Selective Up-regulation of Cytokine-induced RANTES Gene Expression in Lung Epithelial Cells by Overexpression of IκBR*

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Prabir Ray‡, Liyan Yang, Dong-Hong Zhang, Samir K. Ghosh, and Anuradha Ray

From the Department of Internal Medicine, Pulmonary and Critical Care Section, Yale University School of Medicine, New Haven, Connecticut 06520

We previously reported the cloning of a cDNA for IκBR (for IκB-related) from human lung alveolar epithelial cells. IκBR belongs to the IκB family of proteins that function as regulators of the NF-κB family of transcription factors. Here, we investigated the consequence of IκBR overexpression on the expression of NF-κB-regulated chemokine genes in lung alveolar epithelial cells. Chemokines play an important role in many inflammatory diseases such as asthma and AIDS. Overexpression of IκBR in the lung cells resulted in a rapid 50–100-fold greater production of the RANTES (regulated upon activation, normal T expressed and presumably secreted) protein upon cytokine-induction compared with control cells. IκB overexpression, however, did not enhance interleukin-8 or MIP-1α gene expression, despite the fact that the expression of all three chemokine genes are regulated by NF-κB. The up-regulation of RANTES gene expression resulting from overexpression of IκBR correlated with increased amounts of a unique RANTES-κB binding activity and decreased binding of p50 homodimers. Previous studies have shown that p50 homodimers function as repressors of certain κB sites. Our studies suggest that IκBR can aid activation of select NF-κB-regulated genes by sequestering p50 homodimers.

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‡ To whom correspondence should be addressed: Dept. of Internal Medicine, Pulmonary and Critical Care Section, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520, Tel.: 203-785-3620; Fax: 203-785-3826; E-mail: Prabir.Ray@qm.yale.edu.

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Fax: 203-785-3826; E-mail: Prabir.Ray@qm.yale.edu.
up-regulate cytokine-induced expression of the RANTES gene in lung epithelial cells. Furthermore, this up-regulation of RANTES gene expression is associated with increased binding of a unique NF-κB complex to the RANTES κB region and decreased binding of p50 homodimers.

**EXPERIMENTAL PROCEDURES**

**Generation of Stable Transfectants**—The oligonucleotide GACTA-CAGGACGAGTACAAA encoding the FLAP octapeptide epitope (DYKDDDDK, IBI/Kodak) was inserted immediately downstream of the codon for amino acid 479 of IκBα using polymerase chain reaction techniques. The FLAP epitope-tagged IκBα cDNA was subcloned into pcDNA3 (Invitrogen) for expression under the control of the cytomegalovirus promoter in eukaryotic cells and for *in vitro* transcription with T7 polymerase. The cloning was confirmed by coupled *in vitro* transcription-translation reactions using T7 polymerase and the TNT rabbit reticulocyte lysate system (Promega). The *in vitro* translated product was immunoprecipitated with anti-FLAG monoclonal Ab (M2; IBI/Kodak) and analyzed by SDS-PAGE and autoradiography. This plasmid and the insert-free vector pcDNA3 were subsequently used to generate the stable transfectants A549/IκBα and A549/vector, respectively. Briefly, A549 cells (ATCC) were maintained in Dulbecco's modified Eagle's medium/Ham's F-12 medium (Life Technologies, Inc.) supplemented with 10% serum (Life Technologies, Inc.). Cells were transfected using the calcium-phosphate coprecipitation procedure (26), and transfected cells were selected by using 50 μg/ml of active G418 (Life Technologies, Inc.) in culture medium 48 h after transfection. G418-resistant clones were picked after 2 weeks, and pooled clones were used for all analyses.

**Transient Transfection Assays**—For transient transfection assays, cells were transfected by the calcium-phosphate coprecipitation procedure (26). The wt RANTES promoter-reporter (luciferase) plasmid contained promoter sequences between −897 (StuI) and +54 (KpnI) (derived from a −961/+54 construct obtained from A. M. Kremsky and co-workers (27)), and the mutant −897/+54 plasmid was constructed by swapping a fragment (−181/+54) containing mutations in the A and B NF-κB sites (28) with the corresponding wt fragment. 16 h after transfection, cells were washed, suspended in serum-free medium, and treated with recombinant hIL-1α (5 ng/ml) or hTNFa (50 ng/ml) and harvested 6 h later for assaying β-galactosidase and luciferase activity. Luciferase assays were performed using the Luciferase Assay System of Promega (Wisconsin), and the luciferase activity was measured in the LB 9501 luminometer of Berthold, Inc. (Germany). β-Galactosidase assay was performed using chlorophenol red β-D-galactoside as substrate.

