Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5056–5060, May 1994
Biochemistry

Qualitative changes in the subunit composition of κB-binding complexes during murine B-cell differentiation

SHIGEKI MIYAMOTO, MARK J. SCHMITT, AND INDER M. VERMA

Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800

Communicated by Harold Weintraub, February 16, 1994

ABSTRACT **We report here that the major κB-binding complex in murine mature B cells is composed of a p50–Rel heterodimer, whereas the major inducible form in pre-B cells is a p50–p65 heterodimer. Treatment of a pre-B-cell line with lipopolysaccharide changes the subunit composition of κB-binding complexes from p50–p65 to p50–Rel. This change is preceded by the enhanced Rel expression and correlates with the expression of the gene for the immunoglobin κ light chain. The heterodimeric p50–Rel complex binds to the intronic enhancer-κB site in the immunoglobulin κ light chain gene at least 20-fold more stably than does the p50–p65 dimer. These data support a model in which augmentation of Rel expression during the differentiation of pre-B cells to mature B cells leads to an exchange of κB-binding subunits resulting in the transcriptional activation of the gene for the immunoglobulin κ light chain.**

The expression of the immunoglobulin κ light chain (Igκ) gene is restricted to the B-cell lineage, specifically to mature B cells and plasma cells (1). One of the transcription factors that regulates Igκ gene is nuclear factor κB (NF-κB), which binds to the decameric enhancer element, GGGACTTTCC (κB site), located in the κ intronic enhancer (2). Original studies identified NF-κB as a tissue-specific factor present only in mature B cells; however, subsequent studies revealed the presence of inactive NF-κB complexes in the cytoplasm of many cell types (3). NF-κB is sequestered in the cytoplasm by an inhibitor protein IκB (4) by a direct protein–protein interaction through its ankyrin repeat motifs (5–7). Genes encoding κB-binding proteins form a family of related genes that include *NFKB1* (p50/p105), *NFKB2* (p52/p100), v-*rel*, c-*rel*, *relA* (p65), *relAΔ* (p65Δ), and *relB* (8–21). Accumulated data argue (22) for the presence of multiple κB-binding complexes *in vivo* composed of various members of this protein family.

Although the κB-binding complex in mature B cells is generally referred to as a heterodimer of p50 and p65 (8–13), the exact subunit composition has not been determined. Since the expression of Rel, one of the members of the Rel/NF-κB family, has been shown to be augmented in this cell type (15, 23), it may constitute a subunit of κB-binding complexes in these cell types. Furthermore, the Rel-containing complexes may be stage-specific in mature B cells. To examine this possibility, we have characterized the presence of Rel proteins in the κB-binding complexes in mature murine B-cell lines and sought its functional significance in this cell type.

MATERIALS AND METHODS

Cell Culture and Antisera. The murine pre-B cell lines (70Z/3, RAW253, and ABE8.1/2) and B-cell lines (WEHI231, WEHI279, and CH33) were cultured in RPMI medium 1640 (GIBCO) supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 50 μM 2-mercaptoethanol, 1250 units of penicillin G, and streptomycin sulfate (0.5 mg/ml). Rel antiserum 5075 has been described (24). Anti-p65 serum was raised against N-terminal 19-aa peptide (MDDLFPLIFPSEPAQASGP). Anti-p50 sera were raised against a glutathione *S*-transferase–p50 fusion protein, whose gene is constructed by cloning murine *NFKB1*–p50 (*Apa* I digested, Klenow fragment filled-in, *Eco*RI linker added, and finally the 1.4-kb *Eco*RI fragment containing N-terminal Rel homology domain generated) into *Eco*RI site of pGEX-2TK (a gift from W. Kaelin, Dana–Farber Cancer Institute and Harvard University Medical School).

Metabolic Labeling and Immunoprecipitation. Approximately 10^7 cells were incubated with methionine/cysteine-free Dulbecco's modified Eagle's medium (1 ml) containing 10% dialyzed fetal bovine serum, 50 μM 2-mercaptoethanol, nonessential amino acids (GIBCO), and 2 mM glutamine. [^{35}S]Methionine/cysteine was added at 1 mCi/ml (1 Ci = 37 GBq) and further incubated for 2 h. After rinsing with phosphate-buffered saline, the cells were lysed in IP buffer (20 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM EDTA/0.2% Nonidet P-40/0.2% deoxycholate). SDS was added to 0.5% and the cell lysates were then boiled for 5 min and diluted 1:5 with the IP buffer. After preclearing with protein A-Sepharose (Pharmacia), affinity-purified Rel antiserum and protein A-Sepharose were added and further incubated for 4 h. The Sepharose beads were rinsed five times with the IP buffer, and the Rel proteins bound to the Sepharose were released by boiling in SDS sample buffer and resolved by SDS/PAGE.

