diseases, including
chronic arthritis, Crohn’s disease, irritable bowel and other
inflammatory diseases, diabetes, metabolic syndrome, cancer
and ageing [12, 19, 29, 30]. Targeting these proteins
represents an additional new therapeutic strategy for these
pathologies [12, 31].

**Effector and Cytokine Storm**

Inflammation or innate immunity involve signaling
pathways leading to the production of inflammatory
mediators or effectors in a cell-specific manner. Examples
are classical mediators such as histamine, prostaglandins,
leukotrienes, lipoxins, plasmalogens, cytokines, chemokines,
reactive oxygen intermediates (ROI), reactive nitrogen
intermediates (RNI), and anti-inflammatory molecules
(IL-10, A20, IL-1RA). Sometimes a massive production of
proinflammatory cytokines (cytokine storm) occurs leading to
multiorgan irreversible damage and shock [1, 31]. As a
matter of fact, all the effectors are a double-edge sword,
having from one side an exquisite protective role to defeat
invaders and limiting the harm of the host, from the other
hand when their action is excessive or improper they can be
the driving force responsible for tissue/organ damages.
During inflammation a production of numerous ROI, RNI
and chlorine species occurs, in addition to the products of
lipid and sugar oxidation [32,33]. Most of these products are
capable of chemically modifying protein amino acids,
changing the structure, folding and function of proteins.
Increasing evidence demonstrates that such oxidative post-

<table>
<thead>
<tr>
<th>Exogenous Alarms (PAMPs)</th>
<th>Receptors (Ref. 12, 13, 59)</th>
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<tr>
<td>- Viruses</td>
<td>TLR3, TLR7, TLR8, TLR9</td>
<td>[13, 59]</td>
</tr>
<tr>
<td>- Bacteria</td>
<td>CD14, TLR2, TLR4, TLR5, TLR9, TLR11</td>
<td>[13, 59]</td>
</tr>
<tr>
<td>- Fungi and protozoa</td>
<td>TLR2, TLR4, others</td>
<td>[13, 59]</td>
</tr>
<tr>
<td>- Obligate Intracellular pathogens</td>
<td>NOD-like receptors (NLRs) and intracellular TLRs</td>
<td>[19, 58]</td>
</tr>
<tr>
<td>- Intracellular fibers (such as asbestos fibers)</td>
<td>NOD-like receptors (NLRs)</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>Endogenous alarmins</td>
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<tr>
<td>ATP/ADP</td>
<td>P2X7, (other P2X and P2Y receptors?)</td>
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</tr>
<tr>
<td>Oxidized phospholipids and Phosphatidylin-serine Pattern</td>
<td>TLRs (?</td>
<td>[57]</td>
</tr>
<tr>
<td>Crystals (cholesterol, uric acid, etc.)</td>
<td>NOD-like receptors (NLRs) and intracellular TLRs (TLR2, TLR4, CD14)</td>
<td>[19]</td>
</tr>
<tr>
<td>dsDNA – dsRNA virali citosolici</td>
<td>RIG-like helicases (RLHs), TLR9</td>
<td>[55]</td>
</tr>
<tr>
<td>Defensins and cathelicidins</td>
<td>TLR4+CCR6, and FPRL1</td>
<td>[55]</td>
</tr>
<tr>
<td>Heat shock proteins</td>
<td>CD14, CD91, TLR2, TLR4, CD40</td>
<td>[55]</td>
</tr>
</tbody>
</table>

References are indicated and discussed in the text. PAMPs = pathogen associated molecular patterns; DAMPs = damage associated molecular patterns; TLRs = Toll-like receptors; CCR6 = chemokine receptor 6; FRLP1 = formyl peptide receptor-like 1.
translational modifications may generate neo-epitopes capable of eliciting both innate and adaptive immune responses giving origin to autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, chronic bowel diseases and others [25]. Some new epitopes generated by oxidation are conserved along evolution as pathogen-associated molecular patterns (PAMPs) [25].

**IRR Regulation**

Usually inflammatory response is self-limiting and well controlled, but in the presence of improper activation, aberrant amplification, mutated alarmin receptors or inflammasomes, inhibited or delayed resolution results in chronic diseases. Much attention has been focused on pro-inflammatory signaling but the precise mechanisms leading to the inhibitory control of inflammation is still unclear [34].

The IκB kinase (IKK) complex contains two catalytic subunits, IKKα and IKKβ, and controls the activation of NFκB transcription factors, the pivotal actor/controller of the inflammation [34, 35]. NFκB activation and activity persistence are tightly controlled by a number of endogenous mechanisms that limit the excessive and prolonged production of pro-inflammatory mediators and the associated tissue damage [35].

