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Hypoxia Activates NF-κB–Dependent Gene Expression Through the Canonical Signaling Pathway

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Abstract

Hypoxia and inflammation are coincidental events in a diverse range of disease states including tumor growth, ischemia, and chronic inflammation. Hypoxia contributes to the development of inflammation, at least in part through the activation and/or potentiation of NF-κB, a master regulator of genes involved in innate immunity, inflammation, and apoptosis. NF-κB can be activated through two distinct signaling pathways termed the canonical and noncanonical pathways, respectively. The canonical pathway is activated through the IKKα/β/γ complex, while the noncanonical pathway involves NIK–mediated activation of IKKα homodimers. In the current study, we have investigated the relative roles of these two pathways in hypoxia-dependent NF-κB activation. Lymphotoxin α1β2 (LTα1β2) activated both the canonical and noncanonical NF-κB signaling pathways in HeLa cells. Sustained hypoxia enhanced basal and LTα1β2-induced NF-κB activity in a manner that was dependent upon the canonical but not the noncanonical signaling pathway. Intermittent hypoxia activated NF-κB in a manner that was also primarily dependent upon the canonical pathway. Knockdown of the p65 subunit of the canonical NF-κB pathway was sufficient to abolish the effects of hypoxia on LTα1β2–induced NF-κB activity. Furthermore, in synovial biopsies obtained at arthroscopy from patients with active inflammatory arthritis, the canonical pathway was preferentially activated in those patents with lower joint pO_2 values. In summary, we hypothesize that hypoxia enhances NF-κB activity primarily through affecting the canonical pathway. *Antioxid. Redox Signal.* 11, 2057–2064.

Introduction

HYPOXIA OCCURS when the cellular demand for molecular oxygen necessary to maintain normal physiologic function exceeds the vascular supply and is associated with a diverse range of pathological states, including atherosclerosis, chronic inflammatory disease, and cancer (24). Under such conditions, cells activate a transcriptional program which leads to increased expression of >200 genes, the primary function of which is to promote adaptation to low oxygen levels. Genes induced under conditions of hypoxia include angiogenic factors such as vascular endothelial growth factor (VEGF), glycolytic enzymes such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and hematopoietic factors such as erythropoietin (EPO), all of which promote an adaptive response (21). Because of the high therapeutic potential of manipulating this pathway either to promote tissue survival or to prevent tumor growth, the molecular mechanisms underpinning the transcriptional response to hypoxia have become an area of intense investigation (22, 23).

As well as the adaptive response to hypoxia outlined above, a number of studies have shown that a range of inflammatory genes are also induced by hypoxia. Such genes include pro-inflammatory enzymes such as cycloxygenase-2 (COX-2) (9), cytokines such as tumor necrosis factor alpha (TNFα) (25), and chemokines such as interleukin-8 (IL-8) (10). Thus, as well as facilitating adaptation, hypoxia can also impact upon inflammatory processes.

More than 20 transcription factors have been described to have some degree of oxygen-dependent sensitivity (3) and principal amongst these is the hypoxia-inducible factor (HIF). HIF is a heterodimeric transcription factor consisting of an oxygen-dependent alpha subunit (HIFα) and a constitutively expressed beta subunit (HIFβ). The oxygen dependence of the alpha subunit is conferred by a family of hydroxylase enzymes consisting of three prolyl hydroxylases (PHD 1–3) and

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one asparagine hydroxylase (FIH). The identification of these enzymes and how they confer oxygen dependence upon the HIF pathway were seminal discoveries in our understanding of oxygen sensing in metazoans, and has recently been extensively and expertly reviewed (8). The genes under the control of HIF under conditions of hypoxia are broadly adaptive in nature and include VEGF, EPO, and GAPDH.

Another transcription factor which has been shown to be dependent upon local oxygen concentrations is nuclear factor kappaB (NF-κB), a critical regulator of innate immunity, inflammatory, and apoptotic processes (26). NF-κB signaling is complex and may be activated by a number of inflammatory and noninflammatory signals, including cytokines, bacterial products, and cell surface adhesion molecules that typically activate NF-κB signaling through ligation of membrane-bound receptors and initiation of receptor-specific signaling cascades (11, 13). Notably, a number of recent studies have shown that NF-κB can be activated by environmental cues, including ultraviolet light and hypoxia (3, 17). NF-κB is a dimeric complex consisting of a limited number of combinations of p50, p65, p52 RelA and RelB subunits. The NF-κB pathway may be activated via at least two distinct pathways termed the canonical and the noncanonical pathway, respectively. The canonical pathway depends upon the activation of the IκB kinase (IKK) complex consisting of IKKα, IKKβ, and IKKγ (NEMO) which usually results in nuclear localization of p65/p50 dimers. The noncanonical pathway is dependent upon activation of IKKα homodimers leading to nuclear localization of RelB/p52 or p52/p52 dimers. The canonical pathway is preferentially activated by pro-inflammatory cytokines such as TNFα and IL-1β, while the noncanonical pathway is preferentially activated by BAFF (B cell activating factor). Ligation of the lymphotoxin receptor has been reported to activate both canonical and noncanonical signaling (Fig. 1) (4, 12, 14, 16, 27).