Electrophoretic Mobility Shift Assays (EMSAs). Approximately 2×10^8 cells were used to prepare cellular extracts from 70Z/3 and WEHI231 cells (24, 25). The protein concentration of extracts was ≈5 mg/ml. EMSAs were performed as described (24). Briefly, 5 μg of cytoplasmic or nuclear extract was incubated with 1 μg of poly(dI-dC) (Pharmacia) for 20 min on ice and ^{32}P-labeled double-stranded oligonucleotides containing the κB site from the Igκ gene (5′-TCAACAGAGGGACTTTCCGAGAGGCC-3′) or a palindromic human interleukin 2 receptor α κB site (5′-CAACGGCAGGGGAATTCCCCTCTCCTT-3′; ref. 26) were added. The mutated κB oligonucleotides used for competition were 5′-TCAACAGAGCTCACTTTATGAGAGGCC-3′ and 5′-CAACGGCAGATCAATCTCCCTCTCCTT-3′ for Igκ and interleukin 2 receptor α κB site, respectively. Production of bacterially expressed murine Rel and truncated Rel466 has been described (24).

UV-Crosslinking. Crosslinking was done as in ref. 26 with minor modifications. Briefly, the palindromic κB probe (0.5 μg) was annealed to 0.2 μg of complementary 10-mer (5′-AAGGAGAGGG-3′) and filled-in with the Klenow fragment of DNA polymerase I in 10 mM Tris·HCl, pH 7.4/6 mM $MgCl_2$/50 mM NaCl/6 mM 2-mercaptoethanol/gelatin (0.2 mg/ml)/50 μM BrdUTP/5 μM dATP/5 μM dGTP/5 μM dCTP/250 μCi of [α-^{32}P]dATP/250 μCi of [α-^{32}P]dGTP/250 μCi of [α-^{32}P]dCTP. The binding reaction was done with 50

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Igκ, immunoglobulin κ light chain; NF-κB, nuclear factor κB; EMSA, electrophoretic mobility shift assay; LPS, lipopolysaccharide.

μg of extract and BrdUrd-substituted probe (10^6 cpm). The reaction mixture was transferred to a round-bottom 1.5-ml tube (Nunc), covered with plastic wrap, and irradiated from a distance of 5 cm with a UV transilluminator (Photodyne, New Berlin, WI) with the analytical setting for 1 h at room temperature. The resulting DNA–protein adducts were resolved by SDS/PAGE. Northern blot analysis was carried out as described (26). The probes were murine *rel* (1.8-kb *Bam*HI–*Dra* I fragment) and murine Igκ gene (0.5-kb *Bgl* II fragment of the pK construct).

RESULTS

Major κB-Binding Complexes in 70Z/3 and WEHI231 Cells Are p50–p65 and p50–Rel, Respectively. We explored the possibility that Rel is a component of κB-binding complexes in mature B cells, as at least 10-fold more Rel was expressed in mature B cells (Fig. 1, lanes 5–7) compared to pre-B cells (lanes 1–3). 70Z/3 and WEHI231 cells, a pre-B-cell line and a mature B-cell line, respectively, were initially studied in detail to determine the subunit composition of κB-binding complexes by EMSA (Fig. 2*A*). Cytoplasmic (Fig. 2*A*, lane 1) and nuclear (lane 5) extracts from unstimulated 70Z/3 cells showed a very low level of DNA binding activity. The three resulting complexes were termed C_1, C_2, and C_3. When the cells were treated with lipopolysaccharide (LPS) for 2 h, a marked increase in C_2 binding activity was observed (lane 7). Treatment of the cytoplasmic extract from unstimulated 70Z/3 cells with detergents, deoxycholate and Nonidet P-40, resulted in the increased activity of the inactive C_2 complex (lane 2). In contrast, the constitutive κB-binding activity was observed in the cytoplasmic (lane 10) and nuclear (lane 13) extracts of WEHI231 cells in the absence of LPS stimulation. The specificity of the complexes was shown by competition

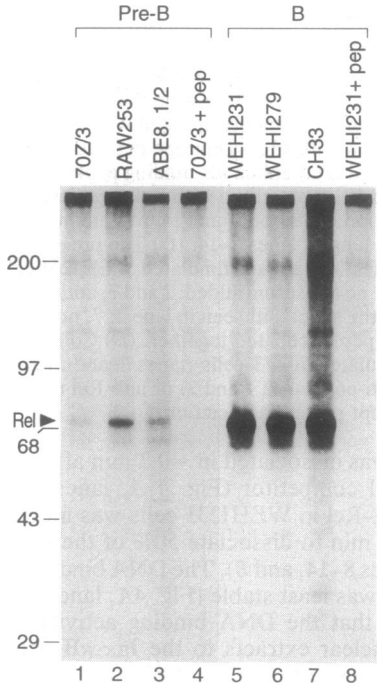

FIG. 1. Augmented expression of Rel proteins in mature murine B cells. Pre-B- (lanes 1–4) and mature B- (lanes 5–8) cell lines were metabolically labeled with [^{35}S]methionine/cysteine and immunoprecipitated with Rel-specific antiserum 5075 (24). The specificity of immunoprecipitation was shown by competition with excess epitope peptide (pep) (lanes 4 and 8 for 70Z/3 and WEHI231 cells, respectively). The molecular mass markers are shown in kDa. Arrowhead, position of Rel proteins.

with a 50-fold excess of unlabeled wild-type (lanes 3, 8, 11, and 14) and mutant (lanes 4, 9, 12, and 15) oligonucleotides.

