In Vivo Suppression of NF-κB and Preservation of IκBα by Interleukin-10 and Interleukin-13

Alex B. Lentsch, Thomas P. Shanley, Vidya Sarma, and Peter A. Ward
Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109

Abstract

IL-10 and IL-13 have powerful antiinflammatory activities in vitro and in vivo. In the IgG immune complex model of lung injury in rats, exogenously administered IL-10 or IL-13 have recently been shown to suppress neutrophil recruitment and ensuing lung injury by greatly depressing pulmonary production of TNFα. Transcriptional control of the TNFα gene is regulated by the nuclear factor kappa B (NF-κB). Activation of NF-κB involves the degradation of its cytoplasmic inhibitor IκBα, allowing the nuclear translocation of NF-κB, with ensuing transcriptional activation. In this study, we sought to determine whether the protective effects of IL-10 and IL-13 in IgG immune complex–induced lung injury were mediated by inhibition of NF-κB activation. Electrophoretic mobility shift analysis of nuclear extracts from alveolar macrophages and whole lung tissues demonstrated that both IL-10 and IL-13 suppressed nuclear localization of NF-κB after in vivo deposition of IgG immune complexes. Western blot analysis indicated that these effects were due to preserved protein expression of IκBα in both alveolar macrophages and whole lungs. Northern blot analysis of lung mRNA showed that, in the presence of IgG immune complexes, IL-10 and IL-13 augmented IκBα mRNA expression. These findings suggest that in vivo, IL-10 and IL-13 may operate by suppressing NF-κB activation through preservation of IκBα. (*J. Clin. Invest.* 1997. 100:2443–2448.)
Key words: alveolar macrophages • TNFα • inflammation • immune complexes • neutrophils

Introduction

Regulation of inflammation by cytokines involves an intricate balance of pro- and antiinflammatory mediators. Intrapulmonary deposition of IgG immune complexes in rats causes alveolar macrophage activation and results in neutrophil-dependent parenchymal cell injury characterized by increased pulmonary vascular permeability and alveolar hemorrhage (1, 2). Progression of these inflammatory sequelae is facilitated by synthesis and secretion of the proinflammatory cytokines, TNFα, and IL-1β. Functional blockade of either TNFα or IL-1β greatly attenuates injury in a manner that is associated with diminished upregulation of lung vascular ICAM-1 (3, 4, 5). The local inflammatory response in this model ultimately resolves, suggesting that intrinsic factors are produced that serve to limit the effects of proinflammatory mediators. Recent studies have identified IL-6, IL-10, and IL-1 receptor antagonist as endogenous regulators in this model of inflammatory lung injury (6–8). Endogenous IL-13 may also play a regulatory role (9). We have shown that exogenously administered IL-4, IL-10, or IL-13 greatly attenuates the lung injury induced by IgG immune complexes, these protective effects being associated with greatly reduced production of pulmonary TNFα (10, 11).

The transcriptional regulation of many early response genes, including TNFα, is largely controlled by the nuclear transcription factor NF-κB. The primary form of NF-κB consists of a heterodimer of NF-κB1 (p50) and RelA (p65), being retained in the cytoplasm bound by inhibitory proteins of the IκB family, including IκBα. In response to inflammatory stimuli, IκBα is phosphorylated, ubiquinated, and degraded in a process requiring the 26S proteasome. Degradation of IκBα allows NF-κB to translocate to the nucleus where it may bind to specific promoter elements and induce gene transcription (for review see reference 12).

While in vitro studies suggest that IL-10 inhibits proinflammatory cytokine production by suppressing NF-κB activation (13), it is unknown whether this effect of IL-10 is operational in vivo. Furthermore, the mechanism(s) of inhibition by IL-13 is(are) largely unknown. In this study, we sought to determine the molecular events associated with the protective effects of IL-10 and IL-13 in the IgG immune complex–induced model of lung injury. We demonstrate that both IL-10 and IL-13 inhibit nuclear localization of NF-κB in alveolar macrophages and lung tissues in a manner associated with preserved expression of IκBα protein. These findings suggest that IL-10 and IL-13 reduce lung inflammation by preventing degradation of IκBα, thus inhibiting the activation of NF-κB.

