Differential roles for NF-κB in endotoxin and oxygen induction of interleukin-8 in the macrophage

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D’Angio, Carl T., Michael B. LoMonaco, Carl J. Johnston, Christina K. Reed, and Jacob N. Finkelstein. Differential roles for NF-κB in endotoxin and oxygen induction of interleukin-8 in the macrophage. Am J Physiol Lung Cell Mol Physiol 286:L30–L36, 2004. First published August 8, 2003; 10.1152/ajplung.00360.2002.—The alveolar macrophage is an important source of interleukin (IL)-8 during pulmonary injury. The IL-8 gene promoter sequence contains nuclear factor (NF)-κB, NF-IL6, and activator protein (AP)-1 binding sequences. These sites may have differing regulatory roles in hyperoxia-exposed macrophages than in those stimulated by bacterial lipopolysaccharide (LPS). U-937 and THP-1 macrophage-like cells were exposed to air-5% CO2 or 95% O2-5% CO2, with or without 1.0 μg/ml of LPS, and transfected with an IL-8 promoter-reporter containing NF-κB, NF-IL6, or AP-1 mutations. Hyperoxia and LPS caused additive increases in IL-8 production by U-937 cells, whereas THP-1 cells responded only to LPS. An NF-κB mutation ablated baseline and O2- and LPS-stimulated reporter activity in both cell lines, whereas NF-IL6 mutations had little effect. An AP-1 mutation had an intermediate effect. LPS, but not hyperoxia, stimulated nuclear translocation of NF-κB in both cell lines. Pharmacological blockade of NF-κB nuclear translocation ablated LPS-, but not hyperoxia-, stimulated IL-8 production. Although an intact promoter NF-κB site is crucial to macrophage IL-8 production, only LPS-stimulated production appears to require additional nuclear translocation of NF-κB. Hyperoxia; U-937 cells; THP-1 cells; gene regulation; monocyte

INTERLEUKIN (IL)-8 is a multifunctional cytokine that has significant attractant and activating actions on neutrophils. IL-8 is produced by a variety of pulmonary cell types in response to injury (2). The alveolar macrophage (AM) is a primary producer of IL-8 in the injured lung (10, 13). IL-8 has been implicated as a participant in several human inflammatory lung diseases, including the acute respiratory distress syndrome (ARDS), idiopathic pulmonary fibrosis (IPF), and bronchopulmonary dysplasia (13, 17, 18). The levels of IL-8 mRNA expression by AM reflect the intensity of the neutrophil infiltration in IPF (17). IL-8 accounts for 30–50% of the neutrophil chemoattractant activity produced by stimulated human AM in vitro (28) and 65–75% of the neutrophil chemotactic activity in a number of lung injuries (3, 4). We have previously described increased IL-8 mRNA expression by AM in rabbits exposed to hyperoxia and have correlated this response to the maximal neutrophil influx (9, 10).

Despite the in vivo role of AM in the response to hyperoxia, the in vitro response of the isolated macrophage to hyperoxia is muted. Both human AM and the human U-937 macrophage-like cell line produce IL-8 in vitro in response to exposure to 100% oxygen (11). However, isolated human AM and macrophage-like cells are much more responsive to stimulation by bacterial endotoxin (lipopolysaccharide, LPS) than to hyperoxia exposure. Indeed, preliminary work in our laboratory suggested that some human macrophage-like cell lines were unable to produce IL-8 in response to hyperoxia alone. This presented an opportunity, using macrophage cell lines with contrasting abilities to produce IL-8 in response to hyperoxia, to understand the molecular mechanisms that allow the in vivo AM to produce IL-8 in response to hyperoxia.

The promoter sequence of the IL-8 gene contains, within the 130 bases proximal to the transcription start site, binding sequences for the transcription factors nuclear factor (NF)-κB, NF-IL-6, and activator protein-1 (AP-1). These sequences have central regulatory roles in IL-8 production by numerous cell types in response to a variety of stimuli. Oxidant stress, whether a result of hyperoxia or other reactive oxygen species, has been implicated in the regulation of NF-κB (6, 14, 25, 27, 29). We hypothesized that the NF-κB, NF-IL-6, and AP-1 sites would have differing regulatory roles in macrophage-like cells exposed to oxygen than in those stimulated by LPS.