While the oxygen dependence of the HIF pathway has been extensively investigated, the sensing mechanisms linking a drop in environmental pO_2 to NF-κB have only recently begun to become understood. Recent studies have indicated that the same hydroxylases which confer oxygen sensitivity to the HIF pathway may also play a role in oxygen sensing in the NF-κB pathway. Specifically, PHD1 and PHD2 appear to act to repress the canonical NF-κB pathway through mechanisms which may include a direct hydroxylation of IKKβ (2), although this remains to be directly demonstrated. Furthermore, FIH has been shown to hydroxylate p105 (a precursor of p50) and IκBα (1). Thus, hydroxylase inhibition, which occurs in hypoxia, may impact directly upon NF-κB signaling. Recent studies have revealed how critical canonical NF-κB signaling is to inflammatory gene expression and to the regulation of HIF (2, 18). However, to date, little is known about how hypoxia impacts upon noncanonical signaling in the NF-κB pathway. In the current study, we have investigated whether hypoxia impacts upon noncanonical NF-κB signaling in cultured cells and in arthroscopic synovial biopsies from patients with active inflammatory arthritis.

Methods

Cell culture and hypoxia

HeLa cells were maintained in Eagle's minimum essential medium (Sigma, St. Louis, MO) containing 2 m*M* glutamine, 100 U/ml penicillin, nonessential amino acids (Sigma), and 10% fetal calf serum (Gibco, Carlsbad, CA). For normoxia, cells were maintained in a tissue culture incubator at atmospheric oxygen conditions (21% O_2) with 5% CO_2 at 37°C in a humidified environment. For hypoxia, cells were placed in a humidified hypoxia chamber (Coy Laboratories, MI) at 1% O_2 (5% CO_2 balance N_2) and the temperature was maintained at 37°C.

Intermittent hypoxia

HeLa cells were exposed to nine cycles of intermittent hypoxia consisting of 5 min hypoxia (1% O_2, 5% CO_2; balance N_2 and water vapor) in a hypoxia chamber (Coy Laboratories), and 10 min of reoxygenation at atmospheric oxygen concentrations (21% O_2, 5% CO_2; 37°C). Instantaneous hypoxia was achieved by incubation with pre-equilibrated

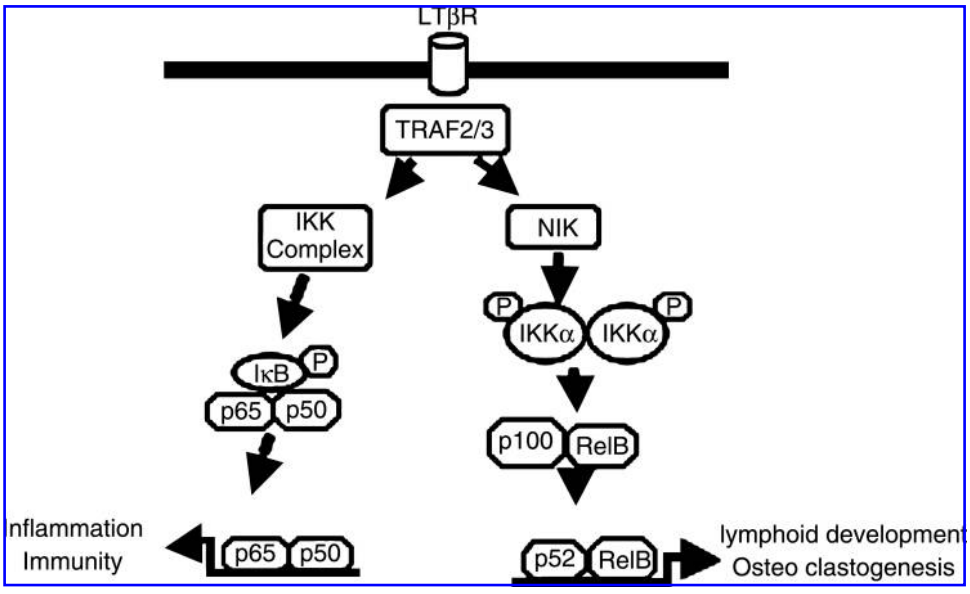

FIG. 1. NF-κB signaling in response to lymphotoxin receptor activation. Lymphotoxin receptor activation leads to activation of canonical and noncanonical NF-κB signaling. The canonical pathway (which may be modulated by hypoxia) is mediated via activation of the IKK complex leading to IκB degradation and liberation of the p50/p65 dimer. The noncanonical pathway involves activation of NIK which leads to the activation of IKKα homodimers and subsequent processing of p100 to allow liberation of the p52/RelB dimer.

hypoxic medium. Control cells were exposed to an equivalent time period of normoxia or sustained hypoxia.