Methods

IgG immune complex-induced alveolitis. Male Long-Evans rats (275–300 g, specific pathogen-free; Harlan Sprague-Dawley Inc., Indianapolis, IN) were anesthetized with Ketamine HCl (150 mg/kg, intraperitoneally). A total of 1.5 mg of rabbit polyclonal IgG rich in anti-BSA Organon Teknika, West Chester, PA) in a volume of 300 μl PBS was instilled via an intratracheal catheter during inspiration. Immediately thereafter, 10 mg BSA in 500 μl PBS (pH 7.4) was injected intravenously. Control rats received PBS intratracheally. Recombinant murine IL-10 or IL-13 were administered intratracheally at a dose of 1 μg. At the indicated time points, rats were exsanguinated, and the pulmonary circulation was flushed with 10 ml saline by pulmonary artery injection. The lungs were surgically dissected and immediately frozen in liquid nitrogen. For studies of alveolar macrophages stimulated in vivo, rats were exsanguinated 30 min after immune complex deposition, and alveolar macrophages were harvested by broncho-

Address correspondence to Peter A. Ward, M.D., Professor and Chairman, Department of Pathology, The University of Michigan Medical School, 1301 Catherine Road, Ann Arbor, Michigan 48109-0602. Phone: 313-763-6384; FAX: 313-763-4782; E-mail: pward@umich.edu
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alveolar lavage (BAL).[1] At this time point, BAL fluids contained > 95% macrophages as determined by microcytometry.

Stimulation of alveolar macrophages in vitro. Alveolar macrophages from normal rat lungs were isolated by BAL. Cells were resuspended in DMEM supplemented with 10% FBS (GIBCO BRL, Gaithersburg, MD), and plated in 100-mm dishes and allowed to adhere for 1 h in a humidified incubator at 37°C with 5% CO_2. Nonadherent cells were discarded, and the adherent cells were determined to be > 99% alveolar macrophages by microcytometry. Alveolar macrophages were stimulated for 30 min with DMEM or IgG-BSA immune complexes (0.1 mg/ml) in the presence or absence of murine IL-10 or IL-13 (10 ng/ml).

Electrophoretic mobility shift assay. Nuclear extracts of whole lung tissues were prepared by the method of Deryckere and Gannon (14). Nuclear extracts of alveolar macrophages were prepared as previously described (15). Purity of nuclear extracts were confirmed by assessing the relative level of lactate dehydrogenase in nuclear versus cytoplasmic extracts. Nuclear extracts contained approximately 10-fold less lactate dehydrogenase than did cytoplasmic extracts as measured by an endpoint assay (data not shown; Sigma Chemical Co., St. Louis, MO). Protein concentrations were determined by bicinchoninic acid assay with trichloroacetic acid precipitation using BSA as a reference standard (Pierce, Rockford, IL). Double-stranded NF-κB consensus oligonucleotide (5′-AGTGAGGGGACTTTCCCAGGC-3′; Promega Corp., Madison, WI) was end-labeled with γ[^{32}P]ATP (3,000 Ci/mmol at 10 mCi/ml; Amersham Corp., Arlington Heights, IL). Binding reactions containing equal amounts of protein (10 μg for whole lung extracts; 5 μg for alveolar macrophage extracts) and 35 fmols (~ 50,000 cpm, Cherenkov counting) of oligonucleotide were performed for 30 min in binding buffer (4% glycerol, 1mM $MgCl_2$, 0.5 mM EDTA, pH 8.0, 0.5 mM dithiothreitol, 50 mM NaCl, 10 mM Tris, pH 7.6, 50 μg/ml poly [dI-dC]; Pharmacia LKB Biotechnology Inc., Piscataway, NJ). Polyclonal rabbit anti-p65 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and AP-1 consensus oligonucleotide (Promega Corp., Madison, WI) were used for supershift and competition assays, respectively. Reaction volumes were held constant to 15 μl. Reaction products were separated in a 4% polyacrylamide gel and analyzed by autoradiography.

