Transcriptional activation of the chicken lysozyme gene by NF-κBp65 (RelA) and c-Rel, but not by NF-κBp50

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The lysozyme gene is expressed at a low level in myeloblasts and is progressively activated to constitutively high expression in mature macrophages. The binding activity of the newly defined NF-κB/Rel family of transcription factors increases during the terminal differentiation of macrophages. In this study, I show that NF-κB/Rel-like proteins bind to the nuclear factor kappa B (κB)-like sequence of the lysozyme promoter. These binding activities were induced by treatment of HD11 cells with lipopolysaccharide. Immunomobility shift assays show that c-Rel is possibly a factor in the complexes that bind to the κB-like sequence lysκB. Binding activity to one of the protein complexes seems to be regulated by phosphorylation. In fact, overexpression of p65 and c-Rel stimulates expression of the chloramphenicol acetyltransferase gene controlled by the lysozyme promoter. Furthermore, co-transfection experiments reveal that the κB-like sequence within the lysozyme promoter mediates the transactivation by p65 and c-Rel. These results indicate that the p65 and c-Rel could be components of the protein complexes that bind to the κB-like sequence and this binding could contribute to the progressively activated expression of the lysozyme gene during the terminal differentiation of macrophages.

INTRODUCTION

The chicken lysozyme gene offers an attractive model for studying the cell- and stage-specific expression of eukaryotic genes. In tubular gland cells of the chicken oviduct, the expression of the lysozyme gene is activated by steroid hormones [1]. The chicken lysozyme gene is also a marker gene for the myeloid lineage of haematopoietic differentiation. In mature macrophages, as part of the bacteriocidal system of the organism, the gene is expressed constitutively [2] and is not influenced by steroid hormones. It is progressively activated during macrophage differentiation: expression is low in myeloblasts and is high during the terminal development from immature to mature macrophages [3,4].

The actively transcribed gene is embedded in a chromatin domain of approx. 19 kb that displays elevated nuclease sensitivity [5]. The 5′ and 3′ borders of this chromatin domain coincide with the matrix attachment regions [6–9]. This domain encompasses several regulatory elements involved in the tissue-specific and developmentally regulated expression of the gene [10–14].

The newly defined nuclear factor kappa B (NF-κB)/Rel family of transcription factors includes p50 (NFKB1), p52 (NFKB2), p65 (RelA), RelB and c-Rel. Members of this family contain at their N-terminal region a Rel homology domain that is required for DNA binding and dimerization [15]. NF-κB was originally found in mature B lymphocytes and is crucial for expression of the kappa light chain immunoglobulin gene [16]. Recent studies indicate that different NF-κB/Rel complexes bind selectively to different κB sites, and play an important role for specific and selective control of gene expression [15,17–21].

During the terminal differentiation of macrophages, NF-κB binding is progressively activated with low binding activity in immature monocytes/macrophages and with full activity in mature macrophages [22].

The chicken lysozyme gene promoter contains a κB-like sequence (lysκB) that does not match the NF-κB binding site consensus 5′-GGGR(C,A,T)TYYCC-3′ [23] because of a thymidine at position 3. In this study I show that the p65 subunit of NF-κB or c-Rel induces expression from the lysozyme promoter, and NF-κB/Rel-like protein complexes bind to the lysκB within the lysozyme promoter region. The role of the lysκB and NF-κB/Rel-like proteins in the activated expression of the lysozyme gene by external stimuli and during the terminal differentiation of macrophages is discussed.

MATERIALS AND METHODS

Plasmid construction

Plasmid constructions were performed by standard procedures [24]. pcPCAT was constructed by insertion of the *Sau*3A fragment from pLYSCAT2100 [10] containing the chicken lysozyme promoter region (−579/+14) into the *Bgl*II site of the promoterless parent plasmid pBLCAT5. p4lysκBCAT and p4mlysκBCAT were constructed by cloning four tandem copies of the double-stranded oligonucleotide lysκB or mlysκB (see oligonucleotides) containing 5′-overhang sequences for *Hin*dIII and *Bam*HI restriction sites into the *Hin*dIII and *Bam*HI restriction sites upstream of the corticotropin-releasing hormone (CRH) promoter of the pCRH, respectively [25]. Plasmids pRcCMV-p50 and pRcCMV-p65 containing the coding sequences for the p50 [26] and p65 subunit [27] of the human NF-κB under control of the cytomegalovirus (CMV) promoter were gifts from Dr. P. A. Baeuerle (Albert-Ludwigs-University, Freiburg, Germany). The c-Rel cDNA [28], kindly provided by Dr. A. J. Capobianco

Abbreviations used: CAT, chloramphenicol acetyltransferase; CRH, corticotropin-releasing hormone; CMV, cytomegalovirus; EMSA, electrophoretic mobility shift assay; LPS, lipopolysaccharide; NF-kB, nuclear factor kappa B; RSV, Rous sarcoma virus; SV, simian virus.

(Boston University, Boston, MA, U.S.A.), was cloned downstream of the Rous sarcoma virus (RSV) promoter to generate pRSV-c-Rel. pSV-Gal from Promega (Heidelberg, Germany) was used as an internal control for transfection efficiency.

All plasmids were amplified in DH5 bacteria. Plasmid preparations were performed by two cycles of CsCl equilibrium gradient centrifugation.

Oligonucleotides

Synthetic oligonucleotides were purchased from Pharmacia Biotech (Freiburg, Germany).

The κB motif oligonucleotide corresponds to the sequence −92 to −79 of the LTR of the human immunodeficiency virus HIV-1 [29], 5′-TGGGGACTTTCCAG-3′. The lysκB oligonucleotide contains the sequence −167 to −155 of the chicken lysozyme promoter [30], 5′-TGTGGTACTTCCC-3′. The mlysκB oligonucleotide contains point mutations at positions −162, −163, and −164 (underlined), 5′-TGTCTCTACTTCCC-3′.

Single-stranded oligonucleotides were annealed and double-stranded oligonucleotides were isolated by using non-denaturing polyacrylamide gels in 1 × TBE (89 mM Tris buffer, pH 8.3, 89 mM boric acid and 2 mM EDTA).

Cell culture and DNA transfections

HD11 cells of an established chicken myelomonocytic line transformed by the *myc*-encoding MC29 virus [31] were grown in Iscove's modified Dulbecco's medium supplemented with 8% fetal calf serum, 2% chicken serum, 100 units/ml penicilin, and 100 μg/ml streptomycin at 37 °C and 5% CO_2. DNA transfections were performed by the calcium phosphate co-precipitation method from Graham and van der Eb [32] as modified by Schöler and Gruss [33]. Briefly, HD11 cells were transiently transfected, usually with 2.5 μg of reporter plasmid and when indicated with 5 μg of expression vectors per 9 cm tissue culture plate; 4 h after transfection, cells were shocked with 3 ml of 15% (v/v) glycerol, 140 mM NaCl, 0.75 mM Na_2HPO_4, 25 mM Hepes, pH 7.13, for 2.5 min, and then supplemented with fresh medium and incubated, and when indicated with 5 μg/ml lipopolysaccharide (LPS) for 24 h at 37 °C before being harvested for preparation of protein extracts and determination of chloramphenicol acetyltransferase (CAT) activity and β-galactosidase activity. Protein concentrations of the cell extracts were determined by the method of Bradford (Bio-Rad Laboratories, Munich, Germany).

CAT and β-galactosidase assays

Cell extracts and CAT assays were performed as described previously [7], with 200 μg of total protein. After autoradiographic exposure, the unmodified chloramphenicol and its acetylated derivative were excised from the TLC plate and counted for radioactivity in a liquid scintillation counter. β-Galactosidase assays were carried out by a standard method [24]. Results were normalized for each extract to provide relative CAT activity. The values shown are representative of three independent transfection experiments with a deviation of less than 20% and were corrected according their β-galactosidase values.

Nuclear extract preparation and electrophoretic mobility shift assay (EMSA)

Nuclear extracts from HD11 cells were prepared by a rapid method described elsewhere [34]. Double-stranded oligonucleotides were usually end-labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. Band shift assays were performed in a total reaction volume of 20 μl containing 0.5 μg of poly(dIdC), 5 μg of nuclear extract, 10 mM HEPES (pH 7.9), 60 mM KCl, 1 mM EDTA, 4% (w/v) Ficoll 400, 1 mM dithiothreitol and 10000–20000 c.p.m. of the radiolabelled double-stranded oligonucleotide. Nuclear extracts were preincubated with poly(dIdC) at 4 °C for 15 min, and, when indicated, with specific competitors before addition of the labelled probe. After 30 min incubation at 4 °C the samples were directly loaded on a 4% (w/v) non-denaturing polyacrylamide gel in 0.25 × TBE. The gel was run for 1 h at 150 V at room temperature, fixed in 10% (v/v) acetic acid, dried and autoradiographed at −80 °C. For immunomobility shift assays, the nuclear extract was incubated with the radiolabelled oligonucleotide for 15 min at 4 °C. Diluted antiserum was then added and the incubation proceeded for an additional 15 min before the sample was loaded on a gel.

Phosphatase treatment of nuclear extracts

Nuclear extracts of DH11 cells were treated with potato acid phosphatase by the method of Williams and Maizels [35].

RESULTS

The κB-like sequence of the chicken lysozyme promoter is a binding site for NF-κB/Rel-like protein complexes

The chicken lysozyme promoter region contains a κB-like sequence (lysκB) located between −164 and −155 relative to the transcription start site. To examine whether nuclear factors could bind to this site, EMSA was performed with nuclear extracts from unstimulated and LPS-stimulated HD11 cells. The radiolabelled probe was a 21 bp oligonucleotide containing the lysκB of the lysozyme promoter. As shown in Figure 1, nuclear extract from unstimulated HD11 cells contained at least three protein complexes binding to the lysκB. These binding activities

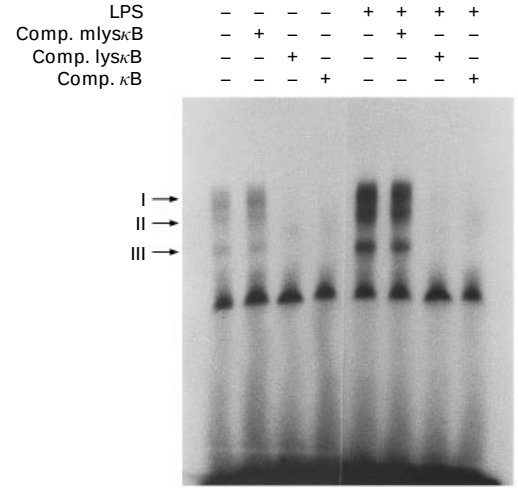

Figure 1 The lysκB within the lysozyme promoter is a binding site of NF-κB/Rel-like protein complexes in HD11 cells

Nuclear extracts were prepared from untreated HD11 cells and from HD11 cells treated with 5 μg/ml LPS from *Salmonella typhimurium* for 5 h; each nuclear extract (5 μg) was analysed by EMSA with the lysκB (20000 c.p.m.) radiolabelled by using T4 polynucleotide kinase and [γ-^{32}P]ATP, and in the presence of 0.5 μg of poly(dIdC) and a 100-fold molar excess of unlabelled competitor oligonucleotides (Comp.). DNA–protein complexes were resolved on a 4% (w/v) polyacrylamide gel. Arrows denote specific complexes competed for by unlabelled lysκB and κB.

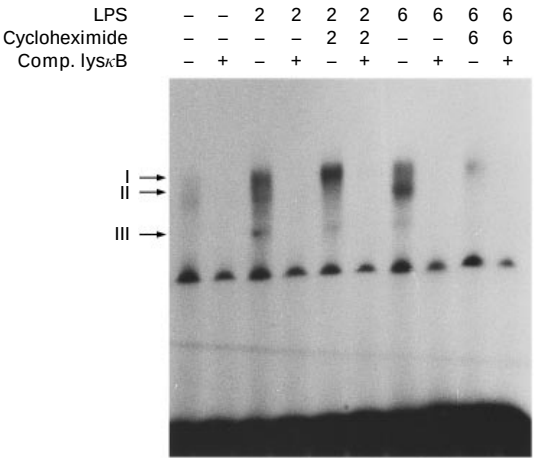

Figure 2 Effect of cycloheximide on binding activities of NF-κB/Rel-like proteins to the lysκB

Nuclear extracts were prepared from untreated HD11 cells and from HD11 cells treated with 5 μg/ml LPS, with or without 70 μM cycloheximide for the indicated times in hours. EMSA was performed with 5 μg of each nuclear extract and 20000 c.p.m. of the radiolabelled oligonucleotide containing the lysκB sequence, in the presence of 0.5 μg of poly(dIdC).

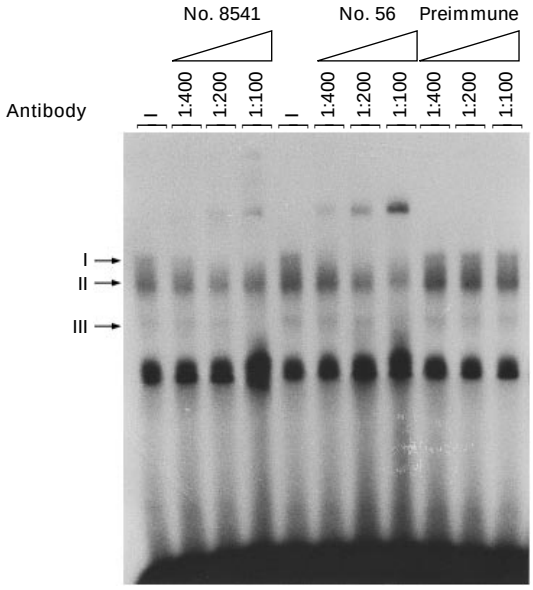

Figure 3 c-Rel is a component of the protein complexes binding to the lysκB

Immunomobility shift assays were performed with the radiolabelled lysκB (20000 c.p.m.) and 5 μg of nuclear extract prepared from HD11 cells activated with 5 μg/ml LPS for 5 h, in the presence of diluted antisera raised against either a recombinant v-Rel protein (no. 8541) or a synthetic peptide of the v-Rel (no. 56) or of a pre-immune serum.

were enhanced in HD11 cells treated with 5 μg/ml LPS from *Salmonella typhimurium* for 5 h. To demonstrate the sequence specificity of DNA-protein complexes and to show that protein complexes that bind to lysκB may be NF-κB/Rel-like proteins, competition assays were performed in the presence of unlabelled lysκB or κB oligonucleotide derived from the κB motif of HIV-1 or mlysκB containing three point mutations in the lysκB. Binding activities to the lysκB from unstimulated HD11 cells or HD11 cells treated with LPS were completely competed for, not only by lysκB, but also effectively by κB. In contrast, mlysκB was unable to inhibit the formation of the DNA–protein complexes even at a 200-fold molar excess. The faster-migrating band represented binding of a non-specific protein complex that was not inhibited by unlabelled lysκB or κB, and was not stimulated by LPS.

Effect of cycloheximide on binding activities of the complexes binding to the lysκB

It has been shown that members of the NF-κB/Rel family are present in an inactive form in the cytoplasm and can be induced to become active and shuttled into the nucleus [16,36]. To determine whether LPS-enhanced binding activities to the lysκB are activated from inactive complexes, HD11 cells were treated with LPS for 2 and 6 h in the presence or absence of cycloheximide, an inhibitor of protein synthesis. As shown in Figure 2, three complexes were present in nuclear extract of HD11 cells. Interestingly, whereas complex II was continuously and progressively activated by LPS, complexes I and III were rapidly enhanced and reached a maximum after 2 h treatment with LPS. Treatment of HD11 cells with cycloheximide, however, increased binding activity to complex I at 2 h and decreased binding activity to complex III at both 2 and 6 h. In contrast, binding activity to complex II in nuclear extract from LPS-activated HD11 cells treated with cycloheximide was reduced to a level comparable to the binding activity in untreated cells. Taken together, these results indicate that complex I may be activated by LPS from an inactive pre-existing complex independent of protein synthesis, and binding activity to complex II may be enhanced by LPS via protein synthesis.

c-Rel is a component of the protein complexes that bind to the lysκB

Next I tried to determine directly the protein composition of protein complexes that bind to the lysκB. To investigate whether c-Rel could be a component of complexes binding to the lysκB, immunomobility shift assays were performed with specific antisera raised against recombinant v-Rel or against synthetic peptide in v-Rel (gifts from Dr. N. Rice). As shown in Figure 3, antisera no. 8541 and no. 56 to v-Rel caused new complexes with lower mobility to form and clearly reduced the binding to complex II (and possibly III) as well as complex I. This indicates that the antisera reacted specifically with the DNA–protein complexes, because no effects were observed with a pre-immune serum. This result suggests that the three complexes binding to the lysκB contain Rel-homologous proteins, possibly c-Rel.

Binding activity to complex I is inhibited by treatment with acid phosphatase

To determine whether binding activity to the lysκB can depend on phosphorylation, binding assays were performed in which the ^{32}P-labelled synthetic oligonucleotide lysκB was incubated with nuclear extracts that had been briefly treated with potato acid phosphatase. Figure 4 shows that binding activity to complex I in untreated and LPS-treated DH11 cells was reduced by treatment with phosphatase, whereas binding activity to complex III, and possibly to complex II, was not affected by phosphatase treatment. Thus the binding activity to complex I seems to be

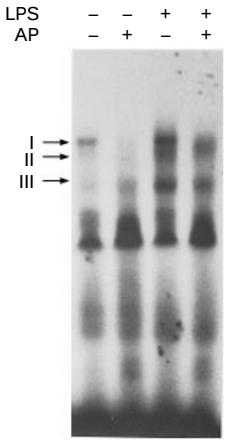

Figure 4 Acid phosphatase treatment of nuclear extracts

Nuclear extracts were prepared from untreated HD11 cells and from HD11 cells activated with 5 μg/ml LPS for 5 h and subsequently briefly treated with potato acid phosphatase (AP) (60 m-units per 5 μg of nuclear extract) for 10 min at room temperature. EMSA was performed with the lysκB (20000 c.p.m.) labelled by using [α-^{32}P]dCTP and the Klenow fragment of the DNA polymerase I. DNA–protein complexes were resolved on a 4% (w/v) polyacrylamide gel. —, No acid phosphatase treatment. The complex I appeared prominently (lane 1) with nuclear extract from HD11 cells maintained in culture for prolonged times.

dependent on phosphorylation. If this were true, one could speculate that binding activity to complex I, which was shown to be quickly activated by LPS, is also regulated by phosphorylation of the phosphoprotein pp40, an inhibitor of Rel and NF-κB. pp40 has been shown to be part of the Rel complex present in the cytoplasm and in the nucleus of WEHI-231 cells, and the activity of pp40 to inhibit the DNA binding of Rel and NF-κB is modulated by phosphorylation [37].

LPS stimulates CAT expression controlled by the lysozyme promoter region

It has been shown above that binding to the lysκB is enhanced by LPS. To examine the effect of LPS on the lysozyme promoter activity, HD11 cells were transiently transfected with the plasmid pcPCAT containing the CAT gene controlled by the lysozyme promoter region ($-579/+14$). The CAT reporter construct, under control of the thymidine kinase promoter from herpes simplex virus (pBLCAT4), and the promoterless parent plasmid pBLCAT5 served as controls. Transfected cells were subsequently treated with 5 μg/ml LPS or with DMSO as control. As shown in Table 1, expression of the pBLCAT4 is only 2-fold inducible by LPS, whereas expression of the pcPCAT is activated 6-fold in LPS-stimulated HD11 cells. This response is due to the presence of the lysozyme promoter region because no induction of CAT expression of the parent plasmid pBLCAT5 was observed in LPS-stimulated HD11 cells.

p65 subunit and c-Rel activate expression controlled by the lysozyme promoter region

To test the transactivation of NF-κB/Rel on the expression of the lysozyme gene, transient co-transfections of pcPCAT were performed with the expression vectors pRcCMV-p50, pRcCMV-p65, and pRSV-c-Rel into HD11 cells. The p65 and p50 subunits of NF-κb encoded by these plasmids are active in DNA binding and transactivation [26,27]. Co-transfections with pRcCMV-0 and pRSV-0 that do not contain the coding sequence for p50, p65 and c-Rel served as controls. Table 2 shows that the expression of transfected pcPCAT was significantly elevated in comparison with controls in response to co-transfection of pRcCMV-p65, pRSV-c-Rel, or pRcCMV-p65 plus pRSV-c-Rel into HD11 cells (36-fold, 5.6-fold and 24-fold, respectively). In contrast, overexpression of the p50 subunit or simultaneous overexpression of p50 and p65 led to reduction of CAT expression from pcPCAT, indicating that p50/p50 homodimers or p50/p65 heterodimers are not necessary for the lysozyme expression. Reduction of expression of the pcPCAT caused by p50 could be explained by the complex formation of overexpressed p50 with endogenous p65. The transactivation by the p65 subunit was greater than that by c-Rel or by simultaneous overexpression of p65/c-Rel. This result may indicate that c-Rel/c-Rel homodimers and p65/c-Rel heterodimers have a lower transactivation potential than p65/p65 homodimers.

Table 1 CAT expression controlled by the lysozyme promoter region is activated by LPS

HD11 cells were transfected with plasmids pcPCAT, pBLCAT4 and pBLCAT5. Transfected cells were treated with 10 μl of DMSO per 9-cm tissue culture dish as control, or with 5 μg/ml LPS from *Salmonella typhimurium* for 24 h before harvesting for preparation of cell extracts. The values shown are relative to the CAT activity of cells transfected with pcPCAT and treated with DMSO (1.0). Standard deviations were less than 20%.

	Relative CAT activity	
	DMSO	LPS
pcPCAT	1.0	6.4
pBLCAT4	7.0	13.5
pBLCAT5	0.5	0.7

The lysκB of the lysozyme promoter mediates high basal expression and transactivation by p65 and c-Rel

To determine the role of the lysκB in transactivation by p65 and c-Rel, the p4lysκBCAT containing four tandem copies of the lysκB cloned upstream of the human CRH promoter and expressing the CAT reporter gene was used for co-transfections with p65 and c-Rel expression vectors. The CAT gene driven by the human CRH promoter was previously shown to be expressed in chicken myelomonocytic cells [25]. The human CRH promoter has no κB-like sequences and is not activated by NF-κB/Rel (results not shown). As shown in Table 3, the lysκB mediates a 5-fold higher basal expression of the plasmid p4lysκBCAT

Table 2 NF-κBp65 and c-Rel activate expression controlled by the lysozyme promoter region

HD11 cells were co-transfected with the reporter plasmid pcPCAT and expression vectors pRSV-c-Rel (c-Rel), pRcCMV-p50 (p50) and pRcCMV-p65 (p65), and control plasmids pRSV-0 (RSV-0) and pRcCMV-0 (CMV-0); 24 h after transfection, cells were harvested and assayed for CAT activity. The values, relative to the CAT activity of cells co-transfected with pcPCAT and RSV-0, represent the average of three independent experiments. Deviations from the averages were less than 20%.

	Relative CAT activity						
	RSV-0	CMV-0	p50	p65	c-Rel	p65 c-Rel	p65 p50
pcPCAT	1.0	1.0	0.07	36.4	5.6	24.2	0.09

Table 3 Functional analysis of the lysκB of the lysozyme promoter

CAT reporter plasmids p4mlysκBCAT and p4lysκBCAT were co-transfected with expression vectors pRSV-c-Rel (c-Rel), pRcCMV-p65 (p65), control plasmids pRSV-0 (RSV-0), or pRcCMV-0 (CMV-0) into HD11 cells; 24 h after co-transfection, cells were harvested for preparation of cell extracts. For LPS induction, HD11 cells were transfected with the two plasmids p4mlysκBCAT and p4lysκBCAT respectively. Transfected cells were treated with DMSO as control or with 5 μg/ml LPS for 24 h before harvesting for preparation of cell extracts. Values shown are relative to the CAT activity of cells co-transfected with p4mlysκBCAT and pRSV-0 or transfected with p4mlysκBCAT alone and treated with DMSO (1.0). Standard deviations were less than 20%.

	Relative CAT activity					
	RSV-0	CMV-0	p65	c-Rel	DMSO	LPS
p4mlysκBCAT	1.0	0.9	1.3	0.8	1.0	1.7
p4lysκBCAT	5.0	4.8	132.4	80.3	4.5	15.7

compared with the expression of the control plasmid, possibly because of binding of NF-κB/Rel-like proteins with the lysκB. Furthermore, overexpression of p65 or c-Rel activated the p4lysκBCAT controlled by the lysκB 27-fold or 16-fold respectively, whereas the mlysκB (see oligonucleotides) failed to mediate transactivation by p65 and c-Rel. These results show clearly that the lysκB within the lysozyme promoter is able to mediate transactivation by p65 and c-Rel.

To determine whether the lysκB can mediate LPS activation, the CAT constructs pm4lysκBCAT containing four tandem copies of the mlysκB and p4lysκBCAT were transfected into HD11 cells. Treatment of transfected cells with LPS revealed a 3.5-fold increase in CAT activity from p4lysκBCAT, but only a 1.7-fold increase with the p4mlysκBCAT (Table 3). Although LPS induction is mediated not only by the lysozyme promoter (6-fold) but also by the lysκB alone (3.5-fold), the difference in the extent of LPS induction (6-fold versus 3.5-fold) should not be left unmentioned. It may be explained by assuming that the lysκB within the lysozyme promoter works more effectively with the transcriptional machinery than in the CRH promoter context or, perhaps more probably, the lysκB acts co-operatively with other elements within the lysozyme promoter to mediate LPS induction.

DISCUSSION

This study demonstrates that NF-κB/Rel-like proteins present in chicken myelomonocytic HD11 cells bind to the lysκB of the chicken lysozyme promoter. The data show that a c-Rel-homologous protein, possibly c-Rel, is a component of these protein complexes. The lysozyme gene is expressed at low level in myeloid cells and is progressively activated to a high expression level in terminally differentiated macrophages [3,4]. This activation seems to correlate temporally with an increase in NF-κB/Rel binding activity during the terminal differentiation of macrophages [22]. The results in this study indicate a possible role of NF-κB/Rel, of the p65 and c-Rel in activation of transcription of the lysozyme gene. It is most likely that this activation is the result of increased NF-κB/Rel binding activity during the terminal differentiation of macrophages to the lyskB within the promoter of the lysozyme gene. Several arguments support this suggestion. First, NF-κB/c-Rel-like protein complexes can bind *in vitro* to the lysκB; this binding activity is weak in unstimulated HD11 cells, but is induced in LPS-activated HD11 cells. Interestingly, the position of lysκB coincides with the HD11-specific footprint *in vitro* (−175/−155) as observed by Altschmied et al. [38]. Secondly, when placed upstream of the human CRH promoter adjacent to the CAT gene, the lysκB can mediate a higher level of basal expression of the CAT gene transfected into HD11 cells. The results shown here are consistent with the observation by Luckow and Schütz [39], who showed that point mutations at positions −165 and −158 within the lysozyme promoter caused a 5–6-fold reduction in the lysozyme promoter activity. Thirdly, overexpression of p65 and c-Rel in HD11 cells clearly induces expression of the CAT gene controlled by the lysκB. This result led to the assumption that p65 and c-Rel could be components of the protein complexes that bind to the lysκB. Because anti-chicken NF-κB antibodies are not available, a development of antisera against chicken NF-κBp50 and NF-κBp65 should help in directly determining the exact composition of the DNA binding complexes. In addition, when overexpressed in HD11 cells, the p65 subunit of NF-κB stimulated cell adherence and growth arrest, whereas c-Rel and p50 had no effect on HD11 cells (results not shown).

Previous studies indicated a role of NF-κB/Rel in the development of macrophages. Interestingly, treatment with antisense oligonucleotides complementary to the p65 subunit caused inhibition of cell adhesion [40,41] and growth inhibition of transformed cells [42]. These results, in the context of the NF-κB/Rel-regulated expression of the lysozyme gene, provide further evidence for functions of NF-κB/Rel proteins in the activation of genes involved in the terminal differentiation of macrophages.

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