To delineate the subunit composition of each of the κB-binding complexes identified by EMSA in pre-B and B cells, UV-crosslinking of DNA–protein complexes was performed (26, 28). The crosslinked κB DNA–protein adducts were immunoprecipitated with antisera specific to Rel, p65, or p50. As seen in Fig. 2*B*, the 80-, 70-, and 55-kDa proteins seen in 70Z/3 cells and the 80- and 55-kDa proteins in WEHI231 cells are Rel (lanes 2 and 9), p65 (lane 4), and p50 (lanes 6 and 13), respectively. The alternate lanes in these figures show the specificity of immunoprecipitation by competition with excess epitope peptides (lanes 3, 5, 10, and 12) or with preimmune serum (lanes 7 and 14). In addition to the Rel proteins, there is a faster-migrating band immunoprecipitated with Rel antiserum (lanes 2 and 9) that probably represents partially degraded Rel protein. These observations were further substantiated by gel supershift experiments using the Igκ κB site (Fig. 2*C*), which showed that the majority of the C_2 complex contained p65 (lane 2) with a small amount of Rel (lane 4; see Fig. 3 for various times after LPS stimulation). In contrast, excess p65 antiserum had no effect (lane 7) but anti-Rel serum showed complete supershift of the Cβ complex (lane 9). The alternate lanes (lanes 3, 5, 8, and 10) show the supershift specificity by competition with excess epitope peptide. In all cases, the C_3 or C_γ complexes were not affected as they contain only p50 proteins as demonstrated by *in situ* crosslinking (data not shown).

Although the data in Fig. 2 *B* and *C* show that the 70Z/3 cells contain complexes containing predominantly p65 and p50 and that WEHI231 cells contain Rel and p50, they do not prove that these proteins exist as heterodimers. We therefore tested whether p50 formed complexes with Rel and p65 by coimmunoprecipitation with Rel and p65 antisera after UV-crosslinking. Each sample was tested with and without boiling in SDS prior to immunoprecipitation. As seen in Fig. 2*D*, p50 coimmunoprecipitated with p65 in 70Z/3 nuclear extract (lane 1) and with Rel in WEHI231 nuclear extract (lane 3). Boiling prior to immunoprecipitation disrupted this association (lanes 2 and 4). These results suggest that the major components of C2 and Cβ are p50–p65 and p50–Rel, respectively. Although these data demonstrate that p50–p65 or p50–Rel and $(p50)_2$ constitute the major κB-binding complexes in 70Z/3 or WEHI231 cells, respectively, they do not exclude the possibility of other minor forms of κB complexes.

Prolonged Stimulation of 70Z/3 Cells with LPS Results in Rel Induction Followed by the Appearance of p50–Rel and Igκ Induction. We next examined how p50–p65 in pre-B cells changed to p50–Rel during differentiation to mature B cells. Prolonged treatment of 70Z/3 cells with LPS has been shown to induce expression of the Igκ gene and subsequent appearance of surface immunoglobulin, a marker of mature B cells (29). Thus, LPS stimulation of 70Z/3 cells partially mimics pre-B-cell to B-cell differentiation. Fig. 3*A* shows that the κB-binding activity appeared in the nucleus 30 min (lane 2) after LPS stimulation, reached maximal level at 2 h (lane 4), and remained active over the 24-h period examined. To examine the subunit composition of these complexes, κB-binding proteins were examined by UV-crosslinking. A gradual change in the composition of κB-binding complexes was observed from p50–p65 at 30 min (Fig. 3*B*, lane 2) to p50–Rel at 24 h (lane 9). Northern blot analysis of total RNA isolated from the LPS-stimulated 70Z/3 cells at the same times showed that the *rel* gene transcription was induced 1 h after LPS stimulation (Fig. 3*C*, lane 3) concomitant with the appearance of p50–Rel and remained highly expressed (lanes 3–9). The data display sequential events that include p50–p65 activation (Fig. 3*A*, lane 2), Rel induction (Fig. 3*C*, lane 3), and the appearance of p50–Rel (Fig. 3*B*, lane 3). As the murine *rel* promoter contains functional κB sites (30), it is