METHODS

Cell culture and hyperoxia/LPS exposure. U-937 histiocytic lymphoma cells [American Type Culture Collection (ATCC), Manassas, VA] and THP-1 monocytic leukemia cells (ATCC) were used as models for macrophages. Cells were maintained in RPMI 1640 media, without phenol red, with 10% serum (GIBCO, Grand Island, NY). For experimental exposures, 5–10 × 10⁶ log phase cells in 10 ml of serum-free RPMI 1640 in a 100-mm tissue culture plate were exposed to either air-5% CO2 or 95% O2-5% CO2 in the presence or absence of 1 μg/ml of LPS (serotype O26:B6; Sigma, St. Louis, MO). Phorbol 12-myristate 13-acetate (1 μg/ml; Sigma) was also used as a positive control condition in some experiments, as detailed below. The hyperoxia exposures were accomplished in an incubator using a sealed, humidified chamber into which the 95% O2-5% CO2 was introduced at ambient pressure. Air exposures were conducted simultaneously in the same humidified incubator. Previous pilot experiments in our laboratory had confirmed that IL-8 mRNA expression and reporter luciferase production in response to an LPS stimulus were most

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marked at 6 h of exposure and that a 1-μg/ml dose of LPS produced near-maximal levels of IL-8 stimulation (unpublished observations). Pilot experiments also showed that IL-8 protein was present in conditioned media by 5 h and increased through 24 h (unpublished observations). As a result, these exposure times were used for the experiments described below, with specific exceptions as noted.

**Enzyme-linked immunosorbent assay.** Conditioned media were collected at 24 h of exposure to experimental conditions, except for experiments paired with electromobility shift assays (EMSA) in MG-132-exposed cells (below), when a 5-h exposure was used. Media were flash-frozen in liquid nitrogen and stored at −70°C until use. IL-8 was measured in conditioned media using an enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

**Ribonuclease protection assay.** The hypoxia/LPS exposure experiment was repeated, and cell pellets were collected at 6 h of exposure. The cell pellets were homogenized in 1 ml of TRIZol (Invitrogen, Grand Island, NY), and total RNA was isolated. Each final RNA pellet was resuspended in 100 μl of diethylpyrocarbonate-treated water. The RNA concentration and purity was quantified using the GeneQuant RNA/DNA Calculator (Pharmacia Biotech, Piscataway, NJ). Quantitation of steady-state cytokine mRNA levels was performed using a multitarget ribonuclease protection assay (RPA). RPAs were performed with a riboprobe template, including a probe for human IL-8, and the constitutively expressed ribosomal protein L32 (hck-5; Pharmingen, San Diego, CA). The riboprobe synthesis reaction consisted of 150 ng of template, 120 μCi [α-32P]UTP (3,000 Ci/mmol; NEN, Wilmington, DE), 5 nmol ATP, 5 nmol GTP, 5 nmol CTP, 150 pmol UTP, 2.5 μg yeast tRNA, 100 mM dTTP, 1 μg bovine serum albumin, 20 nmol spermidine, 10 units of RNase inhibitor, and 50 units of T7 RNA polymerase (Life Technologies) in 1× transcription buffer (40 mM Tris·HCl, pH 7.5, and 6 mM MgCl2). The reaction was incubated for 90 min at 37°C and then diluted to 100 μl with DNase I buffer (50 mM Tris·HCl, pH 7.5, 10 mM MgCl2, and 0.02 U/μl RNase-free RNase A; Promega, Madison, WI). After a 30-min incubation at 37°C, the riboprobe was purified by phenol/chloroform extraction and ethanol precipitation. The dried pellet was then resuspended in 50 μl of hybridization buffer (400 mM NaCl, 40 mM PIPES, pH 6.7, 1 mM EDTA, pH 8.0, and 80% formamide; Sigma), analyzed by scintillation counting, and then diluted to a final concentration of 2.6 × 108 counts per minute (cpm)/μl in hybridization buffer. Five-microgram samples of each RNA were hybridized with 2.6 × 106 cpm in 10 μl of volume, heated to 80°C for 3 min, and then immediately placed at 56°C for 16 h. After solution hybridization, 100 μl of RNase cocktail (0.2 μg/ml RNase A (Sigma), 600 U/ml RNase T1 (Life Technologies), 10 mM Tris·HCl, pH 7.5, 300 mM NaCl, and 1 mM EDTA, pH 8) were added to each sample and incubated for 45 min at 30°C. Eighteen microliters of proteinase K cocktail (0.67 mg/ml proteinase K (Life Technologies), 3.5% sodium dodecyl sulfate, and 100 μg/ml yeast tRNA) were then added to each sample and incubated for 15 min at 37°C. The protected RNA duplexes were purified by phenol/chloroform extraction and ethanol precipitation, and the pellets were resuspended in 5 μl of RPA loading buffer (80% formamide, 0.5× Tris borate-EDTA buffer (TBE), and 0.05% bromphenol blue; Sigma). The protected, radiolabeled RNA fragments were electrophoresed on a 5% acrylamide-8 M urea sequencing gel. Protected fragments were visualized by phosphorimage analysis and/or autoradiography and quantified using ImageQuant (Molecular Dynamics, Sunnyvale, CA) software. IL-8 mRNA abundance was calculated by normalizing the intensity of the protected IL-8 band to the protected L32 band. The mean level of mRNA expression (all conditions combined) in each cell type was arbitrarily defined as one.