Immunoblotting

Whole cell lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) and transferred onto nitrocellulose membranes and Western blotted, as described previously (2). Proteins were labeled following incubation for 1 h at room temperature with primary IκBα antibody (Cell Signaling, Danvers, MA) or NF-κB p65 antibody (Santa Cruz). The blots were then incubated with horseradish peroxidase-conjugated secondary antibody and then developed with an enhanced chemiluminescence (ECL) procedure, according to the manufacturer's instructions (Santa Cruz, Santa Cruz, CA).

NF-κB DNA binding assays

Nuclear protein–DNA binding interactions were determined by a 96-well plate based assay (TransAM®; Active Motif, Carlsbad, CA). Briefly, nuclear lysates were prepared as per the manufacturer's instructions and extracts were incubated with an immobilized oligonucleotide containing an NFκB consensus binding site on a 96-well plate. The activated NF-κB bound to the immobilized oligonucleotide was detected using an antibody against NF-κB family members; p65, p52, or RelB. An HRP-conjugated secondary antibody was used to provide a colorimetric readout quantifiable by spectrophotometry.

Luciferase gene reporter assay

HeLa cells were transfected with an NF-κB promoter reporter construct (Stratagene Cis-Reporting Systems, La Jolla, CA), exposed to the relevant experimental conditions and harvested at room temperature. The NF-κB luciferase reporter construct utilized contains the consensus kB binding site GGGGACTTTCC that binds p65/p50 heterodimers. Luciferase lysis buffer (Promega, Madison, WI) was used to prepare cell lysates. Luciferase substrate (Promega) was added the lysates and luciferase activity was measured using a desktop luminometer (Berthold Technologies, Bad Wildbad, Germany). This assay was performed in duplicate, and luciferase values were normalized to cotransfected β-galactosidase control vector.

RNA Interference by siRNA

HeLa cells were grown to 50% confluence and transfected with specific siRNA targeted against the p65 subunit of NF-κB or equivalent amounts of control (nontarget) siRNA (Dharmacon, Lafayette, CO) using Lipofectamine 2000 transfection reagent, according to the manufacturer's instructions. Cells were maintained in antibiotic-free media for 24 h post-transfection to achieve maximal knockdown of the target gene. Cells were then transfected with an NF-κB-promoter reporter construct as described above, and a luciferase reporter gene assay was performed.

Arthroscopy and sample collection

Prior to commencing biologic therapy, informed consent was obtained from 13 patients with active inflammatory arthritis. The study was approved by the St Vincent's University Hospital medical research and ethics committee. Synovial tissue biopsies were obtained from knee joints with active inflammation under direct visualization at arthroscopy. Briefly, under local anaesthetic, a 2.7 mm needle telescope is inserted into the target joint. Following the first biopsy, a

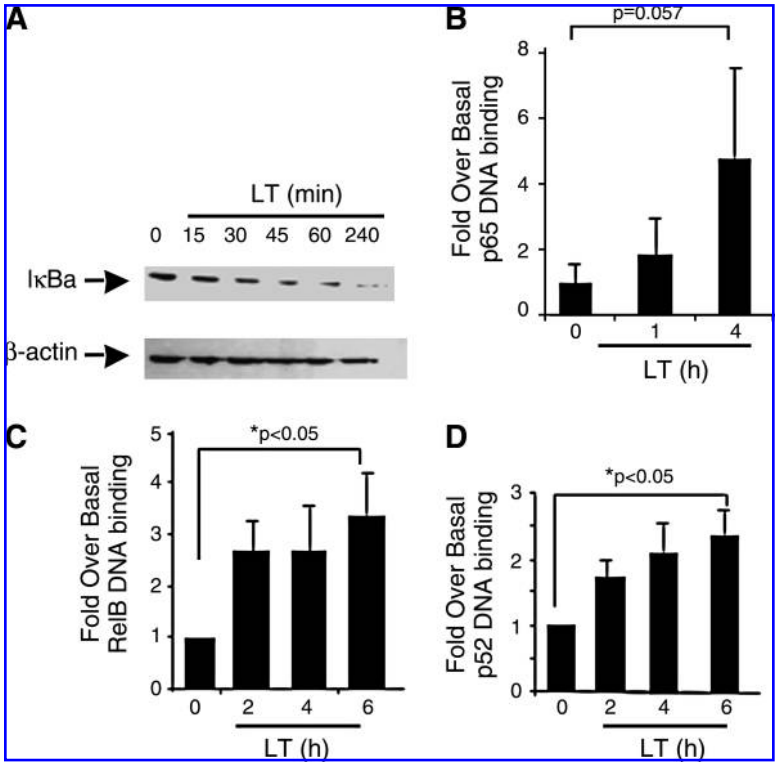

FIG. 2. Lymphotoxin activates both canonical and noncanonical NF-κB signaling in HeLa cells. (**A**) HeLa cells were exposed to LTα1β2 (LT; 100 ng/ml 0–4 h) and total cellular levels of IκBα were determined by Western blot analysis. (**B**) HeLa cells were exposed to LTα1β2 (LT; 100 ng/ml; 0–24 h) and nuclear p65 levels were determined by p65-DNA binding assay. (**C**) HeLa cells were exposed to LTα1β2 (LT; 100 ng/ml; 0–6 h) and nuclear RelB levels were determined by RelB-DNA binding assay ($p = 0.032$). (**D**) HeLa cells were exposed to LTα1β2 (LT; 100 ng/ml; 0–6 h) and nuclear p52 levels were determined by p52-DNA binding assay ($p = 0.044$).