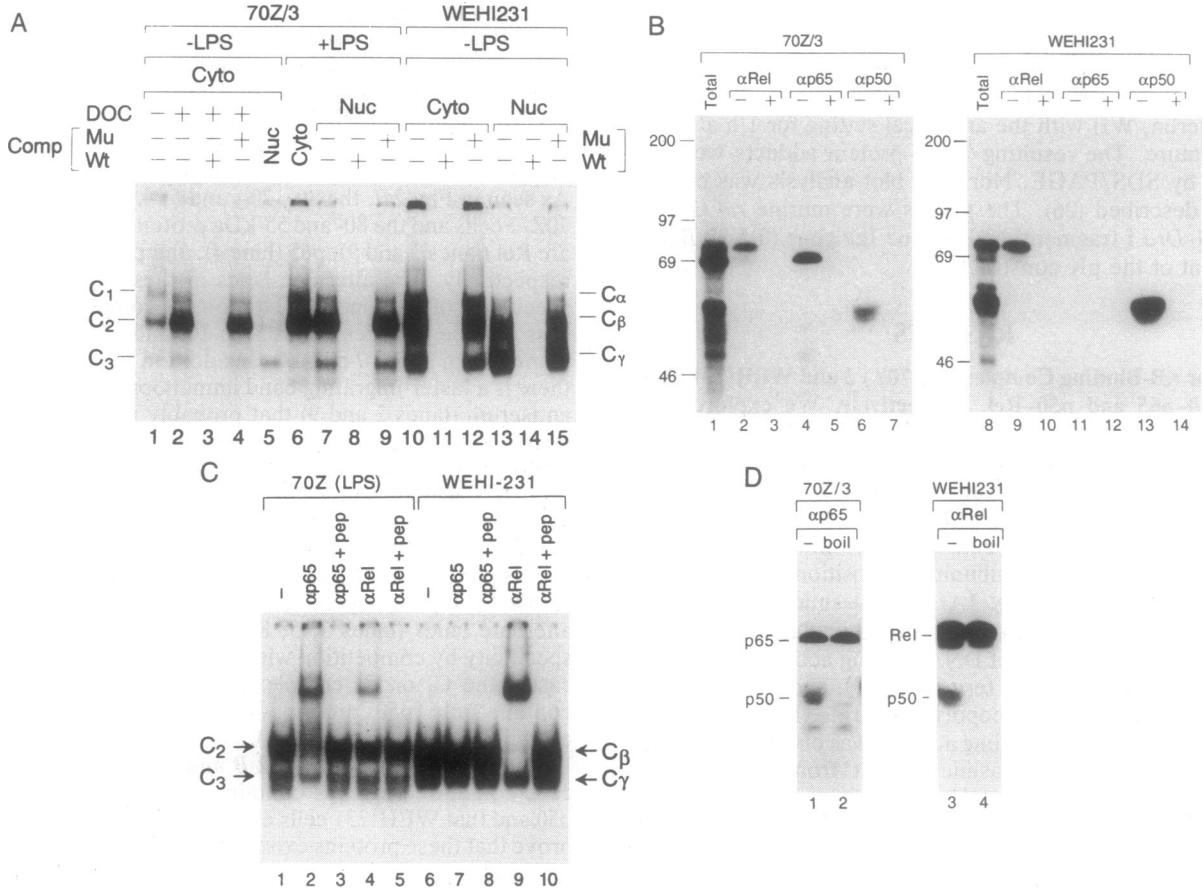

FIG. 2. κB-binding proteins in 70Z/3 and WEHI231 cells. (*A*) EMSA of cell extracts using a palindromic human interleukin 2 receptor α κB site. Cytoplasmic (Cyto) (5 μg) and nuclear (Nuc) (5 μg) extracts were incubated with 1 μg of poly(dI-dC) and ^{32}P-labeled probe (0.2 ng), and the resulting κB complexes were resolved by nondenaturing PAGE on a 4% gel. Lanes: 1, cytoplasmic extract from unstimulated 70Z/3 cells; 2–4, same extract treated with 0.8% deoxycholate (DOC) and 1.2% Nonidet P-40; 5, nuclear extract from unstimulated 70Z/3 cells; 6, cytoplasmic extract from 2-h LPS-stimulated cells; 7–9, nuclear extract from LPS-stimulated cells; 10–12, WEHI231 cytoplasmic extract; 13–15, WEHI231 nuclear extract; 3, 8, 11, and 14, competition with 50× molar excess of unlabeled palindromic (Wt) oligonucleotide; 4, 9, 12, and 15, competition with 50× molar excess of unlabeled mutated (Mu) oligonucleotide. (*B*) Nuclear extracts isolated from LPS-stimulated 70Z/3 cells (lanes 1–7) and unstimulated WEHI231 cells (lanes 8–14) were UV-crosslinked, boiled in 0.5% SDS, and immunoprecipitated with antisera (α) specific to murine Rel, p65, and p50. The specificity of immunoprecipitation was tested by competition with excess epitope peptide or preimmune serum (− and + show the absence and presence of competition, respectively). Lanes: 1 and 8, total crosslinked κB-binding peptide in LPS-stimulated 70Z/3 cells and WEHI231 cells, respectively; 2 and 9, anti-Rel immunoprecipitation; 3 and 10, competition with Rel peptides; 4 and 11, anti-p65 immunoprecipitation; 5 and 12, p65 epitope peptide competition; 6 and 13, anti-p50 immunoprecipitation; 7 and 14, p50 preimmune precipitation. Molecular mass markers are shown on the left in kDa. Lanes 1–7 were exposed twice as long as lanes 8–14. (*C*) The presence of p65 and Rel in NF-κB complexes in the nuclear extracts isolated from LPS-stimulated 70Z/3 cells (lanes 1–5) and unstimulated WEHI231 cells (lanes 6–10) was assayed by gel supershift assays using specific antisera and Igκ κB site. Lanes: 1 and 6, no antiserum added; 2 and 7, antiserum to p65 added; 3 and 8, antiserum to p65 and excess epitope peptides to p65 added to show specificity of the supershift seen in lane 2; 7, no supershift activity; 4 and 9, antiserum to Rel added; 5 and 10, antiserum to Rel and excess epitope peptide (pep) incubated together. (*D*) Coimmunoprecipitation of p50 using anti-Rel (αRel) and anti-p65 (αp65) sera. Nuclear extracts from LPS-stimulated 70Z/3 cells (lanes 1 and 2) and unstimulated WEHI231 cells (lanes 3 and 4) were crosslinked as in *B* and immunoprecipitated with anti-p65 (lanes 1 and 2) or anti-Rel (lanes 3 and 4) sera. Samples in lanes 2 and 4 were boiled in 0.5% SDS prior to immunoprecipitation to disrupt protein association.