**NFκB (nuclear factor kB)** is a transcription factor that controls and modulates the expression of genes involved in both the innate and adaptive immune response [34]. The regulatory ability of NFκB is controlled by post-transcriptional modifications such as phosphorylation and complex interactions with other cytosolic and nuclear proteins. Upon activation of the NFκB signaling pathway, IκB kinases target IκBs for degradation. This allows NFκB to translocate and accumulate in the nucleus, where it binds to DNA, resulting in the expression of target genes [34-36].

One of the genes activated by NFκB is that encoding IκB. Newly synthesized IκB binds to NFκB and attenuates the pathway of response to alarmin receptors, thereby creating a negative feedback loop within this signaling pathway [35]. SOCS gene family is another major regulator of IRR once activated. Seven SOCS members have been identified but in particular SOCS-1 inhibits the cytokine signaling via Janus kinase (JAK) and STAT, efficiently negatively regulating NFκB-directed proinflammatory expression.

Most of the times, the IRR starts locally at the damaged site, and then rapidly eliminate the invading pathogen and/or repair the damaged cell and tissues. However, if the local activation is strong and the release of mediators (such as eicosanoids and cytokines) is abundant, distant leukocytes and cells are recruited, thus amplifying the response at systemic level. The necrosis of damaged tissue and cells are recruited, thus amplifying the response at systemic level. The necrosis of damaged tissue and cells activate the cytokine signaling but the precise mechanisms leading to the inhibitory control of inflammation is still unclear [34].

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**HYPOXIA AND INFLAMMATION IN HUMAN PATHOLOGY**

A large number of evidences suggest that when a tissue environment becomes stably hypoxic, inflammation can be activated causing chronic damage and cell death as well as repair with vessel proliferation and fibrosis. This pathway, with some variations, can occur in many important human pathologies such as cancer, infarction, diabetes, obesity, pathologic hypertrophy, neurodegenerative diseases, chronic and autoimmune diseases and age-related diseases. Interestingly, both HIF-dependent adaptation and proinflammatory gene expression can be observed very early in these conditions and have been shown more evidently in local hypoxic stem cells and progenitors [39].

In the next paragraphs a rapid overview is presented on how hypoxia originates in different pathologic conditions and how this leads to the inflammation.

**Hypoxia and Inflammation in Cancer**

Virchow first noted that inflammatory cells are present within tumors and that tumors frequently arise at sites of chronic inflammation. Today we know that in the site of a chronic inflammation a toxic concentration of free radicals may facilitate the tumorigenesis by favoring DNA mutation accumulation (transformation) [23]. Thereafter, transformed cells rapidly proliferate producing small (400 µm in diameter) tumors in the absence of neoangiogenesis and thus generating a hypoxic microenvironment. Hypoxia has two effects on tumor cells: 1- necrosis of cells in the inner region of the tumor, more distant from host tissue vessels. 2-adaptation of cells to the hypoxic gradient within the tumor through the activation of HIF1α and expression of many genes such as VEGF (which is important in tumor neoangiogenesis), Glut1 and HKII (which explain the metabolic remodeling of malignant tumors) and RAGE, P2X7, Toll-like, etc. (alarmin receptors) (Fig. 1) [1, 7, 8, 11, 20, 24, 40-46]. Importantly, necrotic cells release alarmins (HMGB1, ATP/ADP, membrane debris, nucleic acids, etc.) that, by binding to their receptors, activate NFκB and hundreds of genes of the inflammatory reparative response (IRR) (Fig. 1) [45-60]. Therefore, transformed cells activate a gene response similar to that seen in activated leukocytes [44-46]. In solid human tumors (prostate ca [44], mammary ca [8, 43-46], glioblastoma [24] and thyroid papillary ca [61, 62]) the upregulation of alarmin receptors and the increased expression of major IRR genes has been shown, comparing tumor with host normal LCMD tissue fractions, in the absence of leukocytes (CD45+ cells) [43-46, 61, 62].

Hypoxic treatment of various tumor cell lines reproduces a gene adaptation similar to that seen in solid tumors, leading to the malignant phenotype [44-46]. Inhibition of HIF1α and/or NFκB in the same tumor cell lines prevents invasion and metastasis, suggesting a possibility to control tumor progression, by modulating inflammation through HIF1α.
and NFkB inhibition. An early control of HIF1α and/or NFkB activity could lead to a more efficient and less toxic therapeutic approach, not eradicating the tumor, but rather controlling its progression [36].

Presently, it is unclear if cancer stem cells can be the most susceptible target of these hypoxia-driven changes and if they can be blocked or controlled by modulating HIF1α and NFkB activity.