Western blot analysis. Whole lung tissue or alveolar macrophages obtained by BAL were homogenized in lysis buffer (10 mM Hepes, pH 7.9, 150 mM NaCl, 1 mM EDTA, 0.6% NP-40, 0.5 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotonin, 10 μg/ml soybean trypsin inhibitor, 1 μg/ml pepstatin) on ice. Homogenates were sonicated and centrifuged at 5,000 rpm to remove cellular debris. Interfering IgG anti-BSA in homogenates was removed with Gammabind G sepharose (Pharmacia LKB Biotechnology). Protein concentrations were determined as described for nuclear extracts. Samples (50 μg total protein) were separated in a denaturing 10% polyacrylamide gel and transferred to a polyvinylidene difluoride membrane. Nonspecific binding sites were blocked with TBS (40 mM Tris, pH 7.6, 300 mM NaCl) containing 5% nonfat dry milk for 12 h at 4°C. Membranes were then incubated in a 1:1,000 dilution of rabbit polyclonal anti-IκBα (Santa Cruz Biotechnology) in TBS with 0.1% Tween 20 (TBST). After three washes in TBST, membranes were incubated in a 1:50,000 dilution of horseradish peroxidase–conjugated donkey anti–rabbit IgG (Amersham Corp., Arlington Heights, IL). Immunoreactive proteins were detected by enhanced chemiluminescence (ECL). IκBα protein quantitation was performed on digitized films using image analysis software (Adobe Systems, San Jose, CA).

Northern blot analysis. Total RNA from whole lung tissue was extracted using a guanidinium-isothiocyanate method as described previously (16). Samples (20 μg RNA) were fractionated electrophoretically in a 1% formaldehyde gel and transferred to a nylon membrane (MSI, Westboro, MA). cDNA for murine IκBα (17) was radiolabeled with [^{32}P]dCTP (NEN Research Products, Boston, MA) by PCR. The reaction was primed using the following oligonucleotides: 5′ primer: 5′-ATGTTTCAGCCAGCTGGGCAC-3′ and 3′ primer: 5′-TTATAACGTCAGACGCTG GCCT-3′. Northern blots were hybridized with the cDNA probe at 42°C for 18 h, and an autoradiogram was developed on BioMax-MR film (Eastman Kodak Co., Rochester, NY). Equal loading of samples was confirmed by probing Northern blots with cDNA to β-actin radiolabeled with [^{32}P]dCTP using RediPrime (Amersham Corp.).

Results

Activation of lung NF-κB during IgG immune complex–induced alveolitis. The kinetics of NF-κB activation during IgG immune complex–induced lung injury were determined by electrophoretic mobility shift assays of nuclear extracts from whole lung obtained at various time points after initiation of lung inflammation. Nuclear localization of NF-κB was increased within 30 min after immune complex deposition, and progressively increased thereafter, reaching maximal levels by 4 h (Fig. 1 *A*). To determine whether alveolar macrophages demonstrated a similar pattern of NF-κB activation, alveolar macrophages were obtained by BAL 30 min after immune complex deposition. More NF-κB was present in the nuclei of alveolar macrophages from lungs 30 min after immune complex deposition than in lung macrophages obtained at 0 min (Fig. 1 *B*). Lung instillation of PBS alone did not cause NF-κB activation in alveolar macrophages harvested after 30 min (data not shown). Thus, at least part of the nuclear translocation of NF-κB in whole lung extracts can be ascribed to changes in lung macrophages.

The specificity of the NF-κB consensus oligonucleotide probe was confirmed by experiments using nuclear extracts from whole lungs 4 h after immune complex deposition (Fig. 2). In DNA binding reactions, nuclear extracts from inflamed lungs incubated with only ^{32}P-labeled NF-κB consensus oligonucleotide probe showed typical binding to the labeled oligo-

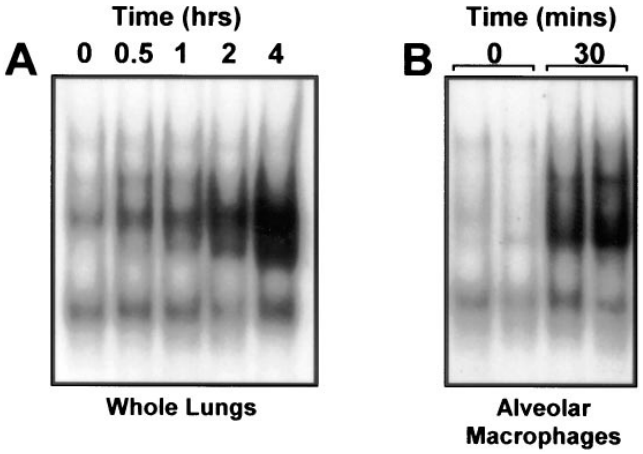

Figure 1. NF-κB activation during IgG immune complex–induced lung inflammation. Nuclear extracts from whole lung tissues or alveolar macrophages harvested by bronchoalveolar lavage were subjected to electrophoretic mobility shift assays. (*A*) Time course of NF-κB activation in IgG immune complex–injured lungs. Results are representative of two separate time-course experiments. (*B*) NF-κB activation in alveolar macrophages harvested 30 min after IgG immune complex deposition. Results are representative of four separate experiments.