likely that induction of NF-κB (p50–p65) leads to the activation of *rel* transcription.

To examine which of the two κB-binding complexes, p50–p65 or p50–Rel, is involved in the transcriptional activation of Igκ gene, the level of Igκ mRNA was measured in 70Z/3 cells stimulated with LPS. As shown in Fig. 3*D*, the Igκ mRNA level increased steadily over 24 h and correlated with the appearance of p50–Rel (Fig. 3*B*; ref. 31). There was little or no overlap with the level of Igκ mRNA expression with p50–p65, which peaked at 2 h and declined thereafter (Fig. 3*B*).

p50–Rel Binds to Igκ κB Site >20-Fold More Stably Than p50–p65. We next compared the stability of p50–Rel and p50–p65 bound to the Igκ κB site by measuring the dissociation rates. Fig. 4*A* shows the EMSA analysis and Fig. 4*B* displays the densitometric tracings of the κB-binding complexes. More than half of the initial p50–p65 in LPS-treated 70Z/3 cells was dissociated in ≈0.3 min after the addition of the unlabeled competitor (Fig. 4 *A*, lanes 1–7, and *B*). In contrast, p50–Rel in WEHI231 cells was much more stable, requiring >7 min to dissociate 50% of the original complex (Fig. 4 *A*, lanes 8–14, and *B*). The DNA binding activity of p50 homodimers was least stable (Fig. 4*A*, lanes 8–14). The data demonstrate that the DNA binding activity of p50–Rel in WEHI231 nuclear extracts to the Igκ κB site was at least 20-fold more stable than that of p50–p65 in LPS-stimulated 70Z/3 cells.

DISCUSSION

Using a variety of methods including EMSA, UV-crosslinking, immunoprecipitation, and gel supershift analyses, we have demonstrated that the κB-binding complex in mature B-cell lines is predominantly p50–Rel and not p50–p65 (NF-

