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The Heat Shock Response Inhibits RANTES Gene Expression in Cultured Human Lung Epithelium

Onsy Ayad,* James M. Stark,† Michael M. Fiedler,† Ingrid Y. Menendez,* Marnie A. Ryan,* and Hector R. Wong‡*

The chemokine RANTES is thought to be involved in the pathophysiology of inflammation-associated acute lung injury. Although much is known regarding signals that induce RANTES gene expression, relatively few data exist regarding signals that inhibit RANTES gene expression. The heat shock response, a highly conserved cellular defense mechanism, has been demonstrated to inhibit a variety of lung proinflammatory responses. We tested the hypothesis that induction of the heat shock response inhibits RANTES gene expression. Treatment of A549 cells with TNF-α induced RANTES gene expression in a concentration-dependent manner. Induction of the heat shock response inhibited subsequent TNF-α-mediated RANTES mRNA expression and secretion of immunoreactive RANTES. Transient transfection assays involving a RANTES promoter-luciferase reporter plasmid demonstrated that the heat shock response inhibited TNF-α-mediated activation of the RANTES promoter. Inhibition of NF-κB nuclear translocation with isohelenin inhibited TNF-α-mediated RANTES mRNA expression, indicating that RANTES gene expression is NF-κB dependent in A549 cells. Induction of the heat shock response inhibited degradation of the NF-κB inhibitory protein, IκBα, but did not significantly inhibit phosphorylation of IκBα. We conclude that the heat shock response inhibits RANTES gene expression by a mechanism involving inhibition of NF-κB nuclear translocation and subsequent inhibition of RANTES promoter activation. The mechanism by which the heat shock response inhibits NF-κB nuclear translocation involves stabilization of IκBα, without significantly affecting phosphorylation of IκBα. The Journal of Immunology, 1998, 161: 2594–2599.

RANTES is a member of the C-C chemokine subfamily. Several lines of evidence indicate that RANTES is an important proinflammatory mediator. RANTES has marked chemoattractant activity for monocytes (1), causes selective migration of CD4+ T lymphocytes, eosinophils, and basophils (1–3), activates eosinophils leading to release of histamine and eosinophil cationic proteins (4), and increases expression of the CD11/CD18 adhesion molecule (2). RANTES expression has been demonstrated in a variety of diseases characterized by inflammation including asthma (5), respiratory syncytial virus bronchiolitis (6, 7), chronic polyposic sinusitis (8), transplantation-associated accelerated atherosclerosis (9), endometriosis (10), and acute lung injury secondary to endotoxemia (11). The RANTES gene is inducible by viruses (e.g., respiratory syncytial virus) and cytokines (e.g., TNF-α and IL-1β). Apart from glucocorticoids and IL-4, very little is known about factors that inhibit induction of RANTES gene expression (12–14).

The heat shock response is a highly conserved genetic response providing cells an adaptive mechanism to survive lethal stresses (for reviews see Refs. 15, 16). The original descriptions of the heat shock response involved the phenomenon known as thermostolerance, whereby subjecting cells to a brief period of sublethal hyperthermia conferred protection against subsequent exposure to otherwise lethal hyperthermia (17). Induction of the heat shock response was also demonstrated to protect against nonthermal forms of injury, such as acute lung injury. Induction of the heat shock response protected rats against acute lung injury secondary to either systemic administration of endotoxin (18, 19) or intratracheal instillation of phospholipase-A1 (20). In vitro induction of the heat shock response protected lung cells against endotoxin and oxidants (21, 22). The mechanisms by which the heat shock response confers protection against acute lung injury are not well understood. One potential mechanism of protection may involve the ability of the heat shock response to inhibit proinflammatory responses in lung cells. The heat shock response inhibited inducible nitric oxide synthase gene expression in cultured lung cells treated with cytokines and in whole lungs of rats treated with endotoxin (23–26). Recent data demonstrated that the heat shock response inhibited nuclear translocation of the proinflammatory transcription factor NF-κB and induced expression of the NF-κB inhibitory protein IκBα in cultured lung cells (25, 27). Collectively, these data indicate that the heat shock response may have a broad antiinflammatory role in the lung. To further explore this concept we determined the effects of the heat shock response on RANTES gene expression. We demonstrate that the heat shock response inhibits RANTES mRNA expression, as well as secretion of immunoreactive RANTES. We further demonstrate that the heat shock response inhibits activation of the RANTES promoter, possibly by preventing nuclear translocation of NF-κB.