Hypoxia and Inflammation in Thrombosis: Role of Arterial Occlusion and Infarction

Thrombus formation is a frequent cause of ischemic hypoxia due to the occlusion of an arterial vessel. Ischemia produces severe cell damage and necrosis in tissues due to shortage of oxygen and nutrients as it occurs in myocardial infarction and cerebral ictus [63, 64]. Similarly to what we have described in tumors, necrotic cells release alarmins, while in surviving cells HIF1α is activated, changing the cell phenotype with the expression of alarmin receptors and activation of NFkB [63, 65-67]. It is well known that a few hours after infarction, HMGB1 and other alarmins are released [64, 68] and myocardial tissue or cerebral tissue can be infiltrated by recruited and activated leukocytes, contributing to the amplification of inflammatory response, to salvage and repair of damaged tissue [64, 68, 69]. However, it is well known that a prolongation of this phase is harmful leading to a pathological remodeling of the myocardium [63, 65].

Hypoxia and Inflammation in Obesity: Role of Adipocyte Hypertrophy

Obesity is probably initiated by chronic overnutrition and decreased energy expenditure (sedentary lifestyle) [70]. At level of white adipose tissue (WAT) this imbalance leads to an accumulation of fat nutrients as lipid droplets in the cytoplasm of adipocytes which progressively undergo hypertrophy, leading to the increase of white fat mass or obesity. Inadequate metabolic homeostasis and insufficient vascular perfusion are responsible for cell stress, local hypoxia, HIF-dependent adaptation and necrosis of adipocytes that are more distant from vessels. Hypoxia progressively occurs as the ratio vessel/adipose tissue area decreases [70-72]. The activation of HIF1α produces metabolic changes favoring insulin-resistance and type II diabetes, production of ROS, and expression of TLRs, NLRs, RAGE and P2X7 all activating NKfB and a non-leukocytic inflammatory response. The gene expression directed by NKfB includes cytokines, adipokines and chemokines, numerous classical inflammatory mediators and production of ROI and RNI. The latter two are responsible for adipocyte necrosis, while mediators activate resident leukocytes and recruits distant and circulating white cells, increasing the local inflammation firstly activated in adipocytes [73]. AT resident macrophages and lymphocytes may increase from less than 10% to more than 40% [73]. The involvement of distant organs (especially hepatocytes and skeletal muscle) creates a stable vicious metabolic circle maintaining local and systemic inflammation, aggravating insulin-resistance and metabolic dysfunctions, strongly contributing in this way to the pathogenesis of type II diabetes and metabolic syndrome [72, 74]. Adipocyte necrosis initially produced by ROI and RNI is relevant for IRR amplification and for AT fibrosis: necrotic adipocytes release alarmins which, acting on alarmin receptors of local adipocytes and resident leukocytes, activate and amplify local inflammation. Additionally IRR chronically stimulates repairing pathways, especially TGF-β pathway, leading to AT fibrosis [70].

Hypoxia and Inflammation in Diabetes Complications: Role of ECM and BM Thickness

Inflammation contributes to the primary pathogenesis of diabetes (i.e. damage of the insulin-producing β-cells and metabolic abnormalities increasing insulin-resistance), but it is as much relevant for the pathogenesis of the devastating complications including diabetic nephropathy, diabetic cardiomyopathy, retinopathy, great vessel involvement, peripheral arm and legs microvasculopathy and cerebral vasculopathy with brain hypotrophy [75]. All of them develop in association with a peculiar microvasculopathy due to an accumulation of AGEs, consequent hypoxia and inflammatory response with tissue damage. In the individual patient the severity of diabetes is not necessarily associated with high levels of hyperglycemia and metabolic abnormalities, but rather with these complications occurring preferentially in a specific organ or tissue, probably depending on individual susceptibility to non-enzymatic glycation and hence to the local accumulation of AGEs [74, 76].

Due to diabetic hyperglycemia, proteins undergo heavy non-enzymatic glycation forming and accumulating AGEs. Slow turn-over, long-lasting proteins forming ECM and basement membranes may be particularly susceptible and relevant for the pathogenesis of microangiopathy, because they rapidly accumulate around the vessels, in the ECM and in basement membranes of tissues, thus increasing the distance between the lumen of vessels and the cells of tissue that need to be fed (nourished) with oxygen and nutrients by simple diffusion (Fig. 2). In this way a hypoxic microenvironment is generated activating HIF1α and inflammatory response. In diabetes a substantial contribution to the activation of NKfB arise from RAGE signaling. RAGE, firstly identified for its binding to AGEs, is an alarmin receptor constitutively expressed by CD45+ cells (leukocytes) or induced by HIF1α [45, 46, 77]. The natural specific ligand of RAGE, under normal conditions, is the cytokine/alarmin HMGB1 having the highest binding affinity [13], but in diabetes a phenomenon of spill-over occurs due to high concentrations of AGEs that may saturate RAGE.