1. *Abbreviation used in this paper:* BAL, bronchoalveolar lavage.

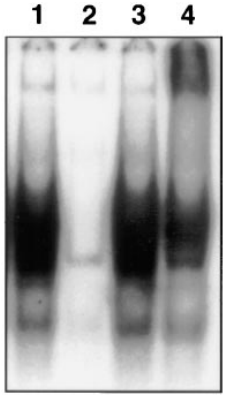

Figure 2. Specificity of NF-κB consensus oligonucleotide and p65 supershift assays. Binding reactions with nuclear extracts from whole lungs harvested 4 h after IgG immune complex deposition were incubated with ^{32}P-labeled NF-κB oligonucleotide (lane *1*). Specificity of the NF-κB oligonucleotide was confirmed by competition experiments with a 50-fold excess of unlabeled NF-κB oligonucleotide (lane *2*) or unlabeled AP-1 oligonucleotide (lane *3*). Supershift assays performed using an anti-p65 (RelA) polyclonal antibody identified p65 as a constituent of the NF-κB complex (lane *4*).

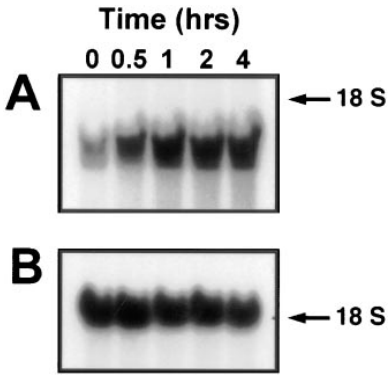

Figure 4. IκBα mRNA expression during IgG immune complex–induced lung inflammation. (*A*) RNA was extracted from whole lung homogenates after anti-BSA/BSA administration at the times indicated in the Northern blot analysis. (*B*) Equal loading of RNA was confirmed by probing the Northern blot for β-actin mRNA.

nucleotide (lane *1*). Competition with an excess (50-fold) of unlabeled NF-κB oligonucleotide completely abolished NF-κB binding to the labeled probe (lane *2*), whereas competition with excess (50-fold) of unlabeled AP-1 oligonucleotide showed no reduction of NF-κB binding (lane *3*). In addition, supershift assays with nuclear extracts from inflamed lungs identified p65 (RelA) to be a constituent of the NF-κB complex (lane *4*).

Changes in lung and alveolar macrophage content of IκBα. To determine whether activation of NF-κB might be a direct result of degradation of IκBα, protein expression in whole lung homogenates and alveolar macrophage lysates was measured by Western blot analysis. In whole lungs, IκBα protein expression showed no evidence of reduction from 0 to 1 h, but there was a decreased level after 2 h, and an even greater reduction 4 h after immune complex deposition (Fig. 3 *A*). In alveolar macrophages, IκBα protein expression showed a very measurable and reproducible decrease 30 min after intrapulmonary deposition of immune complexes (Fig. 3 *B*). These effects occurred despite greatly enhanced IκBα mRNA expression in whole lungs as determined by Northern blot analysis (Fig. 4 *A*). Pulmonary IκBα mRNA appeared to be constitutively expressed, since there was detectable mRNA for IκBα in lung extracts obtained at 0 h. Whole lung IκBα mRNA, however, was rapidly induced, with increases observed at 30 min, resulting in maximal expression after 2 h. This increase was maintained up to 4 h after immune complex deposition. Equal loading of RNA was confirmed by probing the Northern blot for β-actin mRNA (Fig. 4 *B*). Thus, during the course of the lung inflammatory response, even though IκBα mRNA was upregulated, there was loss of IκBα protein as assessed by Western blot analysis of lung homogenates.