Materials and Methods

Cell culture

A human lung adenocarcinoma cell line (A549 cells), representative of distal respiratory epithelium, was used in all experiments. A549 cells were previously demonstrated to express RANTES in response to treatment with TNF-α (13). Cells were grown in plastic tissue culture flasks and maintained in a room air/5% CO2 incubator using DMEM (Life Technologies,
Gaithersburg, MD) containing 8% FBS (Life Technologies), sodium bicarbonate (2 g/ml), and penicillin/streptomycin (Life Technologies).

**Experimental conditions**

The heat shock response was induced by incubating cells at 43°C for 1 h as previously described (27). RANTES was induced by treating cells with recombinant human TNF-α (10 to 100 ng/ml, Boehringer Mannheim, Indianapolis, IN) for 24 h. In transient transfection assays, cells were treated with TNF-α for 4 h. To determine the effects of the heat shock response on TNF-α-mediated RANTES gene expression, one group of cells was heat shocked and allowed to recover at 37°C for 1 h before treatment with TNF-α. To determine the effects of NF-κB inhibition on TNF-α-mediated RANTES gene expression, one group of cells was treated with the NF-κB inhibitor isothiocol compound in 100-mm² dishes. All nuclear extraction procedures were performed according to the manufacturer’s instructions. Miniprep (Promega) using a Berthold AutoLumat LB953 luminometry (260 nM), and 15 μg of total RNA per condition underwent electrophoresis on 15% agarose gels containing 3% formaldehyde. The integrity of the RNA after electrophoresis was confirmed by ethidium bromide staining and brief ultraviolet illumination. RNAs were transferred to nylon membranes (Micron Separation, Westboro, MA) and ultraviolet light cross-linked (UV Stratallinker 1800, Stratagene, La Jolla, CA). After a 4-h pre-hybridization at 42°C, membranes were hybridized overnight with a radiolabeled human RANTES cDNA probe (a kind gift of Dr. Alan M. Krenskey, Stanford University School of Medicine; Ref. 28). The cDNA probe was radiolabeled with [α-32P]dCTP (sp. act. 3,000 Ci/mM, New England Research Products, Boston, MA) by random priming (Pharmacia, Piscataway, NJ). The hybridized filters were serially washed at 53°C using 2× sodium citrate/sodium chloride/0.1% SDS, and 25 mM NaHPO4/l mM EDTA/0.1% SDS solutions. After washing, exposure was conducted overnight using a Phosphor Imager screen (Molecular Dynamics, Sunnyvale, CA). To normalize results for loading differences, membranes were stripped with boiling 5 mM EDTA and rehybridized with an end-labeled [γ-32P]dATP oligonucleotide probe for 18 s rRNA.

**RANTES ELISA**

Immunoreactive RANTES levels in the media of treated cells were quantified using a commercially available sandwich ELISA (R&D Systems, Minneapolis, MN). All procedures were performed according to the manufacturer’s instructions.