**Transcription factor transfection.** Cells were transfected with a promoter-reporter construct containing the 133 bases proximal to the transcription start site of the IL-8 promoter linked to a luciferase reporter (−133 construct). Cells were also transfected with −133 constructs containing point mutations in the NF-κB, AP-1, and NF-IL6 binding sites. These constructs were a kind gift of Dr. R. Phipps with the permission of Dr. N. Mukaida and have been described in detail previously (21). Cells were transiently transfected with 400 ng of promoter-reporter construct using 3.2 μl of Enhancer and 4 μl of Effectene (Qiagen, Valencia, CA) according to the manufacturer’s guidelines. Log phase U-937 (3× 105/well) and THP-1 (6× 105/well) were plated onto 12-well plates in 1 ml of serum-free medium immediately before transfection. To control for intra-assay and interassay variation, cotransfections using a Renilla luciferase reporter (Promega) were performed. Transfection was allowed to proceed for 18 h before experimentation. After transfection, the cells were exposed according to the same exposure protocol used for nontransfected cells. Luciferase output was measured in a luminometer (Tropix, Bedford, MA) after 6 h of exposure to experimental conditions using commercially available luciferase substrates (Promega) according to the manufacturer’s instructions.

**Nuclear extracts.** Log phase cells (5× 106) were seeded onto 100-mm plates and subjected to experimental conditions. Cells were collected after 3, 6, or 9 h of exposure by pipetting and chilling on ice, followed by centrifugation at 300 g. Nuclear extraction was performed using commercially available reagents (Pierce product no. 78833, Rockford, IL) according to the manufacturer’s guidelines. A protease inhibitor cocktail (containing final concentrations of 104 μM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 0.08 μM aprotonin, 2 μM leupeptin, 4 μM bestatin, 1.5 μM pepstatin A, and 1.4 μM EDTA, 1:64, product no. P8340, Sigma) and phosphatase inhibitor cocktail (a proprietary mixture containing sodium orthovanadate, sodium molybdate, sodium tartrate, and imidazole; product no. P5726, Sigma) were included at the recommended concentrations (1:1,000 and 1:100, respectively). Protein was quantified using the bicinchoninic acid assay (Pierce).

**EMSA.** NF-κB, AP-1, and specific protein-1 (Sp1) oligonucleotides were purchased from Promega, and NF-IL6 oligonucleotide was purchased from Geneka Biotechnology (Montreal, Canada). The NF-κB oligonucleotide sequence (sense strand) was 5′-AGTT-GAGGGGACTTTCCCAGG-3′ (area of consensus sequence underlined, and identity to IL-8 promoter in that region denoted by italics), the AP-1 sequence was 5′-CCGTGAGGACGGCAGGAA-3′, and the NF-IL6 sequence was 5′-CTAGGGGTGGAGCAATCATATTCTG-3′ (24). The Sp1 sequence (sense strand) was 5′-ATTCGAGTCGGGGGGCGCGGACG-3′ (area of consensus sequence underlined). Oligonucleotides were end labeled using T4 DNA kinase (Invitrogen, Carlsbad, CA) and 3,000 Ci/mmol (10 μCi) [γ-32P]ATP (NEN, Boston, MA). Probes were purified using affinity chromatography columns (QiQuick, Qiagen). Nuclear extracts (4 μg) were allowed to react with 5–10× 105 cpm (17.5–30.3 fmol) of each probe for 20 min at room temperature in gel shift buffer (containing final concentrations of 4% glycerol, 1 mM MgCl2, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, 10 mM Tris·HCl (pH 7.5), and 0.05 mg/ml poly(dI-dC); poly(dI-dC); Promega). Nonradioactive nonspecific and specific competitors were used at 100-fold molar excess. Nonradioactive AP-1 probe was used as a nonspecific competitor for NF-κB and NF-IL6 experiments, whereas nonradioactive Sp1 probe was used as a nonspecific competitor for AP-1 experiments. For supershift assays, antibodies (2 μg) to the p50, p52, and p65 subunits of NF-κB (Santa Cruz) were added to the reactions after an initial 20-min reaction period for a total of 15 min, and samples were kept on ice until analysis. Each antibody was demonstrated to supershift control HeLa or Jurkat nuclear extracts (4 μg; Promega). Polyacrylamide gels (4%, 37.5:1; Bio-Rad, Hercules, CA) were poured in 0.25× TBE (Invitrogen) and prerun for 5 min at 400 V. Samples were adjusted to 25 mM Tris (pH 7.0), 4% glycerol, and 0.02% bromphenol blue. Resolved species were visualized by autoradiography and/or phosphorimaging (Molecular Dynamics).
Pharmacological inhibition of NF-κB nuclear translocation. The proteasome inhibitor MG-132 (Peptides International, Louisville, KY) was used to block proteasomal degradation of the inhibitor of NF-κB, which prevents the nuclear translocation of NF-κB (22). MG-132 (50 μM) was added to the cell cultures 1 h before the onset of experimental conditions, and cells were exposed for 5 h to the conditions described previously.