Hypoxia and Inflammation in Abnormal Hypertrophy with Insufficient Neoangiogenesis

Cell hypertrophy is an increase of cell volume due to abnormal synthesis of normal molecules and organelles, specific of a cell type. In physiologic myocardial hypertrophy [78, 79], such as in trained athletes, sarcomere components, mitochondria and regulatory proteins harmonically increase leading to myocardiocyte volume increase with normal contractile function. Parallel vessels and stroma increased
and remodeled for an optimal contractile performance [78]. Abnormal hypertrophy, such as that occurring in hypertension or in loss-of-function mutation of sarcomeric proteins, increase of sarcomeric components overcome the mitochondrial, vessel and stroma adaptations, with abnormal organization of contractile fibrils (sarcomeric disarray with abnormal direction of the contractile force) [79]. Also in this case the ratio vessel area/myocardiocyte area is sharply decreased giving rise to a hypoxic condition similar to that seen in obesity. In different types of hypertrophic cardiomyopathy an insufficient vasculature and an increase of leukocytes have been described [80]. In conclusion, in various conditions of abnormal cell hypertrophy, with relative decrease of vascular perfusion the pathway HIF1α-NFκB may be activated primarily by hypoxia, suggesting a role in the long-term damage of the tissue, leading to functional insufficiency and fibrosis. It is unknown if targeting HIF-NFκB pathway may have beneficial effects in slowing or blocking the progression of these conditions.

**Hypoxia and Inflammation in Storage and Neurodegenerative Disease: Role of False Hypertrophy**

False hypertrophy is a cell volume increase due to an abnormal accumulation of molecules and components that normally should be properly eliminated before an evident storage occurs. Adipocyte hypertrophy can be defined false hypertrophy, but a number of other diseases may show false hypertrophy with evident increase in cell volume, in parallel with accumulation of abnormal molecular components, depending on type of disease (Table 2) [71, 74, 81-90]. In storage diseases accumulation occurs in cytoplasm (glycogen) or in lysosomes (mucopolysaccarides, complex lipids and other molecules) and in several neurodegenerative diseases poliglutaminic proteins accumulate into the cytosol as monomeric forms or as polymeric fibrils (Huntington disease, Parkinson disease, etc.) [82-86, 91]. Abnormal proteins may be found as monomers, but more frequently as protease-resistant β-fibrils, both inside the cell (prion diseases) and/or in the extracellular space around the affected cells. In the Alzheimer disease β-fibrils originate from abnormal tau proteins and accumulate into the cytosol as monomeric forms or as polymeric fibrils, forming typical extracellular plaques [87]. In several other conditions, generally named β-fibrillosis, in which an excess of abnormal β-sheet rich protein is synthesized, β-fibrils may polymerize and accumulate in the intracellular and extracellular space.

All these conditions have in common the false cell hypertrophy which establish in the tissue a progressively insufficient perfusion, giving rise to a hypoxic
microenvironment and consequent activation of the HIF1α-NFκB pathway, cell/tissue damage, chronic inflammation, repair and fibrosis. All these features have been described in the various phases of long-duration and slow progression of these diseases. For instance, we have evidences that in Fabry’s disease accumulation of glycolipid bodies causes false hypertrophy in myocardiocytes leading to a hypertrophic heart and in perivascular fibroblast, in vascular smooth muscle cells and in endothelial cells producing an evident reduction or obliteration of the vascular lumen which further contributes to the hypoxia [83].

Hypoxia and Inflammation in Chronic Inflammatory Diseases (Lung Injury, Inflammatory Bowel Diseases, Chronic Arthritis and Autoimmune Diseases)