**Nuclear protein extraction**

Nuclear protein extracts were prepared from treated cells grown to 80% confluence in 100-mm² dishes. All nuclear extraction procedures were performed on ice with ice-cold reagents. Cells were washed twice with PBS and harvested by scraping into 1 ml of PBS and pelleted at 6,000 rpm for 5 min. The pellet was washed twice with PBS, resuspended in one packed cell volume of lysis buffer (10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1.5 mM MgCl2, 0.2% v/v Nonidet P-40, 1 mM DTT, and 0.1 mM PMSF), and incubated for 5 min with occasional vortexing. After centrifugation at 6,000 rpm, one cell pellet volume of extraction buffer (20 mM HEPES pH 7.9, 420 mM NaCl, 0.1 M EDTA, 1.5 mM MgCl2, 25% v/v glycerol, 1 mM DTT, and 0.5 mM PMSF) was added to the nuclear pellet and incubated for 15 min with occasional vortexing. The nuclear proteins were isolated by centrifugation at 14,000 RPM for 15 min. Protein concentrations were determined by Bradford assay (Bio-Rad, Hercules, CA) and stored at −70°C until used for electrophoretic mobility shift assay (EMSA)1.

**EMSA**

The NF-κB oligonucleotide probe used for EMSA (5'-AGT TGA GGG GAC TTC CCC AAG C-3') was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The probe was labeled with [γ-32P]ATP using T4 polynucleotide kinase (Life Technologies) and purified in Bio-Spin chromatography columns (Bio-Rad).

For EMSA, 10 μg of nuclear proteins were preincubated with EMSA buffer (12 mM HEPES pH 7.9, 4 mM Tris-HCl pH 7.9, 25 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM DTT, 50 ng/ml poly[dI-C)] 12% glycerol/v/v, and 0.2 mM PMSF) on ice for 10 min before addition of the radiolabeled oligonucleotide probe for an additional 10 min. Protein-nucleic acid complexes were resolved using a nondenaturing polyacrylamide gel consisting of 5% acrylamide (29:1 ratio of acrylamide:bisacrylamide) and run in 0.5× TBE (45 mM Tris-HCl, 45 mM boric acid, 1 mM EDTA) for 1 h at constant current (30 mA). Gels were transferred to Whatman 3 M paper, dried under a vacuum at 80°C for 1 h, and exposed to photographic film at −70°C with an intensifying screen.

**Western blot analysis**

Treated cells were washed once in PBS and lysed in ice-cold buffer containing 50 mM Tris (pH 8.0), 110 mM NaCl, 5 mM EDTA, 1% Triton X-100, and PMSF (100 μg/ml). Protein concentrations were determined using the Bradford assay. Whole cell lysates were boiled in equal volumes of loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, and 2% 2-ME) and 50 μg of protein loaded per lane on an 8 to 16% Tris-glycine gradient gel (Novex, San Diego, CA). Proteins were separated electrophoretically and transferred to nitrocellulose membranes (Novex) using the Novex Xcell MiniGel system. For immunoblotting, membranes were blocked with 10% nonfat dried milk in Tris-buffered saline (TBS) for 1 h. Primary Ab against human IκBα (polyclonal, Santa Cruz Biotechnology) was applied at a 1:200 dilution for 1 h. After washing twice in TBS containing 0.05% Tween 20 (TTBS), secondary Ab (peroxidase-conjugated goat anti-rabbit IgG, Sigma) was applied at a 1:10,000 dilution for 1 h. Blots were washed in TTBS two times over 30 min, incubated in commercial enhanced chemiluminescence reagents (ECL, Amersham, Buckinghamshire, England), and exposed to photographic film.

**Transient transfections, functional promoter analyses, and luciferase assays**

The effect of the heat shock response on TNF-α-mediated RANTES promoter activation was analyzed by transiently transfecting cells with a RANTES promoter-luciferase reporter plasmid (a kind gift of Dr. Alan M. Krenskey, Stanford University School of Medicine). This plasmid contains a −421 bp 5′ flanking sequence of the human RANTES gene and has been previously described in detail (28).