Northern Blot Analysis and ELISAs—For Northern blot analyses, cells were either left untreated or treated with recombinant hIL-1α (5 ng/ml; R & D Systems) or recombinant hTNFa (50 ng/ml; R & D Systems) for the indicated times in serum-free medium. The culture supernatants were collected and clarified by centrifugation at 12,000 × g for 10 min, and the supernatants were stored at −70 °C until further use for protein estimation by ELISA. Total RNA was prepared from the cell pellets by treatment with Trizol (Life Technologies, Inc.) according to the instructions of the manufacturer. Total RNA (15 μg) from each sample was fractionated on a formaldehyde agarose gel, transferred to nylon membrane, and cross-linked to the membrane by UV light (Stratalinker; Stratagene). Prehybridization of the membranes was performed with QuikHyb (Stratagene) at 50 °C for 2 h. Fragments derived from the RANTES gene, the IL-8 gene, the IκBα gene, and glyceraldehyde 3-phosphate dehydrogenase gene were labeled with [α-32P]dCTP using a Random Primed DNA labeling kit (Boehringer Mannheim) and used as probes to detect expression of the corresponding mRNAs. Hybridizations with individual probes were carried out with ~3 × 106 cpm/ml of hybridization buffer (QuikHyb). The blots were washed twice for 15 min at room temperature in 2 × SSC/0.1% SDS and once for 15 min at 42 °C in 0.2 × SSC/0.1% SDS. The RANTES probe was a 410-base pair EcoRI/HindIII fragment of the RANTES cDNA (14), the IL-8 probe was a 250-base pair cDNA fragment (29), and the MIP1α probe was an EcoRI fragment of the MIP1α cDNA (30).

Preparation of Cell Extracts and EMSAs—For the preparation of nuclear extracts, cells were harvested and suspended in ice-cold phosphate-buffered saline. The cells were centrifuged by centrifugation and suspended in ice-cold buffer A (10 mM Hepes, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, and a mixture of protease inhibitors (Boehringer Mannheim); 100 μl per 2 × 106 cells). After incubation of ice for 10 min, the mixture was diluted with an equal volume of buffer A containing 0.4% Nonidet P-40 and immediately centrifuged at 800 × g for 1 min at 4 °C. The pelleted nuclei were washed in buffer A-Nonidet P-40 and suspended in 50 μl of buffer B (20 mM Hepes, pH 7.9, 420 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 25% glycerol, and a mixture of protease inhibitors). The nuclei were incubated with shaking for 15 min at 4 °C and centrifuged at 14,000 × g at 4 °C. The supernatants were aliquoted and quickly frozen on dry ice and stored at −70 °C until further use. The experiment was performed with different batches of nuclear extracts to ensure reproducibility of data. Binding reactions and gel electrophoresis (using 6% native polyacrylamide gels) were performed as described previously (1).

**Immunoprecipitation and Western Blotting**—Cells in 6-cm plates were washed and starved for 30 min in serum-free Dulbecco's modified Eagle's medium/Ham's F-12 medium without methionine or cysteine. Cells were labeled in 2 ml of the same medium with Express Label (NEN Life Science Products) containing [35S]methionine and cysteine at 100 μCi/ml for 3 h at 37 °C. Cells were washed, and 35S-labeled nuclear and cytoplasmic extracts were prepared. Equal amounts of counts from nuclear and cytoplasmic fractions were diluted 10-fold in buffer containing 50 μM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol, 0.1% Triton X-100, 0.5 mM EDTA, and a mixture of protease inhibitors (Boehringer Mannheim). The extracts were then precleared by incubation with fixed *Staphylococcus aureus* cells (Zosornia; Zymed) for 2 h at 4 °C. The mixtures were centrifuged, and immunoprecipitations were performed with the supernatants using anti-FLAG Ab M2 coupled Sepharose (IBI/Kodak) or with Sepharose coupled control IgG1 by overnight incubation. The beads were washed four times in the same buffer, suspended in 2 × SDS-PAGE sample buffer and analyzed by electrophoresis on 4–20% gradient gels (Bio-Rad). In the sequential immunoprecipitation-Western blot analyses, unlabeled nuclear and cytoplasmic extracts were subjected to immunoprecipitations with the anti-FLAG antibody, and the immunoprecipitates were separated by SDS-PAGE and transferred to polyvinylidene difluoride membrane. The membrane was probed with different anti-NF-κB antibodies, and antibody interactions were detected by chemiluminescence with horseradish peroxidase-conjugated secondary antibody and ECL reagent (Amersham Corp.).

**RESULTS AND DISCUSSION**

To address the effect of IκBα on NF-κB-mediated chemokine gene regulation in lung epithelial cells, we established A549 clones stably expressing epitope-tagged IκBα, named A549/IκBα. IκBα was epitope-tagged to distinguish the protein expressed from the expression vector from other endogenous IκBα proteins. A pool of stable transfectants were incubated with IL-1α or TNFa for various lengths of time and analyzed for expression of different chemokine mRNAs by Northern blotting techniques. In control A549/vector cells, TNFa induced a very low level of RANTES mRNA expression at 24 h post-stimulation, and the level of induction with IL-1α was barely detectable even after 24 h (Fig. 1). In contrast, in A549/IκBα cells, both TNFa and IL-1α caused an up-regulation of RANTES gene expression as early as 2 h post-stimulation, which continued to increase even at 24 h after stimulation. We then tested whether IκBα overexpression specifically up-regulated RANTES gene expression or the expression of other NF-κB-regulated genes such as IL-8 (31–35) and MIP-1α (macrophage inflammatory protein-1α) (36, 37) as well. As illustrated in Fig. 1A, IL-8 gene expression induced by either IL-1α or TNFa was partly inhibited in A549/IκBα cells. We also studied the effect of IκBα overexpression on the expression of MIP1α, whose induced expression is barely detectable in A549 cells. Overexpression of IκBα failed to up-regulate IL-1α- or TNFa-induced MIP1α expression in these cells (data not shown). We tested the culture supernatants for the presence of RANTES and IL-8 proteins. As shown in Fig. 1B, the protein data were in good agreement with the Northern analyses. As 1 h post-stimulation with IL-1α or TNFa, A549/IκBα cells produced 50- to 100-fold more RANTES than the control cells. The results of these experiments showed that IκBα preferentially up-regulated RANTES gene expression in A549 cells.

To determine whether the IκBα-mediated up-regulation of cytokine-induced RANTES mRNA expression was associated with activation of the RANTES promoter, A549/vector and
A549/IkB cells were transiently transfected with a reporter gene (luciferase) construct containing ~900 nucleotides from the 5'-flanking region immediately upstream of the transcription start site of the RANTES gene (27, 28). The 5'-flanking region of the RANTES gene contains two NF-κB-binding sites in tandem, termed A and B (see Fig. 3 and Refs. 27 and 28). In A549/vector cells, the RANTES promoter was activated by IL-1α or TNFα between 1.5–2-fold (Fig. 2). In contrast, in A549/IkB cells, IL-1α and TNFα activated the RANTES promoter between 7–8- and 8–10-fold, respectively (Fig. 2). Mutations in the A and B sites in the context of the 900-base pair promoter abolished reporter gene activity in both cell types. The difference between the level of RANTES mRNA expression and the fold induction of the RANTES promoter in cytokine-induced A549/IkB cells can be attributed to the stability of RANTES mRNA. RANTES mRNA lacks the destabilizing AUUUA sequence in its 3'-untranslated region (14). This probably explains why RANTES mRNA induced by TNFα or IFN-γ was reduced by only 30% after incubation for 4 h in the presence of actinomycin D (25). IL-8, on the other hand, has a half-life of less than 1 h in epithelial cells (38). Because mRNA destabilization appears to play an insignificant role in the regulation of RANTES production, it stands to reason that an up-regulation of RANTES promoter activity by IL-1 or TNF in A549/IkB cells would cause a significant difference in steady-state levels of RANTES mRNA and protein levels between the IkB-overexpressing cells and the control cells.

We next examined the possibility that the IkB-mediated up-regulation of RANTES promoter activity was associated with altered binding of protein(s) to the RANTES κB region. Toward this end, EMSAs were performed with nuclear fractions of cytokine-induced A549/IkB cells and A549/vector cells and a radiolabeled 32-base pair double stranded oligonucleotide containing the two RANTES NF-κB sites as the probe. Although IL-1α and TNFα caused induction of NF-κB DNA binding activity in both cell types, the DNA-protein complexes formed with the activated proteins were not identical with nuclear extracts prepared from the two cell types (Fig. 3). As illustrated in Fig. 3, the binding intensity of complex I was equivalent in the two cell types. The intensity of complex II was 2–3-fold greater with nuclear extracts of A549/vector cells compared with that observed with A549/IkB nuclear extracts. However, the intensity of complex III was at least 10–15-fold (as judged by densitometric scanning) greater with nuclear extracts of A549/IkB cells compared with that observed with extracts of control cells (Fig. 3, lower panel). Two additional minor complexes migrating more slowly than complex I were also obtained with extracts of both cell types. All of these complexes were specific because an excess of the same unlabeled oligonucleotide efficiently competed for formation of the complexes, whereas an oligonucleotide containing mutations in the κB sites failed to compete (Fig. 3, lanes 6 and 7, respectively). Because different members of the NF-κB-Rel family could potentially interact with the κB motifs, we used specific antibodies to determine the composition of the different polypeptide complexes. Formation of complex I was affected with both anti-p50 and anti-p65 antibodies, suggesting that it contained the classic p50-p65 heterodimer (lanes 8 and 9). Complex II and complex III formation were both affected only by the anti-p50 antibody (lane 8). The complex migrating immediately after (slower than) complex I was affected only by the anti-p65 antibody (lane 9). This complex may contain a p65 homodimer. None of the complexes were inhibited/supershifted by the antibodies against c-Rel, p52, and RelB (lanes 10–12). Also, IkB itself did not appear to be present in any of the complexes because a monoclonal antibody against the FLAG epitope (Ab M2; IBI/Kodak) had no effect on the complexes (lane 13).

The ability of the anti-p50 antibody to affect both complex II

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and complex III formation and the selective augmentation of complex III formation with the A549/IκB cells. Nuclear extracts prompted us to further analyze the nature of both II and III. Toward this end, we first tested whether recombinant p50 (Promega) could bind to the RANTES xB region. The recombinant p50 was derived from a full-length cDNA encoding 453 amino acids (39) that was identical to the human p50 cDNA described previously (40). As shown in Fig. 4A, p50 homodimers bound to the RANTES xB region. As expected, the (p50)$_2$-DNA complex could be supershifted with the anti-p50 antibody but not with the anti-p65 (used as control) antibody (Fig. 4A). Also, in accordance with previous findings (31, 34), recombinant p50 did not interact with the IL-8 xB site (Fig. 4A). Interestingly, the (p50)$_2$-RANTES xB complex comigrated with complex II (compare lanes 1, 4, and 5 in Fig. 4A).

Because both complexes II and III could not contain (p50)$_2$ and the data in Fig. 4A suggested that complex II contained (p50)$_2$, we further investigated the composition of the faster migrating complex III with two different anti-p50 antibodies. Although the first antibody (114x; Santa Cruz Biotech) recognizes sequences mapping within the nuclear localization signal (NLS) region of p50, the second antisemurum (119x; Santa Cruz Biotech) was raised against the NH$_2$-terminal of p50. Both antibodies supershifted the RANTES DNA-(p50)$_2$ complex (Fig. 4B). However, the anti-NLS antibody but not the anti-NH$_2$-terminal antibody supershifted complex III (Fig. 4B). The latter antibody did not inhibit complex I (p50-p65) formation either (Fig. 4B). This was consistent with previous reports showing weaker effects of some p50-specific antisera on p50 heterodimers than on p50 homodimers (32, 41). These results demonstrated that complex III did not consist of p50 homodimers.

Because our Northern analyses and ELISAs showed that IκB overexpression actually slightly inhibited IL-8 gene expression, we tested the ability of the IL-8 xB site to compete in the EMSA. At a 100-fold molar excess, the wt IL-8 xB sequence did compete for formation of complex III, albeit incompletely (Fig. 4C, lane 4). The binding of the p50-p65 heterodimer (complex I) was only partially competed for by the wt IL-8 xB sequence (Fig. 4C, lane 4). This was in accordance with previous reports showing the preferential binding of p65-c-rel heterodimers rather than p50-p65 heterodimers to the IL-8 xB site (31, 34, 35), and as shown previously (31, 34), the binding of the p50 homodimers (complex II) was not competed out by the wt IL-8 xB sequence. The formation of complex III with the RANTES probe could not be competed out with 200-fold excess of oligonucleotides containing consensus binding sequences for a few additional families of transcription factors, C/EBP, Oct, CREB/ATF, AP-1, and AP-2 (data not shown). Also, the mobility of complex III is far lower than what might be expected of an NF-κB-HMG-I/Y dimeric complex (42). Taken together, it appears that complex I is a heterodimer of p50-p65 and complex II contains p50 homodimers. Complex III may contain a homodimer of a p50-related protein or more likely a heterodimer containing p50 and an as yet unidentified Rel protein, a splice variant of a Rel protein, or a non-Rel protein. It is unlikely that this complex contains any of the known splice variants of p65.
The samples were analyzed in parallel with binding reactions carried out with nuclear extracts from control and A549/IκB cells. Nuclear extracts of A549/IκB cells were incubated with the wt RANTES probe with or without (lane 1) a 100-fold molar excess of oligonucleotides containing a wt (lane 2) or a mutated (lane 3) IL-8 κB site. Binding reactions were performed with A549/IκB nuclear extracts and the wt (lane 1) or mutant RANTES probes containing either a mutant A site and a wt B site (mAmB; lane 2) or a wt A site and a mutant B site (wtAmB; lane 3). Binding reactions were analyzed by electrophoresis on 6% native polyacrylamide gels (1).

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Fig. 4. The IκB-augmented DNA binding activity is not (p50), but may be a heterodimer containing p50. EMSAs were carried out with either recombinant p50 (rp50; Promega) or nuclear extracts (n.e.) of A549/IκB cells. A, [35S]labeled RANTES oligonucleotide was incubated with rp50 in the presence (lanes 2 and 3) or the absence of the anti-NLS anti-p50 Ab or an anti-p65 Ab (372x; Santa Cruz Biotech., used as a control). The samples were analyzed in parallel with binding reactions carried out with nuclear extracts from control and A549/IκB cells and the RANTES probe (lanes 4 and 5, respectively). The binding of rp50 to the IL-8 κB site was also examined in the same experiment. B, the ability of the anti-NLS Ab and the anti-NH₂-terminal anti-p50 Ab (1191x; Santa Cruz Biotech.) to supershift/inhibit binding of rp50 or A549/IκB nuclear proteins to the RANTES probe was investigated. C, nuclear extracts of A549/IκB cells were incubated with the wt RANTES probe with or without (lane 1) a 100-fold molar excess of oligonucleotides containing a wt (lane 2) or a mutated (lane 3) IL-8 κB site. D, binding reactions were performed with A549/IκB nuclear extracts and the wt (lane 1) or mutant RANTES probes containing either a mutant A site and a wt B site (mAmB; lane 2) or a wt A site and a mutant B site (wtAmB; lane 3). Binding reactions were analyzed by electrophoresis on 6% native polyacrylamide gels (1).

(p65Δ, p65Δ2, or p65Δ3), all of which are larger in size than p50. A TNFα-inducible complex of similar mobility (faster than p50 homodimers) and with reactivity only to anti-p50 antiserum but not to any other Rel-specific antisera was previously detected in primary murine embryonal fibroblasts (41).

Because the RANTES κB site contains two NF-κB sites (A and B) (27, 28), we also examined the involvement of the individual sites for formation of the different complexes using two mutant probes. One of these mutants contained a mutant A site and a wt B site (mAmB), whereas the other contained a wt A site and a mutant B site (wtAmB). As shown in Fig. 4D, both probes formed complex III, although the binding to wtAmB was slightly better. Also, whereas the intensity of complex III appeared to be the same with either probe, it w as less than that obtained with the complete wt probe (Fig. 4D, compare lanes 2 and 3 with lane 1). Complex II containing (p50)₂, however, was only formed with the mAmB probe (lane 2), whereas the p65 homodimeric complex was formed only with the wtAmB probe (lane 3). Thus, both A and B sites need to be intact for stabilization of complex III. Collectively, the EMSAs showed that overexpression of IκB decreases formation of complex II but augments formation of complex III.

To determine whether the observed decrease in binding of (p50), to the RANTES probe with nuclear extracts prepared from A549/IκB cells stemmed from the ability of IκB to interact with p50 in vivo, we investigated the subcellular distribution of IκB in A549 cells. Untreated A549/IκB cells expressing FLAG epitope-tagged IκB protein were metabolically labeled with [35S]methionine, and the cell lysates were fractionated into cytoplasmic and nuclear fractions. The fractions were subjected to immunoprecipitation with the anti-FLAG monoclonal Ab M2 (IBI/Kodak). Two specific bands were detected on the SDS gel resulting from immunoprecipitations of the cytoplasmic fraction. The identity of the upper band was confirmed to be IκB in several Western blot experiments using a polyclonal antisemur against IκB (1). The identity of the upper band was confirmed to be IκB in several Western blot experiments using a polyclonal antisemur against IκB (1). No specific bands were immunoprecipitated from the nuclear fraction of the A549/IκB cells. These results showed that IκB is not (p50) but may be a heterodimer containing p50.
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(43). This probably explains why complex III can only be detected in IkBR-overexpressing cells; by sequestering (p50)2, IkBR causes a shift in the stoichiometry of complex III to complex II from ~1:2 in control cells to ~10:1 in IkBR-overexpressing cells (see Figs. 3 and 4A). In control cells, occupancy of the B site in the RANTES NF-κB region by (p50)2 can be expected to exert a negative regulatory influence on RANTES gene expression because (p50)2 has been shown to act as a dominant repressor of transcription from certain NF-κB binding sites (9). This model is particularly attractive in intact cells where the various NF-κB dimers are in competition to bind limiting amounts of target DNA unlike in EMSAs where a large excess of the probe is present. A similar mechanism, involving a switch from inhibitory p50 homodimers to p50-p65 activators, was proposed in the induction of IL-2 gene expression in activated CD4+ T cells (46). In addition to less competition from inhibitory (p50)2, synergy between complex III and complex I can also be envisaged as a component in the up-regulation of RANTES gene expression in A549/IκBα cells. In our previous studies we showed that IkBα added in vitro to nuclear extracts inhibited the DNA binding activity of p50-p65 heterodimers in EMSAs. In these studies, nuclear extracts prepared from control cells and IkBα-overexpressing cells displayed similar binding of the p50-p65 heterodimer to the RANTES B site region. The difference in the two results can be best explained by the fact that the p50-p65 heterodimer in vitro is normally sequestered by IkBα and IkBβ via the p65 subunit of the complex and therefore unavailable for interaction with excess IkBα in A549/IκBα cells.

Although IkBR overexpression resulted in an up-regulation of RANTES gene expression, it did not stimulate IL-8 gene expression, which is also regulated by NF-κB. It is important to note in this regard that the p50 homodimer does not bind the IL-8 B site as this study and previous studies show (31, 34), and therefore the IL-8 promoter should not be subjected to repression by this dimer. Furthermore, the expression of the IL-8 gene has been shown to require not only the NF-κB site in the promoter but also the adjacent C/EBP site. It is likely that the synergism between the C/EBP site-binding protein C/EBPβ (NF-IL6) and the p65-c-Rel heterodimer, which is the major IL-8 κB binding activity (31, 34), is greater than that between C/EBPβ and the proteins constituting complex III, and therefore the increased availability of the latter does not up-regulate IL-8 gene expression.

The NF-κB region of the RANTES promoter is important for its induction in activated T lymphocytes (28). Our experiments indicate that this region is also critical for RANTES promoter activation in lung alveolar epithelial cells. However, T cells express the RANTES gene only around 3–5 days after activation (28) in contrast to detectable expression in lung epithelial cells ~20–24 h after stimulation that can be shifted to as early as 2 h post-stimulation by overexpression of IkBR. It appears that an up-regulation of RANTES gene expression in lung epithelial cells involves the formation of a unique NF-κB-Rel complex (complex III) with the RANTES κB site. Our data suggest that overexpression of IkBR sequesters (p50)2, which may facilitate the binding of the unique complex to the RANTES κB region. This complex was not detected in EMSAs with nuclear extracts of activated T cells (28). Therefore, the expression of the RANTES gene might follow different kinetics through the involvement of different DNA-binding proteins. In inflammatory disease conditions such as asthma and endotoxemia, where an up-regulation of RANTES gene expression has been reported in lung epithelial cells (20–22, 24), the expression of IkBR and formation of complex III need to be investigated in the disease processes. The present system will allow us to further characterize complex III, which might provide a tool for the specific modulation of RANTES gene expression in different diseases.

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