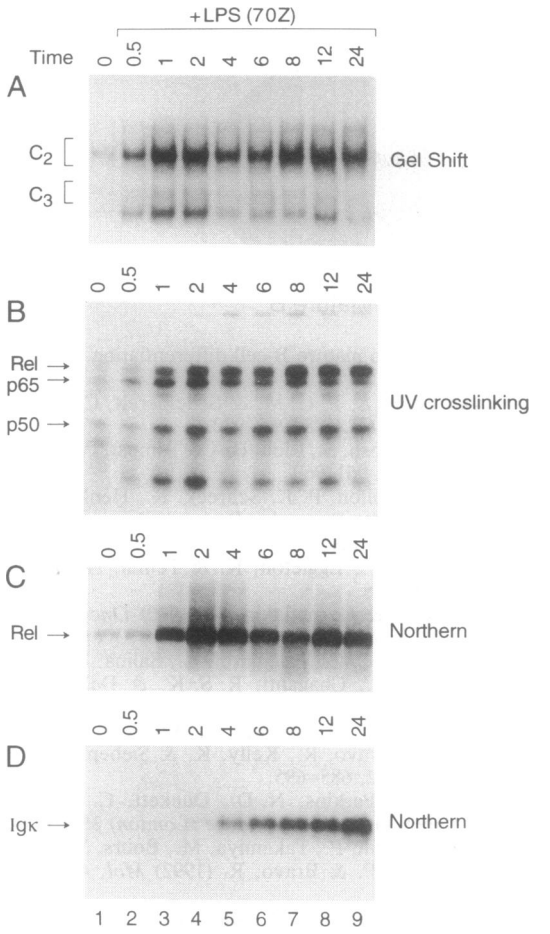

FIG. 3. Time course of the induction of p50–Rel complex, Rel mRNA, and Igκ mRNA in 70Z/3 cells stimulated with LPS for prolonged period. 70Z/3 cells were stimulated with LPS (10 μg/ml) and nuclear extracts and RNA was isolated at the times indicated in hours. (*A*) EMSA of nuclear extracts in which the Igκ κB site is used to show the induction of κB-binding complexes within 30 min of LPS stimulation and its sustained activation thereafter. (*B*) UV-crosslinking of the nuclear extracts as in Fig. 2*B* showing the appearance of increasing amounts of Rel proteins and the appearance and disappearance of p65 in the κB-binding complexes after LPS stimulation. (*C*) Northern blot analysis of Rel mRNA showing its upregulation 1 h after LPS stimulation. (*D*) Northern blot analysis of Igκ mRNA showing its appearance 2 h after LPS stimulation and increased accumulation with time. The equal loading of the RNA for each lane was confirmed by hybridization to the glyceraldehyde-3-phosphate dehydrogenase gene (data not shown).

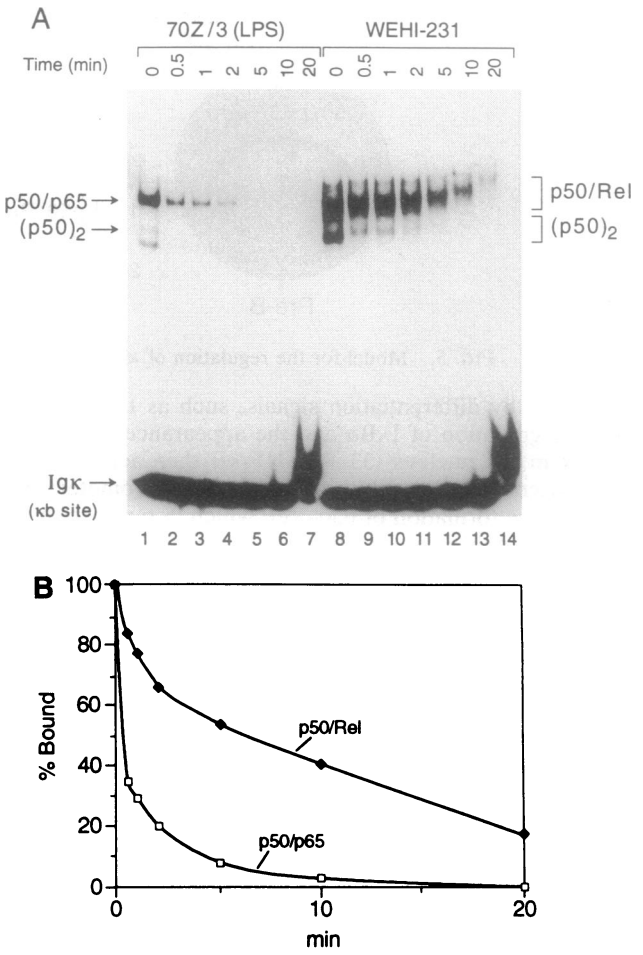

FIG. 4. p50–Rel binds to the Igκ κB site at least 20-fold more stably than does p50–p65. (*A*) Nuclear extracts isolated from LPS (1 h)-stimulated 70Z/3 cells and unstimulated WEHI231 cells were incubated with radiolabeled Igκ κB site oligonucleotide for 20 min then a 50× excess of unlabeled competitor Igκ κB site oligonucleotide was added. After the addition of unlabeled competitor, equal fractions were removed and analyzed by EMSA. Lanes: 1–7, 70Z/3 cells; 8–14, WEHI231 cells. Positions of p50–p65, p50–Rel, and p50–p50 are indicated. (*B*) The intensity of each complex was measured by densitometry and plotted against time. The intensity at the 0 time is set as 100%. More than 50% of p50–p65 at 0 time dissociated in ≈20 sec, whereas it took >7 min to dissociate 50% of p50–Rel.

κB). In contrast, the major inducible form of κB-binding complex in pre-B-cell lines is p50–p65. Thus, during the maturation of murine B cells, the subunits of κB-binding complexes are changed from p65 to Rel, a change that can be directly induced in 70Z/3 cells by prolonged stimulation with a differentiation agent, LPS (Fig. 3).