Another group of cells was transfected with a reporter plasmid in which the human IκBα promoter was cloned upstream from the luciferase reporter gene. Oligonucleotide primers were designed by the University of Cincinnati DNA Core Facility corresponding to the most 5′ and 3′ regions of the published human IκBα promoter sequence (29). Using these primers, a 1.3-kb human IκBα promoter fragment was recovered from a human genomic DNA template using PCR. The PCR product was ligated into the plasmid pCR2.1 (Invitrogen, San Diego, CA) and sequenced by the Sanger dideoxy method. Sequence analysis demonstrated >99% correlation between the PCR product and the published human IκBα promoter sequence (29). A KpnI/Xhol fragment incorporating the 1.3-kb IκBα promoter fragment was excised from pCR2.1 and subcloned into the plasmid pGL2 (Promega, Madison, WI) such that the IκBα promoter regulated expression of the reporter gene firefly luciferase.

Cells were transfected in duplicate, in six-well plates, at a density of 300,000 cells per well by incubation with cationic liposomes (Lipofectin, Life Technologies) for 5 h in Opti-MEM (Life Technologies). After transfection, cells were washed once with PBS and allowed to recover overnight. After exposure to experimental conditions, cellular proteins were extracted and analyzed for luciferase activity according to the manufacturer's instructions (Promega) using a Berthold AutoLumat LB953 luminometer. Luciferase activity is reported as light units corrected for total cellular protein.

**Cell Viability**

Cell viability after exposure to the experimental conditions was determined by MTT assay as previously described (24). This is a colorimetric assay based on the ability of viable cells to reduce the tetrazolium dye 3-(4, 5-dimethylthiazolyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan. Percent cell viability is calculated as (absorbance of treated cells)/absorbance of control cells) ×100.

**Statistical analysis**

Differences in immunoreactive RANTES expression, luciferase activity, and cell viability between the experimental groups were evaluated by one-way ANOVA and Student Newman Keuls test. A p < 0.05 was considered statistically significant.

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1 Abbreviation used in this paper: EMSA, electrophoretic mobility shift assay.
Results

Induction of the heat shock response inhibits TNF-α-mediated RANTES mRNA expression

Treatment of A549 cells with TNF-α for 24 h increased RANTES mRNA expression in a concentration-dependent manner, compared with unstimulated control cells (Fig. 1). These data are consistent with previous studies involving A549 cells (13). We previously demonstrated that incubation at 43°C for 1 h induced the heat shock response in A549 cells, as measured by induction of heat shock protein-70 (27). To determine whether induction of the heat shock response alters RANTES mRNA expression, one group of cells was incubated at 43°C for 1 h, recovered at 37°C for 1 h, then treated with varying concentrations of TNF-α for 24 h. Induction of the heat shock response inhibited subsequent TNF-α-mediated RANTES mRNA expression (Fig. 1). For all doses of TNF-α tested, prior induction of the heat shock response reduced RANTES mRNA levels compared with unstimulated control cells. Cells subjected to heat shock alone had no detectable changes in RANTES mRNA levels compared with unstimulated control cells. Cell viability was greater than 90% after all experimental conditions (data not shown). These data demonstrate that induction of the heat shock response inhibits TNF-α-mediated RANTES mRNA expression in A549 cells.

Induction of the heat shock response inhibits secretion of immunoreactive RANTES

Having demonstrated that the heat shock response inhibited RANTES mRNA expression, we next determined if the heat shock response would alter secretion of immunoreactive RANTES. Treatment with TNF-α for 24 h increased secretion of immunoreactive RANTES into the media of treated cells in a concentration-dependent manner (Fig. 2). Induction of the heat shock response inhibited TNF-α-mediated increases in luciferase activity. Heat shock alone did not affect luciferase activity compared with control cells.

To determine the specificity of this effect, another group of cells was transiently transfected with a human I-kBα promoter-luciferase reporter plasmid. This model was chosen because we previously demonstrated that the heat shock response did not inhibit activity ~14-fold above that of control cells (transfected and treated with basal growth media), demonstrating that TNF-α activated the RANTES promoter (Fig. 3). Induction of the heat shock response inhibited TNF-α-mediated increases in luciferase activity. Heat shock alone did not affect luciferase activity compared with control cells.