Primary chronic inflammation, such that occurring in COPD, in inflammatory bowel diseases, in chronic arthritis and autoimmune diseases all have in common a primary IRR with impaired resolution and tissue damage leading to fibrosis and hypoxia [92, 93]. In these conditions initial inflammation and ROI and RNI production are probably the early events of the disease. Afterwards, the progressive edema, damage and fibrosis are the determinants for the activation of HIF/NFκB pathway and the maintenance of the chronic inflammation leading to the final insufficiency [92, 93]. ROI and RNI are produced mainly by endogenous metabolism (80% of the oxygen is used by mitochondria) and by constitutive or inducible enzymes such as NOS. Hypoxia and stimulation by GFs or cytokines contribute to activate free radical production, together with external factors such as radiations, UV, chemotherapy and activators of metabolism such as diet and physical activity (Fig. 3). Free radicals are highly reactive with all biological molecules and represent damaging agents for our cells. For this reason a number of highly redundant mechanisms and molecules have been selected by the evolution to control their intracellular harmful concentration [26]. Major ROS detoxifying mechanisms are represented in Fig. 3 and include cytosolic and mitochondrial SOD, Glutathione, peroxidases, redoxins and exogenous and dietary antioxidants. Intracellular bilirubin a byproduct of the hemeoxygenase 1 is an efficient scavenger in that 1 mole of bilirubin can inactivate 2000 moles of radicals [94].

At physiological concentrations intracellular radicals are major modulators of several master transcription factors (Fig. 4) such as NFκB, AP-1, STAT3, Nrf2, HIF1α and others all involved in the hypoxia-related proinflammatory pathway involving HIF/NFκB [95-98].

In summary such chronic inflammatory diseases are triggered and maintained by chronic inflammation with the aggravation of an hypoxic condition activating the HIF/NFκB pathway.

Hypoxia and Inflammation in Ageing and Age-related Diseases

Aged tissues may become chronically relatively hypoxic due to ageing of vasculature, especially microvessels, and to the increase of fibrotic and abnormal components of stromal over parenchymal part.

Ageing is considered a progressive degradation of body functions over time, caused by a discrepancy between tissue/cell damage and the homeostatic ability to repair cells and restore the lost function. This results in the accumulation of molecular damage especially on DNA and proteins with the involvement of a number of important functions. For instance in ageing there is an increase in intracellular oxidative stress due from one side to the progressive decrease of the intracellular ROS scavenging, from the other side to an abnormal proinflammatory age-related chronic

### Table 2. Examples of false hypertrophy in which tissues undergo hypoxia for vasculature insufficiency.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Hypertrophy Pathogenesis</th>
<th>Vasculature Alteration</th>
<th>Hypoxic/inflammatory Features</th>
<th>References</th>
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<tbody>
<tr>
<td>Obesity</td>
<td>Lipid droplet accumulation in adipocytes</td>
<td>Insufficient vascular bed in white adipose tissue</td>
<td>HIF1α/NFκB activation; mediator and effector synthesis; increased resident leukocytes; fibrosis.</td>
<td>[37, 41, 49]</td>
</tr>
<tr>
<td>Lysosomal diseases</td>
<td>Glycogen, complex lipid or mucopolysaccharide accumulation into lysosomes</td>
<td>Insufficient vascular bed when vessels are affected. Insufficient vasculature in advanced diseases with unaffected vessels</td>
<td>Increased tissue leukocytes; necrosis; fibrosis</td>
<td>[48, 52]</td>
</tr>
<tr>
<td>Cytosolic storage diseases</td>
<td>Glycogen, cristal (cystine, cholesterol, uric acid), fibril accumulation</td>
<td>Insufficient vascular bed in advanced diseases.</td>
<td>Increased tissue resident leukocytes; fibrosis</td>
<td>[50]</td>
</tr>
<tr>
<td>Neurodegenerative diseases</td>
<td>Neuronal hypertrophy for abnormal polyglutaminic or fibrillar protein accumulation into the cytosol</td>
<td>Insufficient vascular bed only in advanced disease.</td>
<td>Increased tissue leukocytes, fibrosis</td>
<td>[51, 52, 54]</td>
</tr>
<tr>
<td>Amyloidosis or β-fibrillosis</td>
<td>Intracellular and/or extracellular deposits of β-fibrils as polymers of abnormal peptides</td>
<td>Insufficient and compressed vessels by amyloidotic mass</td>
<td>Toll-like receptor activation; fibrosis</td>
<td>[54]</td>
</tr>
</tbody>
</table>

References are indicated and discussed in the text.
systemic state [99, 100]. The last one is produced by several synergic factors including a degraded control of IRR, the insurgence of new antigens (as abnormal/mutated proteins) causing various autoimmune diseases, an increase on AGES which activate the alarmin receptor RAGE, and a systemic sublethal damage releasing HMGB1 and other endogenous alarmins. Due to the chronic and progressive accumulation of tissue damage, inefficient repairing mechanisms (the stem cell compartment is decreased in function and number of cells) privilege the formation of fibrotic healing with an accumulation of fibrous tissue in aged parenchymas. This general tissue degradation together with vasculature ageing may lead to a chronic hypoxic/proinflammatory condition similar to that seen before [101].