Inhibition of NF-κB nuclear localization by IL-10 and IL-13. These studies revealed that, over the 4-h time course studied, IgG immune complex–induced NF-κB activation was maximal at 4 h (Fig. 1). Previous studies demonstrated that IL-10 and IL-13 profoundly suppress lung injury at this 4-h time point (10, 11). Therefore, we assessed whether the protective effects of IL-10 and IL-13 might be related to inhibition of NF-κB activation (translocation) in the lung. Both IL-10 and IL-13, when administered at the beginning of intrapulmonary IgG immune complex deposition, inhibited nuclear localization of NF-κB as assessed in whole lungs 4 h after immune complex deposition (Fig. 5 *A*). There appeared to be nearly

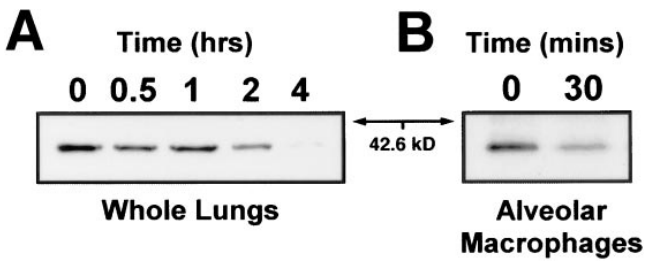

Figure 3. IκBα protein expression during IgG immune complex–induced lung inflammation. Whole lung homogenates and lysates of alveolar macrophages harvested by bronchoalveolar lavage were analyzed for IκBα protein by Western blot technique. (*A*) Time course of IκBα protein expression in whole lungs after IgG immune complex deposition. (*B*) IκBα protein expression in alveolar macrophages obtained 30 min after IgG immune complex deposition. Results are representative of three separate experiments.

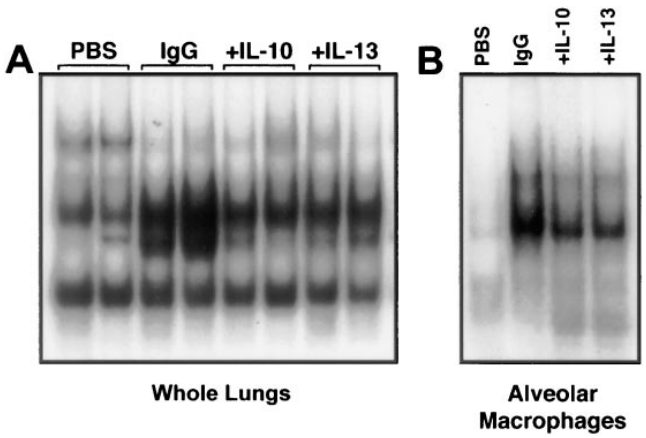

Figure 5. Effects of IL-10 and IL-13 on IgG immune complex–induced NF-κB activation. (*A*) NF-κB activation in whole lung tissues harvested 4 h after intratracheal administration of PBS or anti-BSA (IgG) followed by intravenous infusion of BSA. Some positive controls received 1.0 μg murine IL-10 (+*IL-10*) or IL-13 (+*IL-13*) with the anti-BSA. In each case, results from two separate samples are shown. (*B*) NF-κB activation in alveolar macrophages 30 min after intratracheal administration of PBS or anti-BSA in the absence (*IgG*) or presence of 1.0 μg murine IL-10 (+*IL-10*) or IL-13 (+*IL-13*).

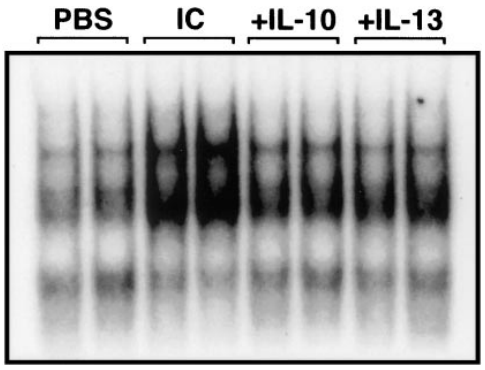

Figure 6. Effects of IL-10 and IL-13 on NF-κB activation in alveolar macrophages stimulated in vitro with IgG immune complexes. Cells were stimulated with media (*PBS*) or preformed IgG-BSA immune complexes in the absence (*IC*) or presence of 10 ng/ml murine IL-10 (+*IL-10*) or IL-13 (+*IL-13*).

complete inhibition in translocation of NF-κB. Similar effects were observed 2 h after initiation of lung injury (data not shown). These results may be associated with suppressive effects of IL-10 and IL-13 on alveolar macrophages, which serve as the source of TNFα (3). The coadministration of either IL-10