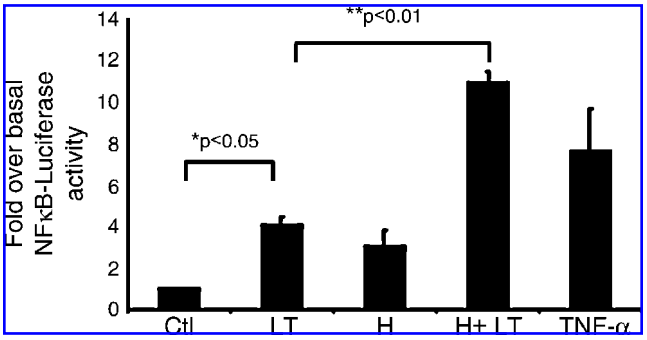

FIG. 3. Sustained hypoxia enhances lymphotoxin-induced NF-κB activity. HeLa cells transfected with an NF-κB-dependent luciferase reporter construct were exposed to LTα1β2 (LT; 100 ng/ml; 24 h) in either normoxia and hypoxia for 24 h prior to measurement of luciferase activity by luminometry. LT activated basal NF-κB activity (4.06 ± 0.4-fold over control, $p = 0.017$) and this was significantly enhanced in hypoxia ($p = 0.03$).

Lycox pO_2 probe was introduced via a 22G hypodermic needle and positioned into the biopsy pocket, allowing direct pO_2 (mm HG) measurements in the synovial membrane lining. Synovial biopsies were immediately snap frozen in liquid nitrogen and stored at −80°C. Three biopsies from each patient were pooled to account for heterogeneity in the joint.

Statistical analysis

Data from *in vitro* experiments were compared by one-way analysis of variance (ANOVA) and paired or unpaired Student's *t*-test as using the commercial software package (SPSS version 12). Values are expressed as mean ± standard error of the mean for a minimum of $n = 3$ independent experiments where *p and **p represent p values of <0.05 and <0.01, respectively. Clinical samples were compared using the Kendall Tau B test.

Results

Lymphotoxin activates canonical and noncanonical NF-κB signaling in HeLa cells

In order to investigate the pathway(s) employed when lymphotoxin activates NF-κB signaling in HeLa cells, we exposed cells to lymphotoxin-α1β2 (LTα1β2; 100 ng/ml) for increasing time periods (0–4 h). Exposure to LTα1β2 induced a time-dependent decrease in total cellular levels of IκBα, reflecting activation of canonical NF-κB signaling (Fig. 2A). A corresponding and temporally consistent time-dependent increase in nuclear p65 was detected in cells exposed to LTα1β2 from which nuclear extracts were generated for a DNA binding assay ($p = 0.057$; Fig. 2B). These data further support activation of the canonical NF-κB pathway by engagement of the lymphotoxin receptor. In separate experiments, a time-dependent increase in nuclear RelB and p52 levels was determined by DNA binding assays, indicating that LTα1β2 also activates the noncanonical pathway (Fig. 2C and D; *$p < 0.05$). In summary, these data demonstrate that both the canonical and noncanonical NF-κB signaling pathways are activated by LTα1β2 in HeLa cells.

Sustained hypoxia enhances lymphotoxin-induced NFκB activity

To determine the impact of hypoxia on lymphotoxin-induced NFκB activity, we performed a luciferase reporter assay. HeLa cells were transfected with a luciferase reporter construct under the control of a promoter containing a concatomer of NF-κB response elements and maintained in normoxia (21% O_2) or hypoxia (1% O_2) and exposed to LTα1β2 (100 ng/ml) or vehicle control. TNFα (10 ng/ml) was used as a