TARGETING HIF AND INFLAMMATION PATHWAYS AND MOLECULES

In this review the pathway HIF-NFkB has been indicated as the highway of signaling bridging hypoxia with inflammation leading to the associated damage, consequently, it appears relatively simple, in principle, to target the molecular component to modify the outcome of all the above mentioned diseases (Fig. 4). However, the problem is much more complex and challenging for many reasons: first, there are many secondary alternative pathways that can be activated if the main one is inhibited; second, we still do not know what is the toxicity and the unwanted side-effects of this therapeutic strategy; finally, some of these agents (especially inhibitors of HIF and NFkB) are natural products or off-label drugs which need to be endorsed on specific clinical trials for these diseases.

Targeting HIF

HIF1α has become the target of an increasing number of inhibitors developed with the aim to block or reduce tumor growth and progression [102, 103] and that could also be useful in other hypoxia-related pathologies described above. The different mechanisms that can be exploited to achieve a significant HIF inhibition have been extensively detailed by us [36]. Here we will review the major molecules inhibiting HIF in vitro and/or in vivo.

HIF activity can be inhibited by preventing protein accumulation as in the case of the PI3 kinase inhibitor wortmannin or the mTOR inhibitor rapamycin [104, 105]. Similarly, HIF protein expression is inhibited by digoxin a cardiac glycoside [102] and by 2 methoxyestradiol (2ME2), a microtubule targeting agent that has been shown to inhibit HIF cytosol to nuclear shuttling [106]. HIF can also be inhibited by altering its post-translational modifications such as acetylation/deacetylation by using modulators of class II and class III, histone deacetylases (HDAC), the latter also known as sirtuins [107, 108]. Molecules falling in this category are trichostatin and resveratrol that modulate the activity of class II and class III HDAC, respectively. However, in this case, while class II HDAC inhibition determines an increased degradation of HIF by increasing its ubiquitination, the mechanism of action of sirtuins activators is more complex and less known. In fact, deacetylation of HIF by sirtuins and in particular SIRT1 and SIRT6 has been shown to deacetylate HIF thereby decreasing its transcriptional activity [107]. In addition sirtuins modulation is complicated by the fact that they also have an antioxidative function with reduction of reactive oxygen species (ROS) production. Since HIF is stabilized by ROS accumulation, sirtuins inhibition of HIF is also achieved by preventing ROS accumulation in the cell [109, 110]. A large number of natural products have been shown to inhibit HIF by different mechanisms such as proteasomal degradation as in the case of curcumin [111], moracin O and P [112]; increased HIF degradation such as in the case of resveratrol [113, 114] and Sibiriquinone A [115]. HIF1α can be inhibited by preventing its dimerization to HIF1β as in the case of the antibacterial agent acriflavine [116], by inhibiting

Fig. (3). Generation of ROS by endogenous or exogenous stimuli (red) and their major detoxifying mechanisms (green). [Modified from 26].
One Special Question to Start with: Can HIF/NFkB Endocrine, Metabolic & Immune Disorders - Drug Targets, 2015, Vol. 15, No. 3

its DNA binding as in the presence of doxorubicin or daunorubicin [117], or, finally, by preventing HIF1α binding to its coactivator p300 as in the case of chetomin or bortezomib [118, 119].

Targeting Alarmin Receptors

Alarmin receptors are localized on the cell surface (plasmamembrane) or on intracellular membranes (endosomes, phagosomes) and recognize repeated structures present in exogenous (microbes) or endogenous (host) molecules [13, 120]. Classic members of the alarmin receptors are: receptor for advanced glycation endproducts (RAGE) and toll like receptors (TLRs). The main role of alarmin receptor is to recognize a molecular pattern associated either to the presence of a pathogen or to necrotic cells. The binding of alarmin receptors to their ligand results in the activation of an inflammatory response through the formation of inflammasome and engagement of NFkB [36, 61]. The observation that alarmin receptors are capable to initiate and propagate inflammation has made them attractive target for the treatment of many inflammatory diseases.

In the case of RAGE, strategies are focused on inhibiting its interaction with advanced glycation endproducts (AGEs), on blocking RAGE or on inhibiting its signaling [121]. In the first strategy, inhibition of AGE accumulation by molecules such as aminoguanidine (Pimagedine®), Pyridoxamine, a vitamin B complex, lysozyme and other AGE blocking agents result in amelioration of vascular functions and reduction of other complications observed in diabetic patients, in prevention of neuropathy and retinopathy in diabetic animal models and in reducing the severity of diabetic retinopathy [122].