Statistical methods. The effects of potentially interacting variables (e.g., LPS and hyperoxia) on outcomes were assessed using two-way ANOVA. Univariate comparisons were made using the Student’s t-test. Log transformation was used for stabilization of variance, as required. Alterations in luciferase output were described qualitatively. Error bars in graphs represent the standard error of the mean.

RESULTS

IL-8 protein secretion. In both U-937 and THP-1 cells, the addition of LPS resulted in increased IL-8 secretion into the media (Fig. 1). In U-937 cells, but not in THP-1 cells, hyperoxia had an independent and additive effect on IL-8 secretion.

IL-8 mRNA expression. In both U-937 and THP-1 cells, the pattern of IL-8 mRNA expression, as measured by RPA, was similar to the pattern of IL-8 secretion into the media in the same cell type (Fig. 2). The independent effect of hyperoxia on U-937 IL-8 mRNA expression, however, was less marked than its effect on IL-8 secretion.

IL-8 promoter-reporter assays. Luciferase output driven by the −133 promoter-reporter construct mimicked the pattern of native IL-8 secretion in both U-937 and THP-1 cells (Fig. 3). Luciferase output was upregulated by LPS exposure in both cell lines, but oxygen had an effect only in the U-937 cell line. Mutation of the AP-1 site of the promoter construct appeared to decrease the amount of luciferase production in both cell lines but did not alter the patterns of response. Mutation of the NF-IL6 site of the construct had little effect on either magnitude or pattern of luciferase expression in either cell line. However, mutation of the NF-κB site completely ablated both baseline and stimulated luciferase expression in both cell lines.

EMSA. In U-937 cells, LPS stimulation consistently produced increased amounts of nuclear protein, which bound to the NF-κB oligonucleotide, after either 3, 6, or 9 h of exposure (Fig. 4, A and B). At none of these times was a similar shift induced by hyperoxia. An excess of nonradioactive NF-κB oligonucleotide (specific competitor) abolished the detectable signal, whereas an excess of nonradioactive AP-1 oligonucleotide (nonspecific competitor) did not. In U-937 cells, the intranuclear abundance of proteins that could bind AP-1 or NF-IL6 oligonucleotides did not vary with either LPS or hyperoxia exposure at 6 h (Fig. 4C). These signals were similarly abolished by specific competitors but not by nonspecific competitors. In U-937 cells, antibody to the p65 subunit of NF-κB was able to induce a supershift of the lower specific, shifted band of NF-κB, whereas antibody to the p50 subunit was able to supershift both specific bands (Fig. 5). Antibody to the p52 subunit had no effect. Hyperoxia exposure had no effect on the intensity or pattern of supershifted bands. LPS exposure resulted in an increased intensity of supershifted bands but no alteration in their pattern.