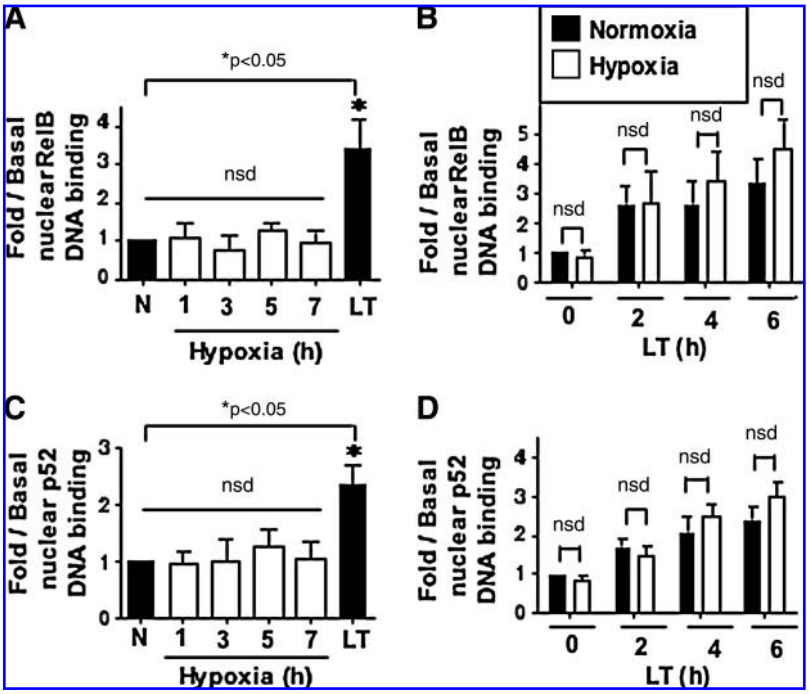

FIG. 4. Hypoxia does not affect basal noncanonical NF-κB signaling nor does it enhance lymphotoxin-stimulated noncanonical NF-κB signaling. (**A**) HeLa cells were exposed to a time course of hypoxia (0–7 h) or LTα1β2 (100 ng/ml), and nuclear levels of RelB were determined by DNA binding assay ($p = 0.032$). (**B**) HeLa cells were exposed to a time course of LTα1β2 treatment (0–6 h) in either normoxia (*full bars*) or hypoxia (*empty bars*), and nuclear levels of RelB were determined by DNA binding assay. (**C**) HeLa cells were exposed to a time course of hypoxia (0–7 h) or LTα1β2 (100 ng/ml), and nuclear levels of p52 were determined by DNA binding assay ($p = 0.044$). (**D**) HeLa cells were exposed to a time course of LTα1β2 treatment (0–6 h) in either normoxia (*full bars*) or hypoxia (*empty bars*), and nuclear levels of p52 were determined by DNA binding assay.

positive control. Promoter activity was assessed by luminometry. LTα1β2 activated NF-κB activity (Fig. 3; $p < 0.05$). Hypoxia significantly enhanced LTα1β2-stimulated NFκB activity (Fig. 3; $p < 0.05$). These data indicate that hypoxia enhances LTα1β2-induced NF-κB activity.

Sustained hypoxia does not enhance basal or stimulated activity of the noncanonical NF-κB signaling pathway

To determine the possible role of the noncanonical pathway in hypoxia-enhanced responsiveness of NF-κB to LTα1β2, nuclear RelB and p52 levels were analyzed following exposure to hypoxia alone and hypoxia in combination with LTα1β2. Hypoxia alone (0–7 h) did not affect nuclear levels of RelB (Fig. 4A). Furthermore, while LTα1β2 increased nuclear RelB activity, this was not affected by concomitant exposure of cells to hypoxia (Fig. 4B). In a manner similar to RelB, neither basal nor lymphotoxin-stimulated p52 levels were affected by hypoxia (Fig. 4C and D). Thus, in the context of the experimental conditions used, hypoxia appears to have a selective effect upon canonical NF-κB signaling over the noncanonical pathway.

Intermittent hypoxia enhances NF-κB signaling via the canonical pathway

We next investigated whether intermittent hypoxia, which has also been shown to activate canonical signaling (19) affected the noncanonical NF-κB signaling pathway. Consistent with previous studies, exposure of HeLa cells expressing an NF-κB luciferase reporter construct to intermittent hypoxia resulted in an increase in basal NF-κB activity ($p < 0.01$; Fig. 5A). Furthermore, intermittent hypoxia caused an increase in nuclear levels of p65 (Fig. 5B). We next investigated the impact of intermittent hypoxia on nuclear levels of p65, p52, and RelB by DNA binding assay. Intermittent hypoxia and sustained hypoxia caused a significant increase in nuclear p65 levels ($p < 0.05$) without affecting the nuclear localization of either p52 or Rel A (Fig. 5C). In summary, these data suggest that, similar to sustained hypoxia, intermittent hypoxia activates NF-κB signaling through the canonical signaling pathway.