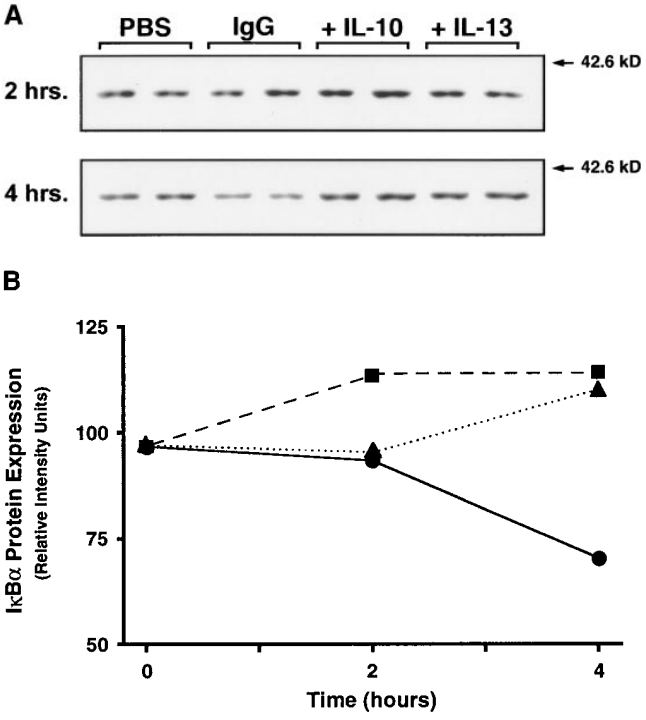

Figure 7. Effects of IL-10 and IL-13 on pulmonary IκBα protein expression during IgG immune complex–induced lung inflammation. (*A*) Western blot analysis of whole lung homogenates obtained 2 and 4 h after intratracheal administration of PBS or anti-BSA (*IgG*). As indicated, some positive controls also recieved 1.0 μg murine IL-10 (+*IL-10*) or IL-13 (+*IL-13*) with the anti-BSA. Results using two separate samples for each condition are shown. (*B*) Quantitation by image analysis of digitized enhanced chemiluminescence (●, IgG; ■, +IL-10; ▲, +IL-13).

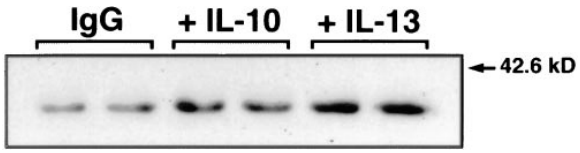

Figure 8. Effects of IL-10 and IL-13 on IκBα protein expression in activated alveolar macrophages obtained 30 min after IgG immune complex deposition. Anti-BSA (*IgG*) was administered intratracheally in the absence or presence of 1.0 μg murine IL-10 (+*IL-10*) or IL-13 (+*IL-13*).

or IL-13 resulted in nearly complete inhibition of NF-κB activation in alveolar macrophages retrieved 30 min after immune complex deposition (Fig. 5 *B*). Similar effects of IL-10 and IL-13 were observed on alveolar macrophages stimulated in vitro. In these studies, both IL-10 and IL-13 greatly reduced the nuclear localization of NF-κB induced by IgG immune complexes (Fig. 6).

Preservation of IκBα protein by IL-10 and IL-13. Since IL-10 and IL-13 inhibited NF-κB activation in alveolar macrophages and whole lungs (Figs. 5 and 6), we designed experiments to determine if the protective effects of IL-10 and IL-13 during lung inflammation might be related to effects on IκBα, the NF-κB regulatory protein. Western blot analysis of whole lung homogenates revealed relatively little change in levels of IκBα after 2 h (Fig. 7 *A*). Image analysis of digitized enhanced chemiluminescence films suggested that in the presence of IL-10 (but not IL-13), IκBα protein levels were elevated when compared to both positive and negative controls (Fig. 7 *B*). 4 h after deposition of immune complexes in the presence of either IL-10 or IL-13, however, IκBα protein levels were clearly higher than in lung homogenates of otherwise unmanipulated positive controls, and also appeared greater than that of negative controls. Expression of IκBα protein in alveolar macrophages obtained 30 min after initiation of lung injury was also preserved in the presence of either IL-10 or IL-13, in contrast to reduced levels of IκBα in macrophages from positive controls not treated with IL-10 or IL-13 (Fig. 8). Similar effects of IL-10 and IL-13 were observed on alveolar macrophages stimulated in vitro. Stimulation with IgG immune complexes greatly decreased the expression of IκBα in cytoplasmic extracts as compared to controls (Fig. 9). These effects were inhibited by IL-10 or IL-13.