Because RANTES expression is primarily regulated transcriptionally, we next determined if the heat shock response alters TNF-α-mediated activation of the RANTES promoter. A549 cells were transiently transfected with a plasmid in which the reporter gene firefly luciferase was under the control of the RANTES promoter. Treatment with 30 ng/ml of TNF-α for 4 h increased luciferase activity ~14-fold above that of control cells (transfected and treated with basal growth media), demonstrating that TNF-α activated the RANTES promoter (Fig. 3). Induction of the heat shock response inhibited TNF-α-mediated increases in luciferase activity. Heat shock alone did not affect luciferase activity compared with control cells.

Average luciferase activities (± SEM) of cells transiently transfected with a RANTES promoter-luciferase plasmid and subjected to the indicated conditions. Data are plotted as mean light units (± SEM) corrected for total cellular protein and represent five separate experiments in duplicate. * indicates p < 0.05 vs control. ** indicates p < 0.05 vs cells treated with TNF-α alone.
pression is dependent on NF-κB (27). Recent evidence supports NF-κB as a mediator of A549 cells with TNF-α, demonstrating that TNF-α facilitates translocation of NF-κB to the nucleus, which is targeted for ubiquitination and subsequent degradation by the proteosome. The specificity of the NF-κB band was previously demonstrated by supershift and competitor assays (27). Isohelenin stock solutions were dissolved in DMSO, and equivalent amounts of DMSO were added to cells not treated with isohelenin.

**FIGURE 5.** Representative EMSA demonstrating the effect of isohelenin on TNF-α-mediated NF-κB nuclear translocation. Lane 1: control cells; lane 2: cells treated with TNF-α (30 ng/ml) for 0.5 h; lane 3: cells were treated with isohelenin (50 μM) for 1 h before treatment with TNF-α. The specificity of the NF-κB band was previously demonstrated by supershift and competitor assays (27). Isohelenin stock solutions were dissolved in DMSO, and equivalent amounts of DMSO were added to cells not treated with isohelenin.

**Effect of the heat shock response on phosphorylation of IκBα**

NF-κB nuclear translocation is regulated by a family of intracellular proteins called IκB (reviewed in Ref. 33). IκBα retains NF-κB in the cytoplasm by physically masking its nuclear translocation sequences. An important early event in the activation of NF-κB involves phosphorylation of IκBα. Phosphorylated IκBα is targeted for ubiquitination and subsequent degradation by the 26S proteosome pathway. Degradation of IκBα unmasks NF-κB nuclear translocation sequences, allowing translocation of active NF-κB to the nucleus.

In these experiments we determined the effect of the heat shock response on phosphorylation of IκBα. Phosphorylated IκBα is inherently unstable and difficult to detect by Western blot analysis. To detect phosphorylated IκBα, cells were pretreated with the relatively specific 26s proteosome inhibitor, MG-132, as previously described (34). MG-132 inhibits proteosome-mediated degradation of IκBα but does not affect its phosphorylation. Treatment with TNF-α caused a rapid disappearance of immunoreactive IκBα within 15 min, consistent with its degradation (Fig. 7, lane 2). Treatment with MG-132 partially inhibited TNF-α-mediated degradation of immunoreactive IκBα and caused the appearance of a slower migrating band corresponding to phosphorylated IκBα (Fig. 7, lane 3, Ref. 34). A similar band was noted when cells were pretreated with both heat shock and MG-132, before treatment with TNF-α (Fig. 7, lane 5). Induction of the heat shock response alone inhibited TNF-α-mediated degradation of IκBα and caused the appearance of a faint, slower migrating band similar to that seen in cells pretreated with MG-132 alone (Fig. 7, compare lanes 4 and 3). Collectively, these data demonstrate that the heat shock response inhibits TNF-α-mediated degradation of IκBα but does not strongly affect phosphorylation of IκBα.