p65 siRNA reverses the effects of hypoxia and lymphotoxin on NF-κB activity

The data presented thus far indicate that the contribution of hypoxia to NF-κB signaling is predominantly mediated via the canonical pathway and involves nuclear localization of the p65 subunit. To confirm that this pathway is the primary target for hypoxia-induced NF-κB activity, we utilized siRNA targeted to the p65 subunit of NF-κB. This strategy silences the majority of canonical NF-κB signaling. First, we demonstrated that p65 siRNA effectively decreased total cellular p65 protein levels in HeLa cells (Fig. 6A). Second, in separate experiments, HeLa cells were transfected with p65 siRNA or nontarget siRNA 24 h prior to transfection with NF-κB luciferase promoter reporter construct. p65 siRNA abolished the hypoxia-enhanced NF-κB response to lymphotoxin treatment, indicating an absolute requirement for the canonical pathway during hypoxia-enhanced activation of lymphotoxin-induced NF-κB activity. It should be noted that the NF-κB reporter utilized contains the consensus κB binding site

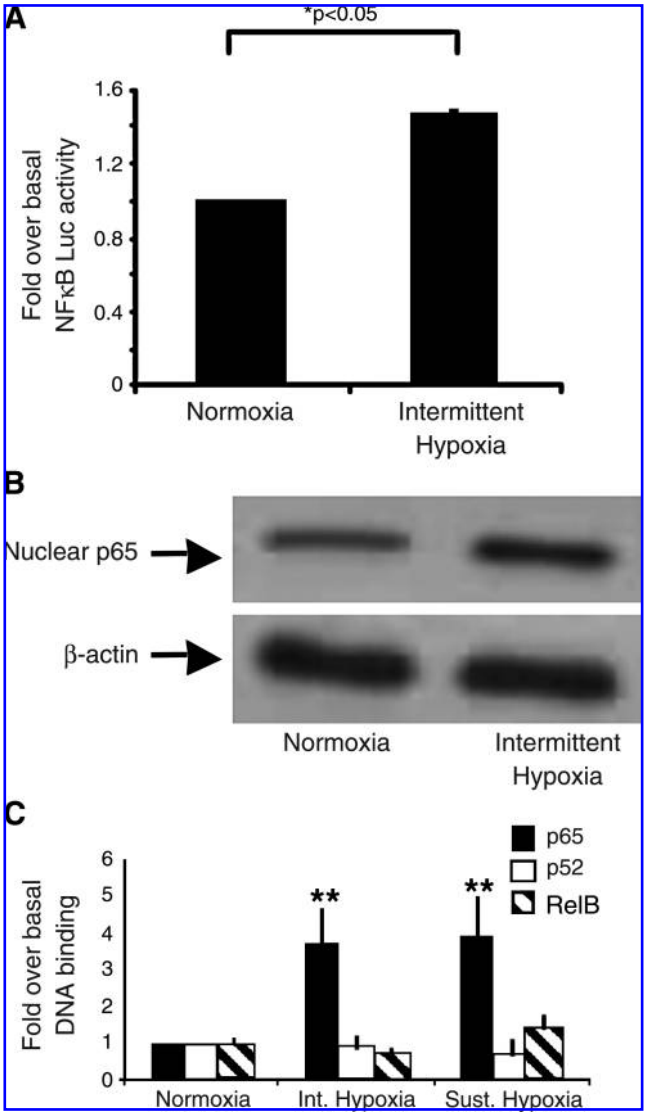

FIG. 5. Intermittent hypoxia activates canonical but not noncanonical signaling. (**A**) HeLa cells transfected with an NF-κB luciferase reporter construct were exposed to nine cycles of intermittent hypoxia and luciferase expression was measured 24 h later by luminometry ($p = 0.01$). (**B**) HeLa cells transfected with an NF-κB luciferase reporter construct were exposed to nine cycles of intermittent hypoxia and p65 nuclear localization was measured by Western blot analysis. (**C**) HeLa cells were exposed to nine cycles of intermittent hypoxia or equivalent time in sustained hypoxia and nuclear levels of p65 ($p = 0.008$), RelB ($p = 0.073$), and p52 ($p = 0.935$) were measured by DNA binding assay.

GGGGACTTTCC that was found to bind p65/50 heterodimers. The affinity of noncanonical heterodimers for this sequence may differ *in vivo*.

Canonical NF-κB signaling is preferentially activated in synovial biopsies with lower in vivo *pO_2 values*

pO_2 values were measured in patients undergoing investigative arthroscopy for active joint inflammation, and synovial biopsies were obtained. Nuclear levels of p65, p52 and RelB were measured by DNA binding assay (Fig. 7). Patients