Blockage of RAGE has been successfully obtained by using the natural presence of a soluble form of RAGE (sRAGE). In fact, sRAGE synthesis is decreased under pathologic conditions such as coronary artery disease,
hypertension, metabolic syndrome, arthritis and Alzheimer’s disease. Therefore, exogenous administration of sRAGE or of drugs such as angiotensin converting enzyme treatment or pioglitazone has shown beneficial effects in diabetic patients [121, 123].

RAGE signaling blockade is a promising therapeutic target that has been observed in drugs such as metformin, an anti-hyperglycemic agent, that reduces protein glycation and also prevents NFkB by activation of RAGE [122]. Other inhibitors of RAGE-axis are the dipeptidyl peptidase 4 (DPP 4) inhibitors Sitagliptin, Alogliptin, and Vildagliptin that preserves the pancreatic β-cell function [124]. Finally, additional agents in clinical use with reported anti-RAGE effects are angiotensin 1 receptor blocker (Replimune), a dihydropyridine based calcium antagonist (Nifedipine) and prostacyclin analog (Beraprost sodium) [121].

In the case of TLRs most of the studies demonstrating their role in human pathologies have been done in vitro or in animal models. In fact few molecules are approved for human use, one of this being imiquimod that has been approved for the treatment of genital warts, actinic keratosis and nonmelanoma skin cancers [125]. Its mechanism of action proceeds through activation of TLR7 and 8 with modulation of the immune response. However, the possibility to prevent or reduce inflammation-associated pathologies is linked to a better understanding of TLRs function that requires more research. In fact, mice lacking TLRs develop inflammatory diseases, such as arthritis, multiple sclerosis and inflammatory bowel disease.

On the other hand, TLR activation occurs early in the cascade of events that give rise to inflammation. Therefore, there might be an advantage in blocking them as they might give rise to chronic inflammation. Importantly, TLRs also detect products released from inflamed tissue, an event that feedback, on TLRs, leading to further activation. Breaking such a, positive feedback loop may be a highly effective strategy, to limit inflammation. From these considerations it is clear that both agonists and inhibitors of TLRs have been developed to prevent diverse human pathologies as extensively reviewed by Hennessy, 2010 [126].

TLRs agonists have been studied to prevent cancer, viral and bacterial infections as well as for autoimmunity. In particular, single stranded RNA-based TLR7 and TLR8 agonists have been used for the treatment of cancer and infectious diseases; CpG-based oligonucleotides that mimic unmethylated bacterial DNA are also being evaluated as potential anticancer drugs [127, 128]. Immune modulatory oligo nucleotides (IMO)s such as IMO-2055 have antitumor activity in a mouse model when used as a monotherapy and this activity was amplified when it was used in combination with chemotherapeutic agents [128].

Noncoding DNA called MGN-1703 and MGN-1706 are double stem-loop immunomodulating adjuvants developed as anticancer TLR9 agonists. A purified, water soluble diphosphorylated and triacetylated lipid-A derived from E. coli known as OM-174 acts as a TLR4 agonist. It reduces tumor growth, increases IFNγ production and prolongs the survival of mice [129, 130]. The TLR2 agonist SMP-105 that consists of cell-wall components, from Mycobacterium bovis (strain BCG/Tokyo), is approved for the treatment of bladder cancer. In mice, SMP-105 activates NFkB in a TLR2-dependent and TLR4-independent manner. Upon administration of the compound, TLR2 knockout mice showed impairment of TNF-α and IL-6 production as well as reduced tumor growth [131]. Resiquimod (R-848) is currently being evaluated for treating hepatitis C and other viral infections [132]. A small-molecule TLR7 agonist, ANA773, is under investigation for the potential treatment of hepatitis C infection and cancer [133]. Finally, vTX-1463 reduced allergic responses through the suppression of a Th2-mediated allergic response. When administered intranasally, it resulted in a decrease in the number of eosinophils and less congestion [126].

Extensive research has been developed to search for TLRs antagonist as novel therapeutics. Derivatives and small-molecule analogues of antimalaria drugs chloroquine and quinacrine act as a TLR9 antagonist suppress activation of an immune response [134]. The quinazoline derivative CPG-52364 is an anti-inflammatory TLR antagonist which specifically inhibits TLR7, TLR8 and TLR9 and inhibits disease progression of autoimmune diseases in animal models [135]. TLR4 antagonistic antibodies are being developed to block immune signaling in diseases associated with an excessive immune response. The most advanced is NI-0101, which interferes with the dimerization of TLR4 [136]. Similarly, 1A6 is an antibody that recognizes TLR4–MD-2 and it delayed the development of colitis and reduced the inflammatory response in a mouse model. However, when the antibody was administered during the recovery stage of the disease it impaired mucosal healing [136].