**FIGURE 6.** Representative Northern blot analysis demonstrating the effect of isohelenin on TNF-α-mediated RANTES mRNA expression. Lane 1: control cells; lane 2: cells treated with TNF-α (30 ng/ml) for 24 h; lane 3: cells were treated with isohelenin (50 μM) for 1 h before treatment with TNF-α. 18s rRNA was used to control for loading differences. Isohelenin stock solutions were dissolved in DMSO, and equivalent amounts of DMSO were added to cells not treated with isohelenin.

**Effect of NF-κB inhibition on TNF-α-mediated RANTES mRNA expression**

The RANTES promoter contains several NF-κB binding sites, and recent evidence supports NF-κB-dependent regulation of RANTES (28, 30, 31). To determine whether NF-κB is involved in the regulation of RANTES mRNA expression in A549 cells, we treated cells with isohelenin, a sesquiterpene lactone recently described to be a specific inhibitor of NF-κB activation (32). Treatment of A549 cells with TNF-α for 0.5 h caused nuclear translocation of NF-κB (Fig. 5, lane 2). The specificity of this band was previously demonstrated by competitor and supershift assays (27). Treatment with isohelenin inhibited TNF-α-mediated nuclear translocation of NF-κB (Fig. 5, lane 3), consistent with a previous report (32).

Having demonstrated that isohelenin inhibits TNF-α-mediated NF-κB nuclear translocation in A549 cells, we next determined the effect of isohelenin on TNF-α-mediated RANTES mRNA expression. Treatment with isohelenin inhibited TNF-α-mediated RANTES mRNA expression (Fig. 6, compare lane 3 to lane 2). These data indicate that TNF-α-mediated RANTES mRNA expression is dependent on NF-κB activation in A549 cells. Because the heat shock response was previously demonstrated to inhibit TNF-α-mediated NF-κB nuclear translocation in A549 cells (27), these data indirectly suggest that the heat shock response inhibits RANTES gene expression by inhibiting NF-κB nuclear translocation.
The heat shock response did not inhibit TNF-α.

The I-κBα gene by preventing nuclear translocation of NF-κB, rather than a generic effect of the heat shock response. Induction of the RANTES promoter contains four NF-κB sequences are unmasked, and activated NF-κB translocates to the nucleus to direct transcription of proinflammatory genes. Inhibition of NF-κB nuclear translocation can occur at several steps along this pathway, including inhibition of I-κBα phosphorylation, inhibition of I-κBα ubiquitination, and/or inhibition of the 26S proteosome.

The heat shock response was previously demonstrated to inhibit degradation of I-κBα after a proinflammatory stimulus (27, 35), thus providing a potential mechanism by which the heat shock response inhibits NF-κB nuclear translocation. In the current study we determined if the heat shock response inhibits phosphorylation of I-κBα using a qualitative assay. Our data confirm that the heat shock response significantly impacts TNF-α-mediated phosphorylation of I-κBα, then we would have expected to not see a slower migrating band in cells pretreated with the combination of heat shock and MG-132 (Fig. 7, lane 5). In cells pretreated with the combination of heat shock and MG-132, before treatment with TNF-α, there is a clear demonstration of a slower migrating band that corresponds to phosphorylated I-κBα. Although it is possible that the heat shock response had a small effect on phosphorylation of I-κBα that we could not detect with our qualitative assay, from these data we can at least conclude that the heat shock response does not have a major impact on phosphorylation of I-κBα. Furthermore, these data indicate that the primary inhibitory effect of the heat shock response on degradation of I-κBα involves a mechanism distal to phosphorylation. Possible mechanisms include inhibition of ubiquitination or direct inhibition of the 26S proteosome. We are currently investigating these issues.