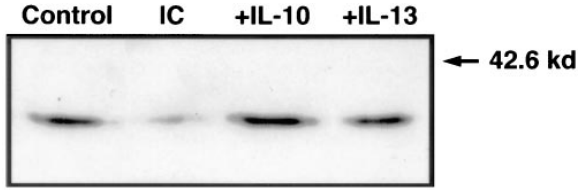

Figure 9. Effects of IL-10 and IL-13 on IκBα protein expression in alveolar macrophages stimulated in vitro with IgG immune complexes. Cells were stimulated with media (*Control*) or preformed IgG-BSA immune complexes in the absence (*IC*) or presence of 10 ng/ml murine IL-10 (+*IL-10*) or IL-13 (+*IL-13*). Cytoplasmic extracts were subjected to Western blot analysis of IκBα. Results are representative of two separate experiments.

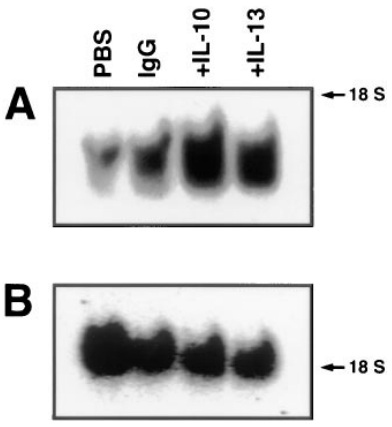

Figure 10. Effects of IL-10 and IL-13 on IgG immune complex–induced expression of IκBα mRNA. (*A*) Northern blot analysis of RNA extracts from whole lung tissues obtained 2 h after intratracheal administration of PBS or anti-BSA (*IgG*) in the absence or presence of 1.0 μg of murine IL-10 (*+IL-10*) or IL-13 (*+IL-13*). (*B*) Equal loading of RNA was confirmed by probing the Northern blot for β-actin mRNA. Results are representative of two separate experiments.

When 1 μg IL-10 or IL-13 was administered intratracheally in negative control rats, no change in IκBα protein levels in lung homogenates was found when compared to otherwise unmanipulated negative controls (data not shown).

Enhancement of IκBα mRNA by IL-10 and IL-13 during lung inflammation. To determine whether the preservation of IκBα protein expression by IL-10 or IL-13 might be related to enhanced IκBα gene activation, Northern blot analysis of IκBα mRNA in whole lung extracts was performed. Lung extracts from rats undergoing 2 h of injury were analyzed, since at this time point IκBα mRNA expression induced by IgG immune complexes appeared to be at a plateau (Fig. 4). Both IL-10 and IL-13 increased IκBα mRNA expression above that of IgG immune complexes alone (Fig. 10 *A*). Image analysis of digitized autoradiograms suggested 34 and 20% increases in mRNA in inflamed lungs in the presence of IL-10 or IL-13, respectively, when compared to IκBα mRNA in extracts from otherwise unmanipulated positive controls. In separate experiments, however, neither IL-10 nor IL-13 had any effect on IκBα mRNA levels in whole lung tissue in the absence of immune complexes (Fig. 11 *A*). Loading of RNA was assessed by probing the Northern blots for β-actin mRNA (Figs. 10 *B* and 11 *B*).

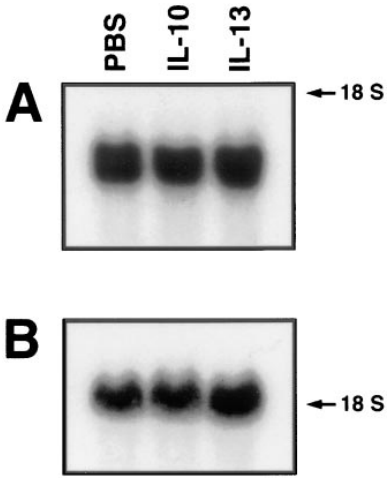

Figure 11. Effects of IL-10 and IL-13 on pulmonary expression of IκBα mRNA. (*A*) Northern blot analysis of RNA extracts from whole lung tissues obtained 2 h after the intratracheal administration of PBS alone, PBS and IL-10 (1.0 μg), or PBS and IL-13 (1.0 μg). (*B*) Equal loading of RNA was confirmed by probing the Northern blot for β-actin mRNA. Results are representative of two separate experiments.