**Targeting Inflammasome**

Pharmacological manipulation of inflammasome activity is under extensive investigation due to the central role of inflammasome components such as NLRs to mediate the response to PAMPs and DAMPs [137]. Currently, the best treatments for inflammasome disorders aim to target the main product of inflammasome activity, IL-1β. Inhibition of IL-1 may be achieved at different levels. Some substances such as monoclonal antibodies target IL-1 molecule directly (canakinumab, rilonacept, gevokizumab) while others are antagonists of IL-1 receptor (anakinra) [138, 139]. Other strategies for treating IL-1 related disease rely on antagonists of P2X7 receptor to treat LES and on caspase-1 inhibitors [140]. Small-molecule inhibitors of NLRP3 such as Glyburide are used in the treatment of T2D, where it inhibits IL-1β production by acting upstream of NLRP3. Parthenolide is a sesquiterpene lactone that has multiple anti-inflammatory properties. Separate to its effects on NFkB activation, parthenolide has now also been shown to inhibit caspase-1 and NLRP3 [141].

Bay11-7082 is another NFkB inhibitor that was also found to specifically inhibit NLRP3 by preventing the ATPase activity of NLRP3 [141]. Currently, available inhibitors of inflammasome function have either not been clinically successful or have multiple targets. Therefore, the development of small molecule inhibitors that directly target the NLRP3 inflammasome could provide a more efficient
therapy for treating IL-1β and inflammasome-related diseases.

In conclusion, our understanding of the precise mechanism of the NLRP3 inflammasome is still scattered. However, given the importance of NLRP3 for inflammatory diseases, more information on its modulation will help our understanding of NLRP3 in disease and its potential therapeutic manipulation.

**CONCLUSIONS**

Hypoxia and inflammation are strictly and sequentially related at the molecular, cellular, and clinical levels. They have a crucial role in pathogenesis of a number of severe and widespread chronic diseases which in Western countries are increasing with the life expectancy. Pathways including oxygen sensing mechanisms, HIF-dependent genes, IRR activation and NFkB-dependent genes are potential therapeutic targets for the treatment of devastating diseases such as cancer, cardiovascular diseases, diabetes, obesity, neurodegenerative diseases and a number of other chronic invalidating inflammatory diseases.

However, such approaches should be clinically tested in all other patients in which hypoxia has been demonstrated to activate chronic inflammation, such as acute lung injury, myocardial ischemia and inflammatory intestinal disease. Finally, targeting hypoxia-dependent signaling pathways could also help attenuate organ failure due to ischemia in patients undergoing major surgery or alleviate hypoxia-driven graft inflammation after solid-organ transplantation.

**CONFLICT OF INTEREST**

The author(s) confirm that this article content has no conflict of interest.

**ACKNOWLEDGEMENTS**

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**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AGEs</td>
<td>advanced glycation end-products</td>
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<tr>
<td>AP1</td>
<td>Activating protein 1</td>
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<tr>
<td>AT</td>
<td>Adipose tissue</td>
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<tr>
<td>COPD</td>
<td>Chronic Obstructive Pulmonary Disease</td>
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<td>DAMPS</td>
<td>Damage-Associated Molecular Patterns</td>
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<tr>
<td>HIF</td>
<td>Hypoxia inducible factor</td>
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<td>HMGB1</td>
<td>high mobility group box 1</td>
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<td>JAK</td>
<td>Janus kinase</td>
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<tr>
<td>LCMD</td>
<td>Laser capture microdissection</td>
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<td>NLR</td>
<td>NOD-like receptors</td>
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<tr>
<td>NFkB</td>
<td>Nuclear factor</td>
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<tr>
<td>PAMPs</td>
<td>Pathogen-Associated Molecular Patterns</td>
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<tr>
<td>P2X7</td>
<td>Purinergic receptor 2X7</td>
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<tr>
<td>RAGE</td>
<td>receptor for advanced glycation end products</td>
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<tr>
<td>ROI</td>
<td>Reactive Oxygen Intermediates</td>
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<tr>
<td>RNI</td>
<td>Reactive Nitrogen Intermediates</td>
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<tr>
<td>SOCS</td>
<td>suppressor of cytokine signaling</td>
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<tr>
<td>STAT</td>
<td>Signal transducers and activators of transcription</td>
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<tr>
<td>TERT</td>
<td>Telomerase reverse transcriptase</td>
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<td>TF</td>
<td>transcription factor</td>
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TD2 = type 2 diabetes
VEGF = vascular endothelial growth factor

REFERENCES