The heat shock response protects against inflammation-associated acute lung injury (reviewed in Ref. 15). The mechanisms of this protective effect are not fully understood, but recent evidence suggests that modulation of lung proinflammatory responses may be involved in protection. The current study supports this concept by demonstrating that the heat shock response inhibits RANTES gene expression. We propose that the heat shock response protects against inflammation-associated lung injury by modulating the expression of various NF-κB-dependent genes (e.g., RANTES) that are involved in the pathophysiology of inflammation-associated lung injury. Models such as the one described here will help to further test this hypothesis and to better understand the mechanisms by which the heat shock response modulates proinflammatory gene expression.

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References


Discussion

Our results demonstrate that the heat shock response is a potent inhibitor of RANTES gene expression in vitro. Induction of the heat shock response inhibited TNF-α-mediated expression of RANTES mRNA and secretion of immunoreactive RANTES. These effects were not secondary to cytotoxicity, and are consistent with previous data demonstrating that the heat shock response inhibited proinflammatory responses of lung cells (23–27). We propose that the observed inhibition is not a generalized effect of the heat shock response. Rather, it appears that the heat shock response has a relatively specific inhibitory effect on lung proinflammatory responses. Evidence to support this assertion includes the observation that the heat shock response did not affect surfactant protein gene expression in cultured respiratory epithelium (24). Moreover, the heat shock response increased I-κBα gene expression in cultured respiratory epithelium, a protein that normally functions to inhibit NF-κB nuclear translocation and is not currently considered to be one of the heat shock proteins (27).

Transient transfection assays involving a RANTES promoter-luciferase reporter plasmid demonstrated that the heat shock response inhibited TNF-α-mediated induction of luciferase activity. These data suggest that the mechanism by which the heat shock response inhibits RANTES gene expression involves inhibition of the RANTES promoter. In parallel experiments involving cells transiently transfected with an I-κBα promoter-luciferase plasmid, the heat shock response did not inhibit TNF-α-mediated induction of luciferase activity. These data allow us to conclude that the observed inhibition of the RANTES promoter was a relatively specific effect, rather than a generic effect of the heat shock response.

The RANTES promoter contains four NF-κB binding sites, and recent data indicate that the RANTES gene is NF-κB-dependent (28, 30, 31). Isohelenin, a recently described and relatively specific inhibitor of NF-κB activity (32), inhibited TNF-α-mediated NF-κB nuclear translocation and TNF-α-mediated RANTES mRNA expression in A549 cells. These data provide evidence that RANTES gene expression is NF-κB dependent in A549 cells. Since the heat shock response profoundly inhibited TNF-α-mediated nuclear translocation of NF-κB in A549 cells (29), it is reasonable to propose that the heat shock response inhibits activation of the RANTES promoter and subsequent induction of the RANTES gene by preventing nuclear translocation of NF-κB.

Under basal conditions NF-κB is retained in the cytoplasm by the I-κB protein family (reviewed in Ref. 33). In response to a proinflammatory signal, I-κBα is rapidly degraded by a phosphorylation- and ubiquitination-dependent pathway. Phosphorylated and ubiquitinated I-κBα is targeted for degradation by 26S proteosome. Upon degradation of I-κBα, NF-κB nuclear translocation

![FIGURE 7](https://example.com/figure7.png)

Representative Western blot analysis demonstrating the effect of the heat shock response on phosphorylation of I-κBα. To induce phosphorylation and degradation of I-κBα, cells were treated with TNF-α (10 ng/ml) for 15 min. To detect phosphorylated I-κBα, some cells were treated with the proteosome inhibitor MG-132 (10 μM) for 1 h before TNF-α treatment. The heat shock response was induced in some cells 1 h before TNF-α treatment. MG-132 stock solutions were dissolved in DMSO, and equivalent amounts of DMSO were added to cells not treated with MG-132.