Figure 6 shows summary data for THP-1 cells after 6 h of exposure to experimental conditions. The pattern was very similar to that seen in U-937 cells, with increased intranuclear NF-κB present after LPS but not hyperoxia exposure. Antibody to the p65 subunit of NF-κB was able to induce a supershift of the lower specific, shifted band of NF-κB, whereas antibody to the p50 subunit was able to supershift both specific bands. Antibody to the p52 subunit had no effect. Hyperoxia exposure had no effect on the intensity or pattern of supershifted bands (data not shown). LPS exposure resulted in an increased intensity of supershifted bands but no alteration in their pattern. AP-1 and NF-IL6 oligonucleotide shifts did not differ with LPS and/or hyperoxia exposure.
The proteasome inhibitor MG-132 was used to prevent nuclear translocation of NF-κB. MG-132 prevented LPS-stimulated NF-κB translocation in both U-937 cells (Fig. 7A) and THP-1 cells (Fig. 7C). MG-132 exposure significantly decreased IL-8 output in U-937 cells exposed to LPS but did not alter the output in air-exposed cells or in those exposed to hyperoxia alone (Fig. 7B). MG-132 treatment ablated LPS-stimulated IL-8 release by THP-1 cells (Fig. 7D).

**DISCUSSION**

We have used two cell lines of monocyte-macrophage lineage with differing oxygen responsiveness to evaluate the mechanisms of hyperoxia-induced IL-8 production in the macrophage. The U-937 line mimicked the hyperoxia-induced IL-8 production seen in the alveolar macrophage, whereas the THP-1 line did not produce IL-8 during hyperoxia exposure. IL-8 mRNA expression and luciferase expression driven by a truncated IL-8 promoter in the cell lines followed patterns similar to those seen in IL-8 protein secretion. This suggests largely transcriptional regulation of IL-8 production, a finding consistent with those of other investigators (2).

Comparison of the responses of the two cell types to two types of stimulation (hyperoxia and LPS) yielded interesting findings. LPS-stimulated nuclear translocation of NF-κB was detectable in low amounts in nuclear extracts at all times of exposure in air-5% CO₂ and did not vary with 95% O₂-5% CO₂ exposure. LPS stimulated increasing, additional nuclear translocation of NF-κB over the time studied in both air-5% CO₂ and 95% O₂-5% CO₂ exposure. A nonspecific, nonradioactive oligonucleotide (AP-1; N) did not prevent association of nuclear protein to the 32 P-labeled oligonucleotide, whereas a nonradioactive NF-κB oligonucleotide (S) did so. Arrows denote NF-κB-specific bands; arrowhead denotes nonspecific band. B: nuclear translocation of AP-1 but did not affect NF-IL6 nuclear translocation. Nonspecific, nonradioactive oligonucleotide (AP-1; N) did not prevent association of nuclear protein to the 32 P-labeled oligonucleotide, whereas a nonradioactive NF-κB oligonucleotide (S) did so. Arrows denote NF-κB-specific bands; arrowhead denotes nonspecific band. C: representative gel electrophoresis shows that neither AP-1 nor NF-IL6 translocation into the nucleus was affected by hyperoxia or LPS exposures over a 6-h period. Stimulation with phorbol 12-myristate 13-acetate (PMA; 1 μg/ml) caused increased nuclear translocation of AP-1 but did not affect NF-IL6 nuclear translocation. Nonspecific, nonradioactive oligonucleotides (AP-1 for NF-IL6, Sp1 for AP-1; N) did not prevent association of nuclear protein to the 32 P-labeled oligonucleotide, whereas a nonradioactive specific oligonucleotide (S) did so. Depicted results are typical of 3–5 experiments.

**Pharmacological inhibition of NF-κB nuclear translocation.**

The proteasome inhibitor MG-132 was used to prevent nuclear translocation of NF-κB. MG-132 prevented LPS-stimulated NF-κB translocation in both U-937 cells (Fig. 7A) and THP-1 cells (Fig. 7C). MG-132 exposure significantly decreased IL-8 output in U-937 cells exposed to LPS but did not alter the output in air-exposed cells or in those exposed to hyperoxia alone (Fig. 7B). MG-132 treatment ablated LPS-stimulated IL-8 release by THP-1 cells (Fig. 7D).

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Comparison of the responses of the two cell types to two types of stimulation (hyperoxia and LPS) yielded interesting
paradoxes. Despite the difference in the ability of hyperoxia to stimulate IL-8 production in the cell lines, there seemed to be a lack of differences at the level of the gene promoter itself in the mechanism of IL-8 transcription between cell lines or between stimuli. A functional NF-κB binding site in the proximal 133 bases of the promoter appeared necessary for IL-8 transcription after either hyperoxia or LPS stimulation. Neither the AP-1 site nor the NF-IL6 site in that portion of the promoter appeared absolutely necessary for gene transcription, although constitutive activity of AP-1 appeared necessary for a full response to either hyperoxia or LPS. Nuclear translocation of NF-κB occurred in both cell lines after LPS stimulation, but in neither cell line after oxygen stimulation. The NF-κB subunits present in the nucleus did not appear to differ between cell lines. The intranuclear abundance of AP-1 and NF-IL6 was unaffected by either hyperoxia or LPS. Pharmacological blockade of NF-κB translocation diminished LPS-stimulated IL-8 production in both cell lines but did not affect hyperoxia-stimulated IL-8 production in the U-937 cell line.