Discussion

We have recently shown that exogenously administered IL-10 or IL-13 powerfully protects against IgG immune complex–induced lung injury in rats in a manner that is associated with nearly complete suppression (≥ 95%) of TNFα levels in BAL fluids (11). In the case of IL-10, this protection is correlated with complete suppression in upregulation of lung vascular ICAM-1 (10), which is an essential requirement for neutrophil recruitment (18). Since it has been demonstrated in vitro that IL-10 is a potent inhibitor of TNFα expression in activated human monocytes by suppressing NF-κB activation (13), these studies were carried out to determine if a similar mechanism might apply in vivo. Our data suggest that nuclear translocation of the transcription factor NF-κB is prerequisite for full development of lung inflammatory injury. The activation of NF-κB is thought to occur secondary to the proteolytic degradation of IκBα, allowing free NF-κB to translocate to the nucleus where it binds to specific promoter sequences and initiates gene transcription (19). In vitro, numerous IκB proteins have been identified, but IκBα has been shown to be the most functionally relevant inhibitor of NF-κB (20). Our findings support a role for IκBα in vivo, since activation of NF-κB during IgG immune complex–induced lung inflammation was accompanied by loss of IκBα in whole lungs (Figs. 1 and 3), presumably through proteolytic degradation.

Alveolar macrophages activated in vivo by IgG immune complex deposition respond by producing TNFα and IL-1β (3, 4). It is known that both of these cytokines are under the transcriptional control of NF-κB (21, 22). Our data show that IκBα degradation and NF-κB activation in alveolar macrophages occurs within 30 min after intrapulmonary deposition of immune complexes. The data implicate NF-κB activation as an in vivo requirement for production of the early response cytokines, TNFα and IL-1β, by lung macrophages. The fact that NF-κB activation in alveolar macrophages occurred before that in whole lungs underscores the importance of events in alveolar macrophages during this inflammatory reaction.

Both IL-10 and IL-13 protect against lung injury by dramatically reducing intrapulmonary production of TNFα (11). We now demonstrate that both IL-10 and IL-13 suppress NF-κB activation in alveolar macrophages and in whole lung tissues (Figs. 5 and 6). These data provide direct evidence that inhibition of pulmonary cytokine production by IL-10 and IL-13 may be due to direct effects on alveolar macrophages, causing suppression of NF-κB activation. Furthermore, these data suggest that the suppressive effects may be responsible for limiting the induction of lung inflammation, since both IL-10 and IL-13 potently inhibited NF-κB activation in whole lung tissues.

The abilities of IL-10 and IL-13 to suppress cytokine production in activated alveolar macrophages have been demonstrated in vitro (23–26). Furthermore, these inhibitory effects of IL-10 are mediated by suppression of NF-κB activation (13). The inhibitory mechanism(s) of IL-13 have not been described. We provide the first in vivo evidence that inhibition of NF-κB activation by IL-10 and IL-13 is due to preservation of IκBα protein expression in both alveolar macrophages and whole lung tissues (Figs. 7–9). In addition, IL-10 and IL-13

augmented IgG immune complex–induced IκBα mRNA, suggesting that these cytokines preserve IκBα protein by inducing gene transcription of IκBα. In the absence of lung deposition of IgG immune complexes, however, neither IL-10 nor IL-13 administered alone had any measurable effect on IκBα mRNA expression in lung. The augmented increases in IκBα mRNA by IL-10 and IL-13 during lung inflammation could also be due to mRNA stabilization, since both IL-10 and IL-13 have been shown to stabilize IL-1 receptor antagonist mRNA (27, 28).

It has been established in the IgG immune complex model of lung injury that IL-10 is an important endogenous regulator of inflammation (7). IL-13 may also function as an endogenous regulator (9). Both IL-10 and IL-13 mRNA and IL-10 protein are increased during the initial phases of this inflammatory response, and blockade of endogenous IL-10 results in increased pulmonary TNFα production, greater recruitment of neutrophils, and enhanced lung injury (7, 9). The assessment of endogenous IL-13 protein has been limited by the lack of availability of antibodies that effectively block the function of this cytokine in vivo. If increased IL-13 mRNA expression during lung inflammation relates to increased protein expression, however, this study suggests that the regulatory effects of endogenous IL-10 and IL-13 may be mediated through inhibition of NF-κB activation.

In summary, activation of the transcription factor NF-κB appears to play a central role in the pulmonary inflammatory response to IgG immune complexes. Exogenous IL-10 and IL-13 inhibit NF-κB activation in alveolar macrophages and whole lung tissues in association with preserved IκBα protein expression. Thus, these studies have identified a potential mechanism for the in vivo antiinflammatory effects of IL-10 and IL-13.

Acknowledgments

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