The change in the composition of κB-binding subunits is concomitant with the augmentation of *rel* transcription. The level of Rel proteins is at least 10-fold greater in mature B cells than pre-B cells (Fig. 1), whereas the expression of the gene encoding p65 does not change (12). Therefore, increased concentration of Rel proteins induces formation of p50–Rel complexes during pre-B- to mature B-cell differentiation. Rel is the major component of the complex and not RelB, as shown in primary B cells of transgenic mice (32), because nearly all the Cβ complex is supershifted with Rel-specific antiserum (Fig. 2*C*). Although the data presented here rely only on two established cell lines, the altered subunit composition of κB-binding complexes is also mirrored in a number of other pre-B-cell (AKAI.GI, RAW253, NFS25.C3, and ABE8.1/2) and B-cell (WEHI279, CH33, and CH31) lines and pre-B cells obtained from bone marrows and mature B cells obtained from spleens of naive mice (S.M. and P. Linton, unpublished observation).

It is puzzling that the constitutive form of κB-binding complex in mature B cells is p50–Rel instead of p50–p65 (Fig. 2*D*), which is the major inducible form in pre-B cells. Furthermore, both p50–Rel and p50–p65 can activate transcription from Igκ intronic enhancer in a heterologous system (S.M., unpublished results). Perhaps one clue is provided by the dissociation rates of p50–Rel, which is >20-fold slower than p50–p65 from the Igκ κB site (Fig. 4). p50–Rel is more stably bound and thus probably a more efficient activator of the Igκ gene *in vivo*.

We propose a model (Fig. 5) to explain the regulation of κB-binding complexes during pre-B- to mature B-cell differentiation based on the above observations and other available data. In pre-B cells, the cytoplasmic p50–p65–IκBα complex

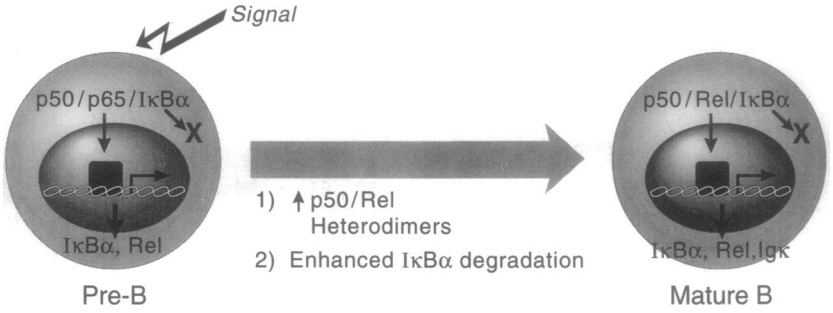

FIG. 5. Model for the regulation of κB-binding activity during murine pre-B- to mature B-cell differentiation.

is activated by differentiation signals, such as LPS, which lead to degradation of IκBα and the appearance of NF-κB complex in the nucleus (33–35). NF-κB then activates *rel* gene transcription through the κB sites in its promoter (30) leading to the formation of p50–Rel, which in turn activates Igκ intronic enhancer, resulting in the production of Igκ proteins, a marker for mature B cells. The p50–Rel complex may also activate other NF-κB target genes, such as genes encoding p105 (the p50 precursor), Rel, and IκBα (a NF-κB inhibitor). Activation of the genes encoding Rel and p105 by p50–Rel ensures the sustained production of p50–Rel in mature B cells. However, augmented Rel expression and subsequent exchange of κB-binding subunits is not sufficient to sustain constitutive activation of p50–Rel and Igκ transcription. There is at least another event during pre-B- to mature B-cell differentiation that causes continuous and rapid degradation of IκBα to sustain constitutive p50–Rel activity in mature B cells (36).

We are grateful to Drs. T. Hunter, R. Wisdom, and E. Bengal for critical reading of the manuscript during its preparation. We thank Dr. P. Chiao for some of the materials, members of the Verma lab and Dr. J.-I. Inoue for discussions and expression constructs, Dr. R. Hymen for some of the pre-B- and B-cell lines, Dr. R. Grosschedl for Igκ enhancer and probe plasmids, Drs. P. Linton and N. Kleinman for primary pre-B and mature B cells, and P. McClintock for preparation of the manuscript. This work was supported by funds from the National Institutes of Health, the American Cancer Society, the H.N. and Frances C. Berger Foundation, and the Council for Tobacco Research–USA, Inc., to I.V. S.M. was supported by a fellowship from the National Institutes of Health. I.V. is an American Cancer Society Professor of Molecular Biology.