The likely conclusion from these data is that although a functional NF-κB site in the IL-8 promoter is necessary for hyperoxia-stimulated IL-8 release from the macrophage, the nuclear translocation of new NF-κB is not. Translocation of additional NF-κB into the nucleus does appear to be required for LPS-stimulated IL-8 production. The identities of the NF-κB species present in the nucleus did not appear to change by condition. One species was a p50/p65 heterodimer, as others have reported to be present in H2O2- or TNF-stimulated U-937 cells (15, 30). The other appeared to be another p50-containing protein, most likely a p50/c-Rel dimer, as seen in LPS-stimulated cells from the RAW 264.7 macrophage cell line (7). Other investigators have reported that either AP-1 or NF-IL6, but not both, are necessary for NF-κB-mediated IL-8 gene transcription (24). Although our experiments did not provide support for a role of NF-IL6 in IL-8 production in our cell lines, AP-1 did appear to be necessary for a full IL-8 response to stimuli.

In the macrophage, NF-κB may be a cofactor for some other driving factor for IL-8 gene transcription in hyperoxia. The effect of oxygen on IL-8 secretion is likely to be regulated by factors other than NF-κB, AP-1, or NF-IL6. These might include other trans-activating factors that bind to additional sites (such as the AP-1-like or antioxidant response element-like sites) present in the proximal portion of the IL-8 promoter (24). Other possibilities include oxygen stimulation of factors such as p38, which has a synergistic effect on NF-κB transcriptional activity, even in the absence of changes in the nuclear abundance of NF-κB (23).

The results obtained in experiments using the nonoxygen-responsive THP-1 cell line highlight several of the lessons learned from the U-937 cell. There appear to be no significant differences in the intranuclear abundance of the transcription factors examined between the two cell lines under any of the conditions tested.
experimental conditions, including hyperoxia exposure. This makes it all the more likely that the difference in oxygen responsiveness between the cell types is dependent on mechanisms other than intranuclear abundance of these transcription factors.

Several investigators have explored the relationship between oxidant stress and the production of proinflammatory cytokines in macrophages. IL-8 mRNA and protein production are increased in human monocytes exposed to 95% oxygen and enhanced by the addition of 0.1% CO₂ or 95% O₂-5% CO₂ in the presence or absence of LPS (1 μg/ml), with or without addition of the proteasome inhibitor MG-132 (50 μM) 1 h before exposure. EMSA were performed on nuclear extracts (A and C), and IL-8 protein secretion into the media was measured by ELISA (B and D). N = 3 experiments. A: a representative gel electrophoresis shows that, in U-937 cells, LPS-stimulated NF-κB translocation (arrow) was blocked by MG-132. B: in U-937 cells, oxygen-related response in IL-8 secretion appeared unaffected by MG-132, whereas LPS-related responses were diminished by MG-132 (*P < 0.01 for LPS, **P = 0.02 for O₂/LPS, by Student’s t-test). C: a representative gel electrophoresis shows that, in THP-1 cells, LPS-stimulated NF-κB translocation (arrows) was blocked by MG-132. D: in THP-1 cells, LPS-related responses in IL-8 secretion were abolished by MG-132 (**P < 0.03 by Student’s t-test). No hyperoxia effect on IL-8 secretion was detectable.

In summary, we have presented evidence that hyperoxia-induced IL-8 secretion in the U-937 macrophage cell line requires the presence of an active NF-κB site in the IL-8 promoter but does not require nuclear translocation of additional NF-κB. This is in contradistinction to LPS-stimulated IL-8 secretion, which requires both additional NF-κB translocation and an active NF-κB site. The data imply that factors other than NF-κB may regulate oxygen-related changes in the transcription of IL-8. These subtle differences in NF-κB regulation may help to explain the disparate and apparently additive effects of hyperoxia and LPS on macrophage IL-8 production.

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NF-κB IN MACROPHAGE LPS- AND O2-RELATED IL-8 INDUCTION

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