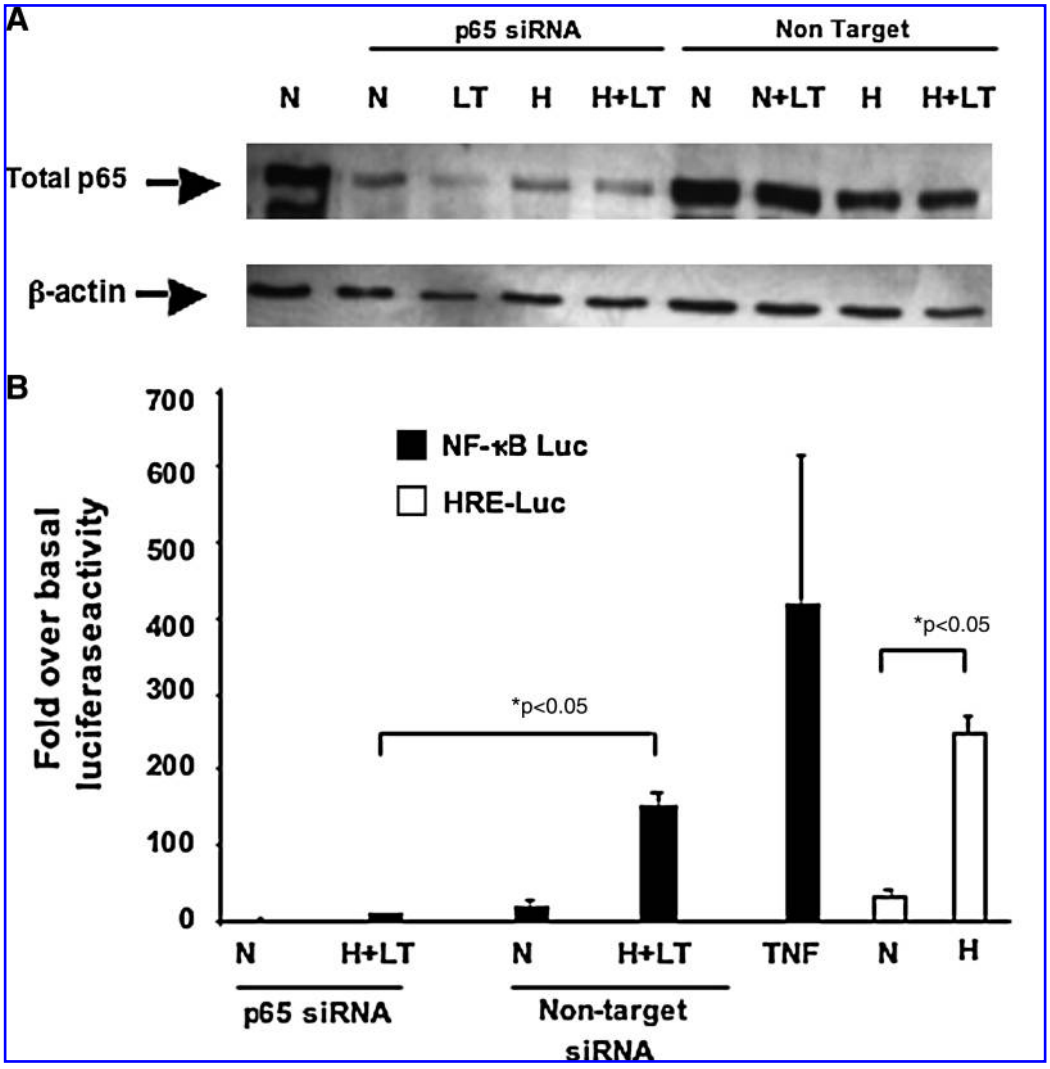

FIG. 6. p65 siRNA reverses the effects of both hypoxia and lymphotoxin on NF-κB-Luciferase activity. (**A**) Total cellular levels of p65 were determined in HeLa cells transfected with siRNA directed against p65 or nontarget control siRNA and exposed to normoxia (N), hypoxia (H), or lymphotoxin (LT). (**B**) HeLa cells expressing a NF-κB -dependent luciferase reporter construct (NF-κB-Luc) or a HIF-response element dependent luciferase reporter construct (HRE-Luc) were transfected with siRNA targeted to p65 or nontarget siRNA and luciferase activity was determined by luminometry following exposure to normoxia or hypoxia and LTα1β2 (100 ng/ml).

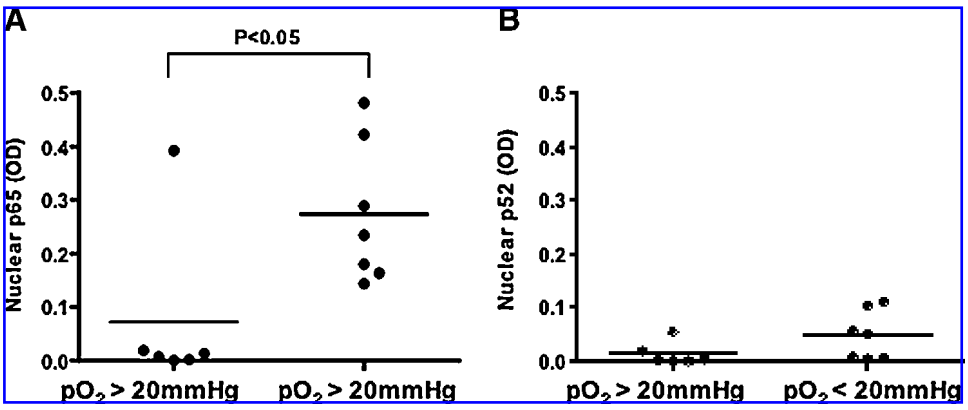

FIG. 7. Preferential activation of canonical NF-κB signaling *in vivo*. (**A**) Nuclear p65 levels were measured in arthroscopic biopsies taken from patients with joint pO_2 values of greater or less than 20 mmHg ($p = 0.044$). (**B**) Nuclear p52 levels were measured in arthroscopic biopsies taken from patients with joint pO_2 values of greater or less than 20 mmHg.

were separated into two groups according to whether the pO_2 value of the joint was below or above 20 mmHg. Of critical interest, we observed a significant association between canonical pathway activation ($p < 0.05$ as determined by nuclear p65 DNA binding assay) in patients with joint pO_2 of <20 mmHg compared with patients with a joint pO_2 >20 mmHg. In contrast to this, there was no significant association between noncanonical pathway activation and joint pO_2 (as determined by nuclear p52 DNA binding assay). Similarly, nuclear RelB levels did not correlate with tissue pO_2 (data not shown). These data support an association of lower tissue oxygen levels with activity of the canonical pathway and not of the noncanonical pathway.

Discussion

Hypoxia and inflammation are coincidental events in a diverse range of pathologic processes that include chronically inflamed tissues and growing tumors (26). A critical transcriptional regulator during both inflammation and tumor development is NF-κB. A number of studies have previously demonstrated that the NF-κB pathway may be activated and/or modulated by hypoxia (26). Thus, we have hypothesized that hypoxia-dependent NF-κB activity may play an important role in pathological processes where inflammation and hypoxia occur together (2).

The NF-κB signaling cascade may be activated through two distinct pathways termed the canonical and the noncanonical pathways, respectively (4, 27). Activation of the canonical pathway by cytokines such as TNFα and IL-1 involves activation of the signalsome complex consisting of IKKα, IKKβ, and IKKγ (NEMO) which targets IκB proteins for phosphorylation-dependent degradation and liberates p65/p50 dimers of NF-κB family members to regulate genes involved in host defense, innate immunity, and inhibition of apoptosis (7). The noncanonical pathway is characterized by the NIK-dependent activation of IKKα homodimers that process the p100 protein and results in the liberation and nuclear translocation of p52/RelB complexes which are involved in the regulation of genes that control lymphoid tissue development, B-cell function, and osteoclastogenesis (27). Lymphotoxin α1β2 is a member of the larger TNF superfamily that activates NF-κB signaling through ligation of the Lβ receptor (LβR) (6, 20). Previous reports have demonstrated that engagement of the LβR by an agonistic antibody or by the addition of LTα1β2 results in the activation of both the canonical and noncanonical NF-κB pathways (5,15, 16). Our current data support these findings and demonstrate that hypoxia increases both basal and lymphotoxin-stimulated activation of the NF-κB pathway. While previous work had demonstrated that hypoxia can impinge on the activity of the canonical NF-κB pathway (1, 2), the impact of hypoxia on noncanonical signaling has remained unknown. Consistent with our previously published work (2; which used TNFα as a ligand), we find that LTα1β2 signaling is enhanced in hypoxia. Here we demonstrate that it does so through activation of the canonical pathway and not via alteration of the noncanonical pathway.

In order to determine whether this association between hypoxia and enhanced NF-κB signaling was also observed *in vivo*, we measured activation of the canonical and noncanonical pathways in arthroscopic biopsies taken from patients with suspected joint inflammation. The canonical pathway (as measured by p65 DNA binding) was preferentially activated in patients with lower joint pO_2 values compared to the noncanonical pathway (as measured by p52 DNA binding). These data support a positive association between lower oxygen levels and activation of the canonical, but not the noncanonical NF-κB pathway.

In summary, hypoxia is a microenvironmental feature in a range of disease processes and can impact upon gene expression via a number of oxygen-sensitive transcription factors including (but not limited to) HIF and NF-κB. In this study, we provide evidence that hypoxia impacts upon basal and lymphotoxin-stimulated NF-κB activity through modulation of the canonical rather than the noncanonical signaling pathway. Furthermore, we report for the first time a significant association between pO_2 levels and the degree of activation of the canonical NF-κB pathway.

Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

ANOVA = analysis of variance
BAFF = B cell activating factor
COX-2 = cyclooxygenase-2
DNA = deoxyribonucleic acid
ECL = enhanced chemiluminescence
EPO = erythropoietin
FIH = factor inhibiting HIF
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
HIF = hypoxia inducible factor
IKK = IκB kinase
IL = interleukin
IL-8 = interleukin-8
LTα1β2 = lymphotoxin α1β2
NEMO = NFκB essential modulator
NF-κB = nuclear factor kappa B
NIK = NFκB inducing kinase
PHD = prolyl hydroxylase
pO_2 = partial pressure of oxygen
siRNA = short interfering RNA
TNF-α = tumor necrosis factor-α
VEGF = vascular endothelial growth factor

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