1. Staudt, L. M. & Lenardo, M. J. (1991) *Annu. Rev. Immunol.* **9**, 373–398.
2. Sen, R. & Baltimore, D. (1986) *Cell* **46**, 705–716.
3. Baeuerle, P. A. & Baltimore, D. (1988) *Cell* **53**, 211–217.
4. Baeuerle, P. A. & Baltimore, D. (1988) *Science* **242**, 540–546.
5. Inoue, J.-I., Kerr, L. D., Rashid, D., Davis, N., Bose, H. R., Jr., & Verma, I. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4333–4337.
6. Leveillard, T. & Verma, I. M. (1993) *Gene Exp.* **3**, 135–150.
7. Hatada, E. N., Naumann, M. & Scheidereit, S. (1993) *EMBO J.* **12**, 2781–2788.
8. Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P. & Baltimore, D. (1990) *Cell* **62**, 1019–1029.
9. Kieran, M., Blank, V., Logeat, F., Vandekerckhove, J., Lottspeich, F., LeBail, O., Urban, M. B., Kourilsky, P., Baeuerle, P. A. & Israel, A. (1990) *Cell* **62**, 1007–1018.
10. Bours, V., Villalobos, J., Burd, P. R., Kelly, K. & Siebenlist, U. (1990) *Nature (London)* **348**, 76–80.
11. Meyer, R., Hatada, E. N., Hohmann, H.-P., Haiker, M., Bartsch, C., Rothlisberger, U., Lahml, H.-W., Schlaeger, E. J., van Loon, A. P. G. M. & Scheidereit, C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 966–970.
12. Nolan, G. P., Ghosh, S., Liou, H.-C., Tempst, P. & Baltimore, D. (1991) *Cell* **64**, 961–969.
13. Ruben, S. M., Dillon, P. J., Schreck, R., Henkel, T., Chen, C.-H., Maher, M., Baeuerle, P. A. & Rosen, C. A. (1991) *Science* **251**, 1490–1493.
14. Wilhelmsen, K. C., Eggleton, K. & Temin, H. M. (1984) *J. Virol.* **52**, 172–182.
15. Grumont, R. J. & Gerondakis, S. (1990) *Oncogene Res.* **5**, 245–254.
16. Neri, A., Chang, C.-C., Lombardi, L., Salina, M., Corradini, P., Maiolo, A. T., Chaganti, R. S. K. & Dalla-Favera, R. (1991) *Cell* **67**, 1075–1087.
17. Bours, V., Burd, P. R., Brown, K., Villalobos, J., Park, S., Ryseck, R.-P., Bravo, R., Kelly, K. & Siebenlist, U. (1992) *Mol. Cell. Biol.* **12**, 685–695.
18. Schmid, R. M., Perkins, N. D., Duckett, C. S., Andrews, P. C. & Nabel, G. J. (1991) *Nature (London)* **352**, 733–736.
19. Ryseck, R.-P., Bull, P., Takamiya, M., Bours, V., Siebenlist, U., Dobrzanski, P. & Bravo, R. (1992) *Mol. Cell. Biol.* **12**, 674–684.
20. Ruben, S. M., Narayanan, R., Klement, J. F., Chen, C.-H. & Rosen, C. A. (1992) *Mol. Cell. Biol.* **12**, 444–454.
21. Mercurio, F., Didonato, J., Rosette, C. & Karin, M. (1992) *DNA Cell Biol.* **11**, 523–537.
22. Grilli, M., Chiu, J. J.-S. & Lenardo, M. J. (1993) *Int. Rev. Cytol.* **143**, 1–62.
23. Brownell, E., Ruscetti, F. W., Smith, R. G. & Rice, N. R. (1988) *Oncogene* **3**, 93–98.
24. Inoue, J.-I., Kerr, L. D., Ransone, L. J., Bengal, E., Hunter, T. & Verma, I. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3715–3719.
25. Dignam, J. D., Lebovitz, R. M. & Roeder, R. G. (1983) *Nucleic Acids Res.* **11**, 1475–1489.
26. Ballard, D. W., Walker, W. H., Doerre, S., Sista, P., Molitor, J. A., Dixon, E. P., Peffer, N. J., Hannink, M. & Greene, W. C. (1990) *Cell* **63**, 803–814.
27. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
28. Lin, S.-Y. & Riggs, A. D. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 947–951.
29. Paige, C. J., Kincade, P. W. & Ralph, P. (1978) *J. Immunol.* **121**, 641–647.
30. Grumont, R. J., Richardson, I. B., Gaff, C. & Gerondakis, S. (1993) *Cell Growth Differ.* **4**, 731–743.
31. Miller, C. L., Feldhaus, A. L., Rooney, J. W., Rhodes, L. D., Sibley, C. H. & Singh, H. (1991) *Mol. Cell. Biol.* **11**, 4885–4894.
32. Lernbecher, T., Muller, U. & Wirth, T. (1993) *Nature (London)* **365**, 767–770.
33. Brown, K., Park, S., Kanno, T., Franzoso, G. & Siebenlist, U. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2532–2536.
34. Sun, S.-C., Ganch, P. A., Ballard, D. W. & Greene, W. C. (1993) *Science* **259**, 1912–1915.
35. Scott, M. L., Fujita, T., Liou, H.-C., Nolan, G. P. & Baltimore, D. (1993) *Genes Dev.* **7**, 1266–1276.
36. Miyamoto, S., Chiao, P. J. & Verma, I. M. (1994) *Mol. Cell. Biol.